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PREFACE

This Special Issue on "Recent Advances in Reproductive Endocrinology and Women’s Health" published by Current Women’s Health Reviews is a two–volume series on both cutting edge and contemporary topics of importance to general gynecologists and specialists alike.

The first volume “Current Concepts in Female Infertility Management” is dedicated to important topics such as endometriosis, PCOS and fibroids, which affect millions of women worldwide. Professor Abdel-Aziz Ismail discusses low-cost infertility management options. His comments— that we should not fail to specify the best cost-effective regimen for our patients and that evidence-based choices can be made without compromising success rates—are very pertinent. Dr. Sekhon has written an excellent and comprehensive chapter analyzing the role that antioxidant supplementation plays in improving female fertility and pregnancy outcomes. This article reviews the current literature on the effects of antioxidant therapy and elucidates whether antioxidant supplementation is useful in preventing and/or treating infertility and poor pregnancy outcomes related to various obstetric and gynaecologic conditions.

There are two articles on PCOS in this special issue by researchers from CASE Medical Center, Cleveland Clinic and Liverpool Women’s Hospital. The article on adolescent PCOS characterizes polycystic ovary syndrome as a heterogeneous endocrinopathy that affects girls and women during their reproductive years. The exact etiology of PCOS is still a topic of debate. This chapter explains why PCOS is a multifactorial syndrome, involving genetic, endocrinologic, metabolic and environmental factors and illustrates that further research on the basic pathophysiology of PCOS and the role of the different etiologic components will aid in the understanding of this condition and help clinicians in their management of adolescents with PCOS. The second article on PCOS, written by Lee et al, substantiates the etiological relationship between PCOS and metabolic syndrome. The authors report a lack of clarity on the role oxidative stress plays in the pathogenesis of PCOS and suggest that there is an association amongst the oxidative microenvironment of the ovarian tissue and ovarian steroidogenesis and follicular development.

The article on Advanced Management Options for Endometriosis focuses on new treatment options for endometriosis while it also briefly describes the pathogenesis, diagnosis and controversies of existing treatment modalities. According to the authors, assisted reproduction holds promise in patients with advanced endometriosis. They highlight that most of the newer therapies are still experimental, but results in animal models show promise, which have served as an impetus for conducting human trials.

Professor Botros Rizk has written an excellent and authoritative chapter on OHSS that explains how this syndrome remains the most serious complication of ovulation induction. According to the authors, OHSS could be successfully prevented in the future if a high index of suspicion is exercised and methodical steps are taken. Newer technologies such as in vitro maturation might completely eliminate its occurrence.

Dr. Tan and colleagues discuss the limitations of current treatment options for women with symptomatic uterine fibroids such as mechanical methods of excision, ablation, and devascularization. According to the authors, increased use of conservative, non-surgical procedures will expand patient eligibility and allow safe and effective long-term resolution of fibroid-related symptoms.

In addition, four articles by leading experts in the field of reproductive health cover various women’s health issues:

- The article on robotics in reproductive surgery, written by Drs. Barakat and Falcone, evaluates the current application of robotics in reproductive surgery. The article highlights the advantages of robotic surgery over conventional laparoscopic surgery.

- Drs. Catenacci and Falcone highlight the pathogenesis of endometriosis and review the current clinical evidence for treatment in regards to improving fertility outcomes. The authors comment that as treatment evolves in this direction, the role diagnostic laparoscopy plays in infertile patients is becoming uncertain. Specifically, the value of diagnostic laparoscopy for patients who do not suffer from pain and have normal imaging studies is in question. Due to the controversial effects that Stage I/II endometriosis has on infertility, recommendations are moving away from performing diagnostic laparoscopies in infertile patients. Ultimately, this will lead to fewer surgeries and increased medical management for patients with infertility-related endometriosis.

- Drs. Bedaiwy and Hurd discuss that the future of fertility preservation for women of reproductive age with cancer is likely to involve removal of ovarian tissue, followed by in vitro follicle culture of the tissue and removal of oocytes. The article highlights that more effective techniques are being developed for cryopreservation of both oocytes and embryos. The authors explain that the surgical approaches for fertility preservation can also be used for reproductive-age women diagnosed with cancer who require pelvic irradiation or systemic chemotherapy.

- Dr. Saman and colleagues highlight the available treatment options for müllerian duct anomalies with a special emphasis on simple and advanced surgical approaches. Surgical options are presented based on a novel treatment plan classification system adapted from the American Fertility Society classification of müllerian duct anomalies. The authors have taken care to include all previously termed unclassified anomalies as well as the important category of longitudinal fusion defects. Important
diagnostic approaches are discussed with special emphasis on detection of associated anomalies of the urinary system and other relevant systems

We hope that the readers will enjoy reading the latest, informative and authoritative articles by some of the most recognized and prolific leaders in reproductive endocrinology from across the globe. We would like to extend our appreciation to all the authors for their hard work and valuable contributions. We are indebted to our colleagues and associates in Cleveland Clinic for their valuable contributions. We gratefully acknowledge the fabulous support of Ms. Amy Slugg Moore (Manager, Medical Editing Services) for her help. We are grateful to Prof. Jose Belizan, Editor in Chief of Current Women’s Health Review, for his constant encouragement and support. We are most thankful to the editorial team of CWHR for their support and hard work. Finally, we extend our sincere thanks for the opportunity to serve as a Guest Editor on the special issue of CWHR. We are confident that readers will benefit from the latest knowledge incorporated in these valuable articles.
Low-Cost Infertility Management

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Abstract: Objectives: To review the evidence regarding the magnitude of infertility as well as the various proposed approaches highlighting the use of the most cost-effective investigatory and treatment regimens. Data Sources and Methods: Medline and Pubmed were searched for all relevant papers published between 1975 and 2009 using a combination of the following keywords: ‘affordable, cost-effective, infertility, IVF, investigations, treatment’. Results: In an era of evidence-based medicine, we often fail to specify the most cost-effective regimen for an infertile couple. Setting a predetermined algorithm can help simplify the management approach. Prevention and education are important as well. Conclusions: A cost-effective approach that does not compromise success rates should be offered to all couples seeking help for infertility. This includes making evidence-based choices when choosing investigatory tools and treatment options. The “patient-friendly” regimen should not necessarily be equated with “minimal stimulation IVF” because to provide the best medical care for patients, it should be evidence-based and without bias. The ESHRE Task Force is working to tackle the challenge of providing a cost-effective simplified assisted reproduction program in developing countries.

Keywords: Infertility, low cost, cost-effective, cheap, investigations, treatment, IVF.

LOW-COST INFERTILITY MANAGEMENT

Magnitude of the Problem

Infertility is defined as the inability to conceive after at least 1 full year of unprotected sexual intercourse [1-3]. It is estimated that worldwide, between 70 and 80 million couples suffer from infertility, and most of these are residents of developing countries, including the Middle East [4, 5].

The prevalence of subfertility and infertility differs tremendously between developing countries. The figures are as low as 9% in some African countries such as Gambia [6] and as high as 35% in Nigeria [7, 8]. The reported international prevalence of infertility ranges from 4% to 14% with a consensus estimate of 10% among married and cohabiting couples [9-11].

What accounts for the variation in infertility levels? It is important to understand that there is a core of about five percent of all couples who suffer from anatomical, genetic, endocrinological, and immunological problems that cause infertility [10]. The remaining couples are infertile largely because of preventable conditions such as sexually transmitted infections (STIs), parasitic diseases, health care practices and policies, and exposure to potentially toxic substances in the diet or the environment.

Worldwide, STIs are the leading preventable cause of infertility. A World Health Organization (WHO) multinational study found that 64% of infertile women in sub-Saharan Africa had some sort of infection (vaginal and/or cervical), which is about double the rate of other regions. Tubal problems and other infection-related diagnoses also are associated with postpartum and post-abortion complications. The results of the WHO study suggest that repeated pregnancies play a greater role in the etiology of infertility in Africa and Latin America, while repeated abortions are more important in Asia and developed countries. Health care practices and policies also contribute to infertility, most notably unhygienic obstetric practices, which can lead to postpartum infections. Septic abortions and their complications are another important factor [12].

Inappropriate gynecological practices also may also lead to infertility. In Egypt, for example, physicians routinely misdiagnose cervical erosion and then treat it inappropriately with cervical electrocautery, potentially causing infertility in the process [13].

In the Middle East, the prevalence of infertility varies between 10% and 15% in married couples because of a high prevalence of post-partum infection, post-abortive infection, iatrogenic infertility, schistosomiasis and tuberculosis (TB) [14, 15]. Bilateral tubal occlusion is the most common underlying cause of infertility following such infections [12, 16].

Tubal and pelvic infertility are the leading causes of female infertility in many countries in the Middle East. Other infectious and parasitic diseases—and the medications used to treat them—contribute to infertility. For example, in India, where 40% of the population is exposed to TB, genital TB contributes to female infertility [17]. In Africa, schistosomiasis, malaria, and sickle-cell disease all contribute to
Infertility [18]. It has been proposed that the success of malaria-control programs may help explain a reduction in infertility rates seen in Tanzania over the past 20 years [19].

In Nigeria, where hernia repairs are routinely performed by inexperienced surgeons, there is a pattern of male infertility due to vascular injuries sustained during these procedures [20]. Increasingly, men and women in developing countries face exposure to environmental and workplace pollution, which can play a role in infertility.

Infertility is a major problem in these countries and causes extensive social and psychological suffering. Providing infertility treatment in resource-poor countries should be part of an integrated reproductive care program that includes family planning and motherhood care [21].

It is important to note that the problem of infertility is not limited to developing countries. Nearly all European countries are currently experiencing long-term downturns in fertility and, consequently, a reduction in the proportion of working-age individuals [22]. As a result, many governments around the world are currently providing incentives to their citizens to promote parenthood [23]. However, to date, there has been little recognition of the role of infertility services in these programs. Therefore, there is mounting pressure on governments to enhance their “baby-friendly” policies as a measure to reverse future reductions in fertility [24].

The limited availability of resources mandates their judicious use. The definition of “better care” should not be equated with “aggressive care.” More aggressive care may result in a quicker establishment of pregnancy and higher pregnancy rates per treatment attempt. However, they may also result in a higher incidence of multiple implantations. Better care should be defined as a balance between attempts to achieve pregnancy quickly and efficiently with as low of a multiple implantation rate as possible [25].

Cost-effective care must also satisfy patient demands. High-quality patient care may not necessarily lead to patient satisfaction if the patients’ expectations are not met. Once these expectations are defined, then they can be met by the provider or if not, addressed with the patient in the hopes that the expectations can be redirected. Failure to do so will result in high drop-out rates from treatment- a wasteful use of resources [26].

The ultimate goal is to create an approach that provides the greatest chance for pregnancy and birth while using limited resources in the most cost-effective fashion. To fulfill that goal, simplified treatment algorithms that attempt to minimize costs at every step of the management process have been proposed. Norbert Gleicher has proposed an algorithm that would help 80% of the couples who proceed through all the treatment steps to conceive, provided there are no drop-outs during any of the treatment steps (see Fig. 1) [27].

Interestingly, a prospective randomized trial that compared this algorithm to the use of in vitro fertilization (IVF) as an initial infertility treatment showed that it was more cost effective and efficient, largely due to a larger number of “treatment independent” pregnancies that occurred during use of the algorithm than in between IVF cycles [28]. Although not universally acceptable, this algorithm has proven acceptable to many providers in the United States and has been accepted by the insurance industry in states with mandated insurance coverage as the basis for contractual agreements [29].

Preliminary results from a prospective study analyzing a cohort of patients who used this algorithm support the outcome data in Fig. (1), although there are considerable drop-out rates at each treatment step. Obviously, this decreases the chances of conception [26].

To design a cost-effective, medically appropriate evaluation and treatment plan, we must consider the patient’s age. While there is little necessity to initiate aggressive therapy for the 20 year old with unexplained infertility, those older than 35 years deserve a more aggressive approach.

**LEVEL 1 OF CARE**

**1. Prevention**

It is often argued that in the Middle East, where there are many low income and middle income countries, the solution to the problem of infertility is in the prevention of postpartum infection, unsafe abortion, iatrogenic infertility, TB, schistosomiasis and STIs, which are preventable causes of infertility [14]. Reducing the incidence of postpartum infections can be achieved through safer birth practices, including the training of traditional birth attendants on how to used hygienic practices during deliveries, and by developing mechanisms to help women with potentially complicated deliveries to deliver in clinics.

The most effective ways to reduce postabortion infections are:

1. Promoting family planning, because effective contraception eliminates the need for abortion;
2. Providing treatment for postabortion complications at a variety of health facilities.

Where other diseases are a common cause of infertility, aggressive campaigns to control their spread may have an impact. For example, reducing the incidence of TB or treating affected men before TB spreads to the genital tract would prevent many cases of female infertility in India [17].

Likewise, testicular biopsies of Nigerian and Ghanaian men, which found a high incidence of inflammatory lesions, suggest that efforts to control and treat schistosomiasis would reduce levels of both male and female infertility in these countries [18]. While preventing reproductive tract infections may be the most effective way to reduce infertility problems in developing countries, this long-term strategy does not address the need for immediate infertility treatment.

**2. Judicious/Cost-Effective use of Diagnostic Work Up/ Monitoring**

Any one of a long list of tests can be used to determine the cause of infertility during the diagnostic evaluation of
infertile couple. Lack of agreement exists, however, among trained infertility specialists in regards to which tests have good prognostic utility and the criteria of normality of many of these tests i.e. a universally accepted range of normality, whether it is for a hormonal level or an imaging technique. Only those tests that are cost effective and correlate directly with the likelihood of conception should be used. These tests include conventional semen analysis, documentation of ovulation by measuring midluteal progesterone levels and assessing uterine factor and tubal patency with hysterosalpingography (HSG) or sono-hysterography.

A comprehensive semen analysis following WHO guidelines is fundamental at the primary care level if one is to make a rational initial diagnosis and select the appropriate clinical management [30]. Despite its limitations, conventional semen analysis is the cornerstone for assessment of male factor infertility; computer assisted semen analysis (CASA) is not superior. A study conducted by Krause W. in 1995 concluded that the determination of elaborate motility characteristics via CASA is of limited value when optimizing the evaluation of male fertility [31].

Previously, the postcoital test (PCT), which assesses sperm motility in a sample of postcoital cervical mucus, was considered an integral part of the basic infertility evaluation. However, past investigations revealed a poor correlation between postcoital sperm motility and pregnancy outcome [32]. In addition, a 1995 blinded, prospective study found that there was poor test reproducibility amongst trained observers, further questioning the validity of the PCT as a diagnostic tool [33].

In 2000, Oehninger, et al., conducted a meta-analysis to determine the diagnostic accuracy and predictive value of various sperm function assays in couples undergoing IVF. They assessed the following tests: CASA, acrosome reaction testing, the zona-free hamster egg penetration test or sperm-penetration assay (SPA) and sperm-zona pellucida binding assays. The results showed that the sperm-zona pellucida binding test and the induced-acrosome reaction assays for
fertilization outcome had the highest predictive power. On the other hand, the findings indicated that the SPA had a poor clinical value when used as a predictor of fertilization. Furthermore, the authors stated that there was a real need for standardization and further investigation of the potential clinical utility of CASA systems. The authors concluded that basic semen analysis remains the cornerstone in the evaluation of the male partner and validated sperm functional tests should expand the initial work up as indicated [34].

Female factor infertility is usually assessed by tracking ovulation, examining the uterus for malformations/polymorphs/fibroids, etc. and determining tubal patency and ovarian reserve. When assessing ovarian reserve, patient’s age is one of the main determinants; with advancing age, fertility declines. This is due to progressive follicular depletion and increased abnormalities in the aging oocytes (oocyte aneuploidy) [35]. Testing includes obtaining a cycle day 3 serum follicle-stimulating hormone (FSH) and estradiol level and performing a clomiphene citrate (CC) challenge test and/or an ultrasonographic ovarian antral follicle count challenge test and/or an ultrasonographic ovarian antral follicle count [36].

A patient with menstrual abnormalities should be investigated for underlying causes such as polycystic ovarian syndrome, thyroid disease, hyperprolactinemia, and hypothalamic causes secondary to weight changes. It is worth mentioning that a group of researchers from Australia conducted a cost-savings analysis of a weight loss program for obese infertile women (in Australian dollars). Their results showed that weight loss improved the reproductive outcome for all forms of fertility treatments and cost considerably less. Prior to the programme, 67 women had treatment costing a total of A$550 000 for two live births, a cost of A$275 000 per baby. After the programme, the same women had treatment costing a total of A$210 000 for 45 babies, a cost of A$4600 per baby [37].

Eumenorrhea—normal menstrual cycles by history—is a highly accurate marker of ovulation, and anovulatory levels of serum progesterone (< 3 ng/mL) are found in only a very small minority of eumenorrheic patients [38]. Obviously, if a pregnancy occurs or if an oocyte can be isolated from the reproductive tract, it means that a patient is ovulating. But neither can be used clinically as reference methods for predicting or confirming ovulation in infertile women [39].

Although it is now well accepted that the basal body temperature (BBT) graph is an unreliable marker for the prediction of ovulation [40], it still could be used as a simple method for retrospective identification of the presumptive day of ovulation [41]. Among the numerous parameters used to detect the day of ovulation, the identification of the luteinizing hormone (LH) surge appears to be the most reliable indicator of impending ovulation [42].

In a 2001 study assessing reliability of ovulation tests in infertile women, Guermend and et al. concluded that urinary LH was accurate in predicting ovulation with ultrasonography as the standard for detection, but time varied widely (LH surge was detected in urine from 72 hours before ovulation to the same day of ultrasonographic disappearance of the follicle). The nadir of BBT predicted ovulation poorly. The BBT chart was less accurate at confirming ovulation than urinary LH testing and serum progesterone assessment. A single serum progesterone assessment in the midluteal phase seemed as effective as repeated serum progesterone measures [43].

In a comparison of low-tech and high-tech methods of monitoring CC ovulation induction, it was shown that urinary detection of the LH surge and vaginal ultrasound offered no advantage over BBT charts alone in achieving pregnancy [44].

Although endometrial biopsy results were previously used to diagnose luteal phase defects, they do not correlate with fertility status and hence are no longer recommended [45]. From the above data, it can be concluded that midluteal serum progesterone and ultrasound may be the two most cost-effective means of documenting ovulation.

In a study assessing the feasibility and acceptability of an out-patient-based investigation of infertile couples (ultrasound, diagnostic hysteroscopy and culdoscopy), the average time needed to perform these three procedures was 41.2 minutes. Most patients appreciated the fact that only 1 hospital visit was needed and that the results were immediately available. However, this “One Stop” approach to the investigation of infertility is not suitable for or desired by all infertile couples [46].

3. Judicious/Cost-Effective use of Medical Treatment/Surgery (Endoscopy)

Proper utilization of surgical procedures, usually endoscopic procedures, represents the single most significant factor in providing cost-effective infertility care [47]. Assessment of the uterine contour and tubal patency is an integral part of the basic infertility evaluation [36]. Hysterosalpingography is the gold standard for the assessment of tubal and uterine factors. Along with laparoscopic dye perturbation, it can best assess tubal patency: the concordance of HSG with laparoscopic dye perturbation is estimated to be near 90% [48].

Severi F.M. et al. showed that hydrosonography can accurately evaluate the uterine cavity and any malformations, particularly in young women, reaching a diagnostic accuracy similar to that of hysteroscopy. They also found that the accuracy of hydrosonography is similar to that of HSG, when the two techniques are compared with laparoscopic chromoperturbation [49].

Moreover, Goldberg found that in the evaluation of patients with infertility or recurrent pregnancy loss and uterine abnormalities, hydrosonography was more accurate than HSG and provided additional information about uterine abnormalities, particularly on the relative proportion of the intracavitary and intramyometrial components of submucus myomas [50].

In a study to determine the feasibility and acceptability of an out-patient based infertility investigation that used a screening test for tubal occlusion called hysterosalpingo-contrast sonography (HyCoSy), the results showed that the former was a valuable and cost effective alternative to laparoscopy and the dye test [51].
The Practice Committee of the American Society for Reproductive Medicine (ASRM 2006) has published guidelines for standard infertility evaluation. It includes a semen analysis, assessment of ovulation, a hysterosalpingogram, and, if indicated, tests for ovarian reserve and laparoscopy.

The role of laparoscopy in the investigation of infertility has changed over the past decade. Whereas laparoscopy used to be part of the basic infertility workup, it is now reserved for selected cases. According to the guidelines of the ASRM, laparoscopy should be performed in women with unexplained infertility or signs and symptoms of endometriosis or when reversible adhesive tubal disease is suspected [36].

The idea of a ‘one-stop shop’ for subfertility investigation is certainly an attractive one for both patients and clinicians alike. It is simply aimed at checking the “Seed, Soil and Passage” involved in conception and can be performed within an hour. There is evidence to suggest that the use of an ultrasound-based system is not only more acceptable to couples, but it is also more cost-effective and provides diagnostic information of a caliber comparable with that of more traditional investigative methods. It is diagnostically accurate, expeditious and reliable. The HycoSy test can also be performed at the same time if necessary [52]—it is minimally invasive and provides both the patient and clinician with useful prognostic information. The male partner can have a detailed sperm test at the same time.

In agreement with the ‘one stop approach’, Ekerhovd E, et al. also proposed the use of the ultrasound for the assessment of infertility, including the evaluation of tubal patency [53].

In the end, it would be fair enough to say that the feasibility of transvaginal ultrasound use, in the infertility clinic, for the assessment of female factor infertility makes it the most cost-effective tool; i.e. transvaginal ultrasound replaces the need for assessing ovarian reserve by measuring the ovarian volume and the antral follicular count, replaces the need for tubal and uterine factor assessment by performing hysterosonography, documents ovulation by follicular scanning and finally, replaces the need for performing hysteroscopy, documents ovulation by follicular development in an IVF cycle, as well as the timing of hCG administration, can be done using sonographic criteria with basic inexpensive ultrasound equipment, thereby avoiding the need for expensive endocrine investigations [55, 56].

When the results of a standard infertility evaluation are normal, practitioners assign a diagnosis of unexplained infertility. Although estimates vary, the likelihood that all such test results for an infertile couple are normal (ie, that the couple has unexplained infertility) is approximately 15% to 30% [57].

In the algorithm proposed by N. Gleicher, in level 1, CC is given for 3 cycles without monitoring (ovulation kits may be used). As previously mentioned, in the study assessing reliability of ovulation tests in infertile women conducted by Guermandi E., et al. in 2001, it was concluded that urinary LH was accurate in predicting ovulation. In another study conducted by Luciano AA et al. [58], the temporal relationship and reliability of the clinical, hormonal, and ultrasonographic indices of ovulation in infertile women were assessed. Urine LH testing correlated well with the serum LH peak, particularly in the evening urine, and predicted ovulation in all patients. In addition, the use of urinary LH surge for the timing of intrauterine insemination (IUI) in CC-IUI cycles resulted in a higher pregnancy rate compared with hCG-induced ovulation [59]. Lastly, it remains to be mentioned that the average cost of the ovulation kits is approximately $0.5-0.8, which highlights its cost effectiveness.

A prospective multicenter randomized trial compared in a parallel design the efficacy of CC with rFSH for ovarian hyperstimulation in an IUI program for couples with unexplained or male subfertility of at least 24 months. There was no significant difference in live birth rates and multiple pregnancy rates between the two groups. It was concluded that unless larger studies demonstrate otherwise, for economic reasons, CC should still be the drug of choice for ovarian stimulation in IUI cycles [60].

Patients who fail to conceive after level 1, despite adequate ovulation (unexplained infertility) or due to failure of ovulation with CC, should proceed to level 2 where they will be given gonadotrophins for 3 cycles based on the assumption that the efficacy of gonadotrophins decreases after 2-4 cycles [61].

A. M. Case, in the Table 1, compared the cost of various treatment regimens for infertility and their success rates. It is clear that the more complicated and expensive treatments are more successful although they may not be as cost-effective [62].

In another comparison of the costs of infertility treatments, IUI, CC-IUI, and hMG-IUI had a similar cost per delivery of between $7,800 and $10,300. All 3 of these treatments were more cost-effective than IVF-ET, which had a cost per delivery of $37,000. The use of IVF in women with blocked fallopian tubes was more cost-effective than tubal surgery via laparotomy, which had a cost per delivery of $76,000 [67]. This study seems to support the proposed algorithm, previously described in Fig. (1); i.e. the use of IUI, CC-IUI, and hMG-IUI before IVF in women with open fallopian tubes. For women with blocked fallopian tubes, IVF-ET appears to be the best treatment from a cost-effectiveness standpoint.

In a recent review by J. Collins on the current best evidence for the advanced treatment of unexplained subfertility, he concluded that IVF is superior to FSH/IUI treatment, but this benefit is achieved only at considerable cost, and the evidence is not robust, comprising only a few trials. The small increase in effectiveness with IVF over FSH/IUI treatment is achieved only at considerable incremental cost, whether it is measured per cycle or per couple. Current best evidence is consistent with a progression from low-tech to high-tech treatment, but it is not convincing enough to support a rigid management protocol; thus a large multi-center factorial trial is needed to
evaluate the relative value of existing empiric treatments for unexplained infertility [68].

In agreement with this, another study assessing conventional treatment in normogonadotrophic anovulatory infertility (WHO 2) (CC followed by exogenous gonadotrophins [FSH] and IVF), showed that using CC → FSH →-IVF compared with FSH→- IVF generated more pregnancies against lower costs but when compared with CC →-iIVF, it also produced more pregnancies, but at higher costs. The average costs per cycle were €53 ($72), €1108 ($1,515), €1830 ($2,502) for CC, FSH and IVF, respectively, and the costs per ongoing pregnancy were €544($743), €8584($11,737), €7686($10,510) [69].

Recently, the validity of evidence used by the Royal College of Obstetricians and Gynecologists in recommending ovarian stimulation with IUI as an effective treatment for couples with unexplained infertility has been questioned, reigniting the debate on what the initial treatment for idiopathic infertility should be. The current best available evidence, using the results of randomized controlled trials, is that the initial treatment for idiopathic infertility should be IUI as opposed to IVF [70]. This was supported by a prospective, randomized, parallel trial that concluded that in idiopathic or male subfertility, IUI offers the same likelihood of successful pregnancy as IVF and is a more cost-effective approach [71]. Cost-effectiveness studies showed that three IUIs were as successful, but much cheaper, than one IVF/ICSI cycle [67,71-75].

**THE CONCEPT OF FRIENDLY IVF / NATURAL CYCLE IVF**

Keeping things simple without altering the success rate of IVF is the idea behind “Friendly IVF”. Friendly IVF aims to reduce the burden of the IVF procedures and its related complications, thereby giving a couple the chance to conceive using procedures that are less costly in terms of physical, emotional, social and financial costs. The rationale behind natural cycle IVF (probably the “gold standard” of friendly IVF) is that it is more nearly natural. The body itself selects its own "best egg" for that cycle. The ovaries do not blister full of multiple follicles, and neither the body nor the endometrium are exposed to supra-physiological levels of estradiol. Natural cycle IVF is safe and less stressful, results in fewer multiple births and is cost effective (one–fifth of the price of the current standard stimulation regimen) [76,84].

In a study conducted by M.J. Janssens, et al., the authors concluded that Natural IVF is an easy, inexpensive and realistic method to achieve pregnancy for patients with tubal infertility. Ongoing pregnancy rates approach 5.3% per cycle, 6.5% per oocyte retrieval, 11.4% per embryo transfer and 11.4% per embryo [77].

In 1995, Daya et al. reported that despite the high failure rate seen with each step in the process, natural cycle IVF was more cost-effective than stimulated-cycle IVF, which incurred an incremental cost per live birth of $48,000. The total cost for one live birth was five times lower with Natural IVF. In Daya’s study, a pregnancy rate of 12% was confirmed [78]. Mild approaches to ovarian stimulation promise to be more science-based and patient-friendly and they may also help improve the health of the offspring, through reduced perinatal morbidity, mortality, multiple pregnancies and the need for fetal reduction. Although a mild stimulation protocol resulted in a lesser number of embryos retrieved when compared to a high dose conventional protocol, it was associated with a significantly higher proportion of chromosomally normal embryos [79].

A multi-center study published in 2005 by Groen et al., compared the effects and costs of conventional IVF with those of Manipulated Natural Cycle-(MNC) IVF. Full treatment costs of MNC-IVF, including costs of pregnancy and delivery, ranged from 1,329 ($971) to 1,465Euro ($1071) per cycle, depending on the treatment phases completed and the number of pregnancies achieved. Medication costs ranged between 265 ($193) and 275 Euro ($201) per cycle versus 885 Euro ($647) for conventional IVF. The cost per live birth after three cycles of MNC-IVF was 17,197 Euro ($12,571), which is comparable to the costs per live birth after a single cycle of conventional IVF. It was concluded that three cycles of MNC-IVF achieve pregnancy rates similar to those of conventional IVF but with much

### Table 1. Indications, Costs, and Success Rates of Commonly Used Infertility Treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Indication</th>
<th>Cost Per Cycle($)</th>
<th>Success Rate Per Cycle (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>Oligo-ovulation</td>
<td>50-150</td>
<td>10-15 [63]</td>
</tr>
<tr>
<td>CC</td>
<td>Unexplained</td>
<td>4-6 [64]</td>
<td></td>
</tr>
<tr>
<td>CC &amp; IUI</td>
<td>Unexplained</td>
<td>150-300</td>
<td>8-10 [64,65]</td>
</tr>
<tr>
<td>SO &amp; IUI</td>
<td>Unexplained</td>
<td>750-2000</td>
<td>18-20 [65]</td>
</tr>
<tr>
<td>IVF</td>
<td>Tubal factor</td>
<td>5000-8000</td>
<td></td>
</tr>
<tr>
<td>IVF</td>
<td>Male factor</td>
<td></td>
<td>40 (&lt; 30 years) [66]</td>
</tr>
<tr>
<td>IVF</td>
<td>Endometriosis</td>
<td></td>
<td>35 (30-35) [66]</td>
</tr>
<tr>
<td>IVF</td>
<td>Unexplained</td>
<td></td>
<td>25(35-39) [66]</td>
</tr>
<tr>
<td>IVF and ICSI</td>
<td>Male factor</td>
<td>8000-10000</td>
<td>&lt;15 (≥ 40) [66]</td>
</tr>
</tbody>
</table>

lower twin pregnancy rates. Thus, MNC-IVF may be a cost-effective alternative for conventional IVF [80]. Alternatively, low-dose hCG can be administered in the later stages of controlled ovarian stimulation. This results in a significantly reduced dose of recombinant FSH/hMG while the outcome is comparable to that of traditional Controlled Ovarian Hyperstimulation (COH) regimens [81, 82].

A non-randomized clinical trial of minimal ovary stimulation compared CC and gonadotropin outcomes and direct costs to those of a conventional GnRHa-gonadotropin stimulation protocol for infertile patients undergoing IVF. The pregnancy rate per oocyte retrieval cycle in the GnRHa-gonadotropin protocol was similar to the minimal stimulation protocol (13.1% vs 13.0%). However, the cost per pregnancy of the minimal stimulation protocol was less than that of the GnRHa-gonadotropin protocol ($6,021.95 vs. $10,785.65) [83]. The use of CC stimulation seems to be superior to natural or minimal stimulation IVF [84, 85].

On the other hand, CC may be no better than natural cycle IVF, which has repeatedly been shown to be inefficient (<10% clinical pregnancy per cycle) [86-89]. Repeating one procedure that has a 10% chance of success four times is not mathematically equivalent to performing a single procedure with a 40% chance of success. Cumulative pregnancy rates after three cycles of minimal stimulation have been disappointingly low, yielding per-cycle success rates of only 8%, similar to the expected rate of CC–IUI rates, limiting its utility [90].

In a study evaluating the acceptability of stimulated versus natural cycle IVF among couples attending one infertility clinic, with respect to cost and pregnancy outcome, 15% (16/107) of the patients who were indicated for IVF cancelled, mostly due to financial reasons (12/16). Most patients who completed their IVF treatment (82/91, 90.1%) believed that the price of the medical service offered was high, and 68.1% (62/91) accepted the idea of using less expensive drugs with fewer side effects but with possibly a lower chance of pregnancy [91].

A policy of elective single embryo transfer (eSET) is the most efficacious measure of reducing the incidence of multiple pregnancies in ART [92-98]. This highlights the importance of natural cycle and minimal stimulation IVF and the lesser need for the production of many embryos per cycle in decreasing the burden imposed by multiple pregnancies. On the other hand, a systematic review of studies looking at the cost-effectiveness of IVF-SET versus IVF with double embryo transfer (DET) used in a health economic model compared three strategies: (1) IVF-SET, (2) IVF-DET, and (3) IUI with gonadotropin stimulation (sIUI). IVF-DET was the most cost-effective strategy at $35,144/live birth, followed by sIUI at $66, 960/live birth, and IVF-SET at $109,358/live birth. The results were sensitive both to the cost of IVF cycles and to the probability of live birth [99].

“Patient-friendly” IVF must be associated with a healthy newborn achieved in a safe, cost-effective, and timely manner. Patients are best served when physicians provide honest appraisal of treatment techniques and outcomes using the evidence available from scientific study [100]. The “less is better” approach has tremendous emotional appeal, because patients do not like taking medications, viewing them as unnatural. Minimal stimulation protocols thrive on that appeal. But a recent review of abstracts presented at the First World Congress on Natural Cycle/Minimal Stimulation reports inconclusive supporting evidence and the availability of procedures that “might be superior” [101].

MAKING IVF AFFORDABLE

In 2006, the European Society of Human Reproduction and Embryology (ESHRE) created a Special Task Force whose mission was to focus on infertility in developing countries; the Arusha-project looks for ways to make IVF affordable for African couples by vastly simplifying conventional IVF technologies. This task force is also attempting to: document the problem of infertility in developing countries; develop and test the effectiveness of a simplified ‘one-step clinic’ for the diagnosis of infertility; and develop and test the effectiveness of simplified IVF-related procedures. It plans to begin offering IVF at clinics in Cairo and Alexandria, Egypt, for around $360. In the US and the UK, the price of one round of treatment can cost as much as $12,000 and £5000 ($8000), respectively, and is rarely covered by health insurance.

One of the aspects of IVF the task force is looking at is the stimulation protocol. The recombinant form of FSH can cause women to release a large number of oocytes per cycle and thus, some embryos can be frozen. However, this has the disadvantage of being enormously expensive. On the other hand, clomiphene costs just $11 for one round of treatment. It can induce the maturation of up to four viable eggs per cycle. That is far fewer than seen with the use of FSH. And because low-cost IVF facilities are unlikely to have the equipment or liquid nitrogen for freezing extra embryos, fewer eggs are needed anyway. Using clomiphene, the ESHRE group plans to transfer no more than two embryos to the woman’s uterus whereas the Low Cost IVF Foundation (LCIF) initiative plans to transfer only one. As clomiphene has fewer side effects than recombinant FSH, women may be more likely try further rounds of IVF if earlier attempts fail. The ESHRE group estimates this approach will achieve a pregnancy rate of 15% to 20%, lower than the European rate of 25% and US rate of 35%. The ESHRE group plans to transfer the embryo on the first or second day after fertilization [102].

Another aspect of assisted reproduction that is being assessed is cutting down on the incubator expenses. Simple portable table-top incubators cost less than $1000. LCIF is counting on the use of warm water baths to incubate embryos. The use of a ‘humidicrib’, a plastic box that is commonly used for keeping newborns snug, instead of expensive laminar flow hoods, has also been proposed [103, 104]. Others argue that incubators can be avoided completely since women themselves can act as a natural one. Intravaginal culture was described approximately 20 years ago [105-108]. A tube filled with 3 ml of culture medium containing 1–5 oocytes with 10 000–20 000 washed spermatozoa per millilitre was hermetically closed and placed in the vagina. It was held in place by a diaphragm for incubation for 44–50 h. Comparable success rates with conventional IVF were reported [106].
The INVOcell is a small plastic capsule into which fertilized eggs are placed together with culture media. The capsule, encased in a protective shell, is then inserted into a woman's vagina for three days, which keeps the embryos at the desired temperature. Fertilization of the oocyte(s) and early embryo development occur in the INVOcell, which is placed into the maternal vaginal cavity for incubation. The vaginal cavity replaces the complex IVF laboratory. After removal, the two best embryos are selected and transferred to the woman's uterus. It costs between $85 in Africa and $185 in Europe and can cut the cost of IVF by half. The INVOcell overcomes the disadvantages of the previously used prototype and makes the procedure simpler and reproducible. Over 800 cycles have been published worldwide that showed a clinical pregnancy rate of 19.6%. The INVO technology can be performed in an office setting with minor capital equipment. INVO is a simple low-cost procedure that can be available almost everywhere [109].

Bicarbonate-free media can be used to maintain the pH, obviating the need for cylinders of CO2, which are expensive and unnecessary if an embryo is incubated for only one or two days. Also, the need for CO2 cylinders can be overcome simply by exhaling across the culture medium before sealing it in a plastic bag. This bag, containing the Petri dish with the embryos, can be dropped into a warm bath without the need for expensive incubators. This technique has been successfully used for more than 10 years for cow embryos in veterinary IVF [110,111].

Less expensive microscopes for confirming cell division can be easily adapted for a minimal cost, as can portable digital ultrasound machines that sell for less than $5000 - far below the typical $400,000 price tag for machines used in western IVF clinics [102].

CONCLUSIONS

In an era of evidence-based medicine, we often fail to specify the best cost-effective regimen for an infertile couple. Setting a predetermined algorithm, though inefficient in some cases, can help simplify the management approach. The value of prevention and education should not be underestimated. Our goal should be to offer not necessarily a low-cost approach, but rather a cost-effective one that does not compromise success rates—a balance that is difficult to achieve. This can be done by making “wise choices” (evidence-based choices) amongst investigatory tools and treatment options.

The availability and implementation of low-cost, effective infertility management protocols is needed in developed countries as much as it is needed in developing countries. Many European countries are experiencing long-term downturns in fertility, and there is increasing pressure on governments to enhance their baby-friendly policies as a measure to reverse future reductions in fertility.

The idea of a “patient-friendly” treatment regimen sounds appealing, but it should not necessarily be confused with “minimal stimulation IVF” because in order to provide the best medical care for patients, it should be evidence-based and without any personal bias. The ESHRE Task Force experience in developing countries will probably be a first step in tackling the challenge of providing a cost-effective simplified assisted reproduction program. However, studies on a wide scale must be a part of that experience.

FIVE YEAR REVIEW

Infertility is a major problem in low-resource countries and causes extensive social and psychological suffering. Providing infertility treatment in resource-poor countries should be part of an integrated reproductive care program that includes family planning and motherhood care. Access to preventive treatment in terms of detection and treatments of STIs is an important preventative aspect, and it should be available to all patients in developing economies. Patients in low-resource and developing countries have a right to infertility treatment including ART.

KEY POINTS

1. The incidence, severity and the gravity of infertility is highest in many low-resource countries.
2. The most common cause of infertility is tubal damage, which is preventable through early detection and treatment of STIs.
3. In practicing medicine, we should not fail to specify the best cost-effective regimen for our patients. Evidence-based choices can be made without compromising success rates.
4. Setting a predetermined algorithm, though inefficient in some cases, helps to simplify the management approach.

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Female Infertility and Antioxidants

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Abstract: Aim: Many studies have implicated oxidative stress in the pathogenesis of infertility causing diseases of the female reproductive tract. The aim of this study was to review the current literature on the effects of antioxidant therapy and to elucidate whether antioxidant supplementation is useful to prevent and/or treat infertility and poor pregnancy outcomes related to various obstetric and gynecologic conditions.

Methods: Review of recent publications through Pubmed and the Cochrane data base.

Results: Antioxidant supplementation has been shown to improve insulin sensitivity and restore redox balance in patients with PCOS. Supplementation with RU486, Curcuma longa, melatonin, caffeic acid phenethyl ester (CAPE) and catechins may induce remission and halt disease progression in endometriosis. Selenium therapy may improve pregnancy rates in unexplained infertility. Currently there is no evidence to substantiate the use of antioxidants to prevent or treat preeclampsia. Up to 50-60% of recurrent pregnancy loss may be attributable to oxidative stress. Observational studies have confirmed a link between antioxidant-poor diet and recurrent pregnancy loss.

Conclusion: Although many advances are being made in the field of antioxidants therapy, there is a need for further investigation using randomized controlled trials within a larger population to determine the efficacy and safety of antioxidant supplementation.

Keywords: Oxidative stress, antioxidants, polycystic ovarian syndrome (PCOS), endometriosis, unexplained infertility, preeclampsia, spontaneous abortion.

INTRODUCTION

Reactive oxygen species (ROS) can modulate cellular functions, and oxidative stress (OS) can impair the intracellular milieu, resulting in diseased cells or endangered cell survival. Reproductive cells and tissues remain stable when free radical production and the scavenging antioxidants remain in balance. The role of ROS in various diseases of the female reproductive tract has been investigated. ROS can affect a variety of physiological functions in the reproductive tract, and excessive levels can result in precipitous pathologies affecting female reproduction. The oxidant status can influence early embryo development by modifying the key transcription factors, hence modifying gene expression.

The review will focus on ROS homeostasis and generation of OS in the female reproductive processes. Our review elucidates the role of ROS in physiological processes such as folliculogenesis, oocyte maturation, endometrial cycle, luteolysis, implantation, and embryogenesis and the role of antioxidants in various reproductive pathologies. This review encapsulates the role of OS, which is becoming increasingly important as new evidence of its role in conditions such as polycystic ovarian disease and abortions is discovered. The review highlights how OS modulates natural and assisted fertility and the importance of antioxidant strategies to intercept OS to overcome its adverse effects.

WHAT IS OXIDATIVE STRESS?

Oxidative stress arises from an imbalance between pro-oxidant molecules generated from aerobic metabolism and protective antioxidants. OS influences the entire reproductive lifespan of a woman. Reactive oxygen species may act as key signalling molecules in physiological processes but at excess, uncontrolled levels they may also mediate pathological processes involving the female reproductive tract. There is a body of literature providing clinical evidence that substantiates the link between OS and female infertility.

Pro-Oxidants

Under physiological conditions, biomolecules are comprised of stable bonds formed by paired electrons. Weakened, disrupted bonds allow for the generation of free radicals- unstable and highly reactive species with one or more unpaired electrons. They gain stability by acquiring electrons from nearby nucleic acids, lipids, proteins, and carbohydrates, initiating a cascade of chain reactions that may result in cellular damage and disease [1-4].

Reactive oxygen species are formed endogenously during aerobic metabolism and as a result of various metabolic pathways of oocytes and embryos or as part of the body’s defense mechanisms. ROS also can arise from exogenous sources, such as alcohol, tobacco, and various environmental...
pollutants. ROS include hydroxyl radicals, superoxide anion, hydrogen peroxide, and nitric oxide (NO) [5]. Several biomarkers indicative of redox status, including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), lipid peroxides, and nitric oxide, have been identified within the ovary, endometrium, fallopian tubes, embryo, placenta, and the peritoneal fluid of women. At controlled levels, free radicals are capable of exerting physiological effects and mediating processes such as tissue remodelling, hormone signalling, oocyte maturation, folliculogenesis, tubal function, ovarian steroidogenesis, cyclical endometrial changes, and germ cell function [6, 7]. However, when ROS increase to pathological levels they are capable of inflicting significant damage to cell structures.

Antioxidants

Under normal conditions, antioxidants act to oppose ROS production, scavenge existing free radicals, and promote the repair of ROS-induced damage to cell structures [8]. Non-enzymatic antioxidants include vitamin C, vitamin E, selenium, zinc, beta carotene, carotene, taurine, hypotaurine, cysteamine, and glutathione. Enzymatic antioxidants include SOD, catalase, GSH-Px, glutaredoxin and glutathione reductase [5]. The degree of antioxidant defense present is often expressed as total antioxidant capacity (TAC) [6].

A disruption in the delicate balance between antioxidants and pro-oxidant molecules can result in OS. OS arises when the generation of reactive oxygen species and other radical species overrides the scavenging capacity by antioxidants, either due to the excessive production of ROS or an inadequate availability of antioxidants. Thus, oral antioxidant supplementation may serve to prevent and alleviate OS and its contribution to the pathogenesis of obstetrical disease such as preeclampsia and recurrent pregnancy loss and gynecological disorders such as polycystic ovarian syndrome (PCOS) and endometriosis.

OS IN THE FEMALE REPRODUCTIVE TRACT – PHYSIOLOGICAL ROLE OF OS

Follicle

The expression of various markers of OS has been demonstrated in normally cycling ovaries [9, 10]. The follicular fluid microenvironment contains leukocytes, macrophages, and cytokines, all of which are known sources of ROS. ROS within the follicular fluid plays a role in modulating oocyte maturation, folliculogenesis, ovarian steroidogenesis, and luteolysis [11]. Follicular development involves the progression of small primordial follicles into large pre-ovulatory follicles. Studies have implicated the nitric oxide radical in the follicular growth and programmed follicular cell death that occur during folliculogenesis [12, 13]. Moderate OS levels are required for ovulation. The final stages of oocyte maturation are associated with fluctuations in cytokines, prostaglandins, proteolytic enzymes, nitric oxide, and steroids, which increase the level of ROS, influencing ovarian blood flow and eventually facilitating follicle rupture [14]. A degree of oxidative enzyme activity is exhibited by thecal cells, granulosa lutein cells, and hilus cells, illustrating the role of OS in ovarian steroidogenesis [11].

ROS is controlled and kept at physiological levels within the ovary by various antioxidant systems, including catalase, vitamin E, glutathione and carotenoids [4]. SOD, a metal-containing enzymatic antioxidant that catalyzes the decomposition of superoxide into hydrogen peroxide and oxygen, has been characterized in the theca interna cells in the antral follicles. Therefore, the theca interna cells may protect the oocyte from excess ROS during its maturation [15]. Another antioxidant factor important for healthy follicle development is transferrin, an iron-chelating glycoprotein that suppresses ROS generation [16]. Vitamin C also is known to have a protective effect within the follicle as vitamin C deficiency has been reported to result in ovarian atrophy, extensive follicular atresia, and premature resumption of meiosis [15].

The overall ROS scavenging ability of antioxidants within the follicular fluid microenvironment may diminish with reproductive aging. Carbone et al. demonstrated decreased levels of follicular fluid catalase and SOD in older women, whose oocytes were seen to exhibit lower fertilization rates and decreased blastocyst development compared with oocytes of younger women [17]. Therefore, the redox status of the follicle is closely related to oocyte quality and fertilization capacity.

Endometrium

The relationship between OS and cyclical changes in the endometrium is well-established. OS-promoting alterations in ROS and SOD levels have been demonstrated just prior to menstruation, during the late-secretory phase [18]. Estrogen and progesterone withdrawal in endometrial cells in vitro has been associated with a decrease in SOD activity, resulting in the unopposed activity of ROS [18]. Elevated lipid peroxide and decreased SOD in the endometrium during the late-secretory phase may modulate endometrial breakdown, leading to menstruation. NO is known to regulate the endometrial microvasculature and is produced by endothelial NO synthase (NOS), which is distributed in the glandular surface epithelial cells of the endometrium [19]. NO is thought to mediate endometrial decidualization and menstruation as endothelial NOS mRNA expression has been detected in the mid-secretory and late-secretory phase. Endothelial NOS is also implicated in the changes seen in the endometrium in preparation for implantation [20]. ROS may mediate the physiological processes of shedding and implantation by its activation of nuclear factor KB within the endometrium, leading to increased cyclooxygenase-2 mRNA and prostaglandin F2α synthesis [18].

Infertility

Approximately 1.3 million American couples receive medical advice or treatment for infertility every year [21]. Infertility is a disease defined as the inability to conceive following 12 or more months of unprotected sex [22]. In general, an estimated 84% of couples conceive after 1 year of intercourse, and 92% of the couples conceive after 2 years
[23]. A primary diagnosis of male factor infertility is made in 30% of infertile couples. High levels of ROS biomarkers have been detected in semen samples of 25-40% of infertile men [5]. Although ROS have a physiological role in normal sperm function, mediating the acrosome reaction, hyperactivation, motility, and capacitation of spermatozoa, excessive levels of ROS may arise from immotile or morphologically abnormal spermatozoa and leukocytes. Spermatozoa lack the necessary cytoplasmic antioxidant enzymes and are vulnerable to OS-induced DNA damage and apoptosis [5, 24]. Substantial evidence exists that implicates OS in many causes of male infertility. Oral antioxidant supplementation has become standard practice for male infertility [5].

Combined female and male factor infertility is responsible for 20%–30% of cases. If the results of a standard infertility examination are normal, a diagnosis of unexplained or idiopathic infertility is assigned [25]. OS has a well-established role in pathogenesis of unexplained infertility, which is seen to affect 15% of couples [25]. Although the frequency and origin of different forms of infertility varies, 40%–50% of the etiology of infertility studied is due to female causes [26].

OS induces infertility in women through a variety of mechanisms. Excess ROS in the follicle may overwhelm follicular fluid antioxidant defense and directly damage oocytes. The DNA of oocytes and spermatozoa may be damaged, leading to defective fertilization when the peritoneal cavity microenvironment is plagued with severe OS. Even when fertilization is achieved, OS-induced apoptosis may result in embryo fragmentation, implantation failure, abortion, impaired placentation, and congenital abnormalities [27]. Excess ROS may hinder the endometrium, which normally functions to support the embryo and its development [28]. OS may induce luteal regression and insufficient luteal hormonal support for the continuation of a pregnancy [8]. The association of OS with various gynecologic and obstetric conditions related to infertility suggests a potential role for oral antioxidant supplementation (Fig. 1). Additional research is needed to determine whether such supplementation can ensure successful fertilization and pregnancy by controlling the OS experienced by patients with endometriosis, PCOS, unexplained infertility, preeclampsia, and recurrent pregnancy loss.

THE USE OF ANTIOXIDANTS IN TREATMENT OF GYNECOLOGICAL CONDITIONS

Polycystic Ovarian Syndrome

PCOS is an anovulatory cause of infertility affecting 6-10% of premenopausal women [29-32]. PCOS often can be characterized by hyperandrogenism, hirsutism, and oligomenorrhea or amenorrhea. Metabolic, endocrinologic, and cardiovascular disorders may also coexist. Oxidative stress has been implicated in mediating the insulin resistance and increase in androgens seen in these patients [33].

A recent study by Kuscu et al. demonstrated increased MDA levels and upregulated SOD activity in patients with PCOS compared to controls. MDA levels were highest in patients who exhibited insulin resistance [34]. Insulin resistance and hyperglycemia are established as factors that increase oxidative stress. Fulghesu et al. evaluated the effect of N-acetyl-cysteine (NAC), known to replenish stores of the antioxidant glutathione, on insulin secretion and peripheral insulin resistance in subjects with PCOS. Patients were treated for 5-6 weeks with a 1.8g oral NAC per day. Massively obese patients were given a higher dose of 3g per day. NAC treatment was found to improve parameters of glucose control in hyperinsulinemic patients. Insulin levels were reduced, with increased peripheral insulin sensitivity. Therefore, the anti-oxidant effects of NAC may serve as a therapeutic strategy to improve the level of circulating insulin and insulin sensitivity in PCOS patients with hyperinsulinemia [35].

Non-obese PCOS patients without insulin resistance also have been reported to have elevated total oxidant and anti-
oxidant status [36]. Verit et al. demonstrated that total anti-oxidant status in these types of PCOS patients was correlated with raised luteinizing hormone levels and free androgen and dehydroepiandrosterone (DHEAS) levels [36]. Yilmaz et al. studied the effects of 12 weeks of treatment with oral hypoglycemic agents on OS in lean patients with PCOS [37]. Before treatment, PCOS patients exhibited OS with significantly raised serum MDA and homocysteine and significantly decreased serum TAS. PCOS patients treated with rosiglitazone showed an increase in TAS and a decrease in MDA levels, compared with a metformin-administered patient group in which these parameters did not change [37]. Therefore, rosiglitazone may be useful in combating OS in hyperinsulinemic PCOS patients.

Zhang et al. used methods of chemical calorimetry to measure and compare levels of serum lipid peroxides (LPO), MDA, SOD, vitamin E, and vitamin C in patients with PCOS and normal women [38]. Levels of serum LPO and MDA in patients with PCOS were significantly higher than those found in normal women. Levels of vitamin E, vitamin C, and SOD were lower in patients with PCOS than in the control group. After 3 months of therapy with oral ethinylestradiol and cyproterone acetate tablets (Diane-35®; Merck, Whitehouse Station, N.J.), an anti-androgenic oral contraceptive often used to treat hirsutism associated with PCOS, MDA and LPO levels decreased, while vitamin E, vitamin C, and SOD levels increased in patients with PCOS [38]. Therefore, this therapy may alleviate the symptoms of PCOS through both its anti-androgenic and anti-oxidant actions.

Endometriosis

Severe cases of endometriosis are thought to render a woman infertile by mechanical hindrance of the sperm-egg union by adhesions, endometriomata, and pelvic anatomy malformations. However, in women with mild-to-moderate forms of endometriosis and no pelvic anatomical distortion, the mechanism by which their fertility is reduced is poorly understood.

ROS production may be amplified in the setting of endometriosis due to menstrual reflux, which subjects the peritoneal cavity to pro-inflammatory hemoglobin and heme molecules released from transplanted erythrocyte debris. Peritoneal fluid containing ROS-generating iron, macrophages, and environmental contaminants such as polychlorinated biphenyls may disrupt the prooxidant/antioxidant balance, resulting in increased proliferation of tissue and adhesions [39-42]. ROS are thought to promote the growth and adhesion of endometrial cells in the peritoneal cavity, contributing to the pelvic anatomical distortion known to cause infertility in endometriosis [43]. OS may have a role in promoting angiogenesis in ectopic endometrial implants by increasing vascular endothelial growth factor (VEGF) production [44]. This effect is partly mediated by glycodecin, a glycoprotein whose expression is stimulated by OS. Glycodecin may act as an autocrine factor within ectopic endometrial tissue by augmenting VEGF expression [44].

Altered molecular genetic pathways may contribute to the effects of OS in the pathogenesis of endometriosis and endometriosis-associated infertility. Differential gene expression of ectopic and normal endometrial tissue has been identified, including differential gene expression of glutathione-S-transferase, an enzyme in the metabolism of the potent antioxidant glutathione [45]. This suggests that altered molecular genetic pathways may determine the development of OS and its ability to induce cellular proliferation and angiogenesis in women with endometriosis.

Peritoneal fluid of women with endometriosis has been reported to exhibit increased ROS generation by activated peritoneal macrophages [46]. Increased macrophage activity is accompanied by the release of cytokines and other immune mediators such as NO. NO is a pro-inflammatory free radical that exerts deleterious effects on fertility by increasing the amount of OS in the peritoneal fluid, an environment that hosts the processes of ovulation, gamete transportation, sperm-oocyte interaction, fertilization, and early embryonic development [2, 47, 48]. However, the results of further studies with large patient numbers failed to confirm an antioxidant or oxidant imbalance as ROS levels in peritoneal fluid of patients with endometriosis were not reported to be significantly higher than controls [49, 50].

After adjusting for confounding factors such as age, BMI, gravidity, serum vitamin E, and serum lipid levels, Jackson et al. reported a weak relationship of elevated levels of thiobarbituric acid reactive substances (TBARS), an overall measure of OS, in women with endometriosis [51]. Increased NO production and lipid peroxidation have been reported in the endometrium of women with endometriosis [2, 52]. However, several studies failed to find significant differences in the peritoneal fluid levels of NO, lipid peroxide, and ROS in women with and without endometriosis-associated infertility.

The failure of some studies to confirm alterations in peritoneal fluid NO, lipid peroxide and antioxidant status in women with endometriosis may be explained by the fact that OS may occur locally, without affecting total peritoneal fluid ROS concentration. Also, markers of OS may be transient and not detected at the time endometriosis is diagnosed.

An imbalance between ROS and antioxidant levels may play an important role in the pathogenesis of endometriosis-associated infertility. Increased concentrations of oviductal fluid ROS may adversely affect oocyte and spermatozoa viability and the process of fertilization and embryo implantation. Also, pro-inflammatory macrophages and activated neutrophils in the oviductal fluid may significantly amplify ROS production by endometriotic foci [43]. Increased ROS production may inflict oxidative damage to the sperm plasma and acrosomal membranes, resulting in a loss of motility and decreased spermatozoa ability to bind and penetrate the oocyte. The various possible consequences of OS-induced DNA damage include failed fertilization, reduced embryo quality, pregnancy failure, and spontaneous abortion.

Modest levels of OS have been shown to induce the proliferation of endometrial stromal cells in vitro, which has been shown to be inhibited by antioxidants [53]. Several studies have shown that the peritoneal fluid of women with endometriosis-associated infertility have insufficient antioxi-
dant defense, with lower total antioxidant capacity (TAC) and significantly reduced SOD levels [2, 47, 54].

An early study used a simple rabbit model to demonstrate the beneficial effect of antioxidant therapy in halting progression of the disease [55]. SOD and catalase were instilled in the rabbit peritoneal cavity and were shown to significantly reduce the formation of intraperitoneal adhesions at endometriosis sites by blocking the toxic effects of the superoxide anion and hydrogen peroxide radicals [55]. More recently, RU486- a potent antiprogesteronal agent with antioxidant activity, has been shown to decrease the proliferation of epithelial and stromal cells in endometriosis [56].

Another drug being investigated for its potential use in the treatment of endometriosis-associated infertility is pentoxifylline, a 3',5'-nucleotide phosphodiesterase inhibitor. Pentoxifylline has potent immunomodulatory properties and has been shown to significantly reduce the embryotoxic effects of hydrogen peroxide [57]. Zhang et al. conducted a recent randomized control trial in which pentoxifylline treatment failed to demonstrate significant reduction in endometriosis-associated symptoms such as pain. Furthermore, there was no evidence of an increase in the clinical pregnancy rates in the pentoxifylline group compared with placebo [58]. Currently, there is not enough evidence to warrant the use of pentoxifylline in the management of premenopausal women with endometriosis-associated pain and infertility.

Curcumin is a polyphenol derived from turmeric (Curcuma longa) with antioxidant, anti-inflammatory, and antiproliferative properties. This compound has been shown to have an anti-endometriotic effect by targeting aberrant matrix remodelling in a mouse model. Matrix metalloproteinase-9 (MMP-9) has been shown to correlate with severity of endometriosis. In randomized controlled trials, curcumin treatment was seen to reverse MMP-9 activity in endometriotic implants near to control values. Furthermore, the anti-inflammatory property of curcumin was evidenced by the fact that the attenuation of MMP-9 was accompanied by a reduction in cytokine release. Decreased expression of tumor necrosis factor alpha (TNF-α) was demonstrated during regression and healing of endometriotic lesions within the mouse model. Pretreatment of endometriotic lesions with curcumin was shown to prevent lipid peroxidation and protein oxidation within the experimental tissue, attesting to its therapeutic potential to provide antioxidant defense against OS-mediated infertility in endometriosis [59].

MMP-9 also was identified as a therapeutic target in the treatment of OS-mediated endometriosis in another study evaluating the effectiveness of melatonin in treating experimental endometriosis in a mouse model [60]. Melatonin is a major secretory product of the pineal gland with anti-oxidant properties. Melatonin was shown to arrest lipid peroxidation and protein oxidation, while downregulating MMP-9 activity and expression in a time- and dose-dependent manner. Tissue inhibitors of metalloproteinase (TIMP)-1 were found to be elevated. Regression of peritoneal endometriotic lesions was seen to accompany the alteration in metalloproteinase expression [60]. Guney et al. confirmed these findings in that treatment with melatonin was also shown to cause regression and atrophy of endometriotic lesions in rats [61]. Endometrial lesions treated with melatonin demonstrated lower MDA levels and significantly increased SOD and catalase activity [61], corroborating the usefulness of this hormone in neutralizing OS.

Guney et al. conducted another study that evaluated the effects of antioxidant and anti-inflammatory caffeic acid phenethyl ester (CAPE) on experimental endometriosis in a rat model, and the levels of peritoneal SOD and catalase activity, and MDA [62]. Treatment with CAPE was seen to decrease peritoneal MDA levels and antioxidant enzyme activity in rats. Endometriotic lesions treated with CAPE were histologically demonstrated to undergo atrophy and regression, compared with untreated controls [62].

As previously mentioned, OS stimulates factors that increase VEGF expression and promote angiogenesis of endometriotic lesions. The green tea-containing compound, epigallocatechin gallate (EGCG) has been evaluated as a treatment for endometriosis due to its powerful antioxidant and anti-angiogenic properties. Xu et al. conducted a study in which eutopic endometrium transplanted subcutaneously into a mouse model was used to compare the effects of EGCG treatment on endometriotic implants to the effects seen with vitamin E treatment or untreated controls [63]. Lesions treated with EGCG exhibited significantly downregulated VEGF-A mRNA. While the control endometrial implants exhibited newly developed blood vessels with proliferating glandular epithelium, the EGCG group demonstrated significantly smaller endometriotic lesions and smaller and more eccentrically distributed glandular epithelium. Despite its widely studied benefits as a potent antioxidant in the treatment of female infertility, vitamin E was not shown to control or decrease angiogenesis compared with baseline controls [63]. As EGCG was shown to significantly inhibit the development of experimental endometriotic lesions in a mouse model, its effectiveness as an oral supplement in female patients to limit progression and induce remission of their endometriosis should be further investigated.

A recent study by Mier-Cabrera et al. utilized questionnaires to compare the dietary intake of antioxidant vitamins and minerals by women with and without endometriosis. Relative to healthy control subjects, women with endometriosis were found to have a significantly lower intake of vitamins A, C, E, zinc, and copper. However, intake of selenium was not significantly different between the two groups studied [64]. A randomized control trial in which the effect of antioxidant supplementation was studied revealed a significant increase in the concentrations of serum retinol, alpha-tocopherol, leukocytes, and plasma ascorbate after 2 months of treatment. Antioxidant supplementation was also observed to increase the activity of antioxidant enzymes (SOD and GPx), while decreasing markers of oxidative stress such as malondialdehyde and lipid peroxides [64]. These effects were not observed in the control group, suggesting a role for antioxidant supplementation in decreasing the levels of oxidative stress afflicting patients with endometriosis-associated infertility.
Unexplained Infertility

Elevated levels of ROS that disturb the redox balance within the body may be the root cause of infertility in women who do not have any other obvious cause. The ovum released from the ovary, the zygote or embryo, and spermatozoe are very vulnerable to damage inflicted by OS [8]. Wang et al. compared ROS levels in peritoneal fluid between women undergoing laparoscopy for infertility evaluation and fertile women undergoing tubal ligation and demonstrated that higher levels of ROS exist in the peritoneal fluid of patients with unexplained infertility compared to that measured within the peritoneal fluid of fertile women [65]. Polak et al. analyzed peritoneal fluid samples obtained at laparoscopy and found that women with unexplained infertility had increased MDA concentrations and TAS, implicating the role of redox imbalance in its pathogenesis [66].

Elevated ROS levels in patients with unexplained infertility implies exhausted antioxidant defense, resulting in the inability to scavenge ROS and neutralize their toxic effects [65]. This was substantiated by the results of a study in which antioxidant concentrations were seen to be significantly lower in the peritoneal fluid of patients with unexplained infertility compared with antioxidant levels in fertile patients [47]. The link between decreased antioxidant status and lowered fecundity suggests a potential use for antioxidant supplementation to treat the high levels of ROS seen in patients with idiopathic infertility.

Howard et al. described a group of patients with a history of unexplained infertility and abnormal red blood cell magnesium (RBC-Mg) levels. These patients’ RBC-Mg levels were unresponsive to oral magnesium supplementation and shown to be associated with deficient red blood cell glutathione peroxidase (RBC-GSH-Px) activity [67]. Treatment with 200 μg of oral selenium as selenomethionine and oral magnesium for a period of 2 months was shown to normalize RBC-Mg and RBC-GPx levels. This correlated with 100% of previously infertile women in the treatment group successfully achieving clinical pregnancies within 8 months of normalizing their RBC-Mg [67].

Badawy et al. conducted a prospective, randomized, double-blind, controlled trial comparing the effects of using clomiphene citrate combined with glutathione-replenishing N-acetyl cysteine versus clomiphene citrate alone in inducing ovulation in women with unexplained infertility [68]. Despite the proposed benefits of strengthening the antioxidant defense of women with unexplained infertility, no difference was seen in the rates of successful pregnancy between both groups [69]. Therefore, the use of N-acetylcysteine to improve outcome during ovulation induction in women with unexplained infertility is not justified.

THE USE OF ANTIOXIDANTS TO PROMOTE HEALTHY PREGNANCY

Preeclampsia

Preeclampsia complicates 5% of all pregnancies and 11% of first pregnancies and is associated with high maternal and fetal morbidity and mortality [70]. Although the exact mechanism by which preeclampsia develops is not known, there is increasing evidence that corroborates the role of OS in its etiopathogenesis. Reduced antioxidant response [71, 72], reduced levels of antioxidant nutrients [73], and increased lipid peroxidation [72, 73] have been observed in patients with preeclampsia.

Preeclampsia is associated with defective placenta, in which the dislodging of extravillous trophoblast plugs in the maternal spiral arteries leads to the onset of blood flow into the intervillous space, causing an oxidative burst that generates ROS. Abnormal placenta leads to reduced fetoplacental circulation secondary to decreased NO-mediated vascular relaxation. Placental ischemia and hypoxia leads to ischemic reperfusion injury to the placenta in which there is release of cytokines and prostaglandins, which triggers the endothelial cell dysfunction seen in preeclampsia. Hypoxia and reperfusion injury leads to increased expression of xanthine oxidase and NADPH oxidase and increased generation of SOD.

Poorly perfused placental tissue, abnormal lipid metabolism, and resultant lipid peroxidation and redox imbalance are established factors that promote the development of preeclampsia. Numerous studies have demonstrated insufficient antioxidant defenses and overwhelming degrees of ROS in women with preeclampsia [73]. Oxidative stress has been evaluated by measuring elevated lipid peroxidation in patients with preeclampsia, as well as elevated protein carbonyl concentrations, plasma MDA levels, and SOD activity. Placental oxidative stress has been proposed as a promoter of lipid peroxidation and endothelial cell dysfunction [74-78]. Increased lipid peroxidation may result in the consumption of antioxidants and depletion of vitamin A, C, and E, erythrocyte thiol, glutathione, and SOD.

There currently is no accepted method of preventing the development of preeclampsia. Some trials have evaluated the use of supplementation with antioxidants vitamin C and vitamin E for prevention. Early intervention at 16–22 weeks of pregnancy with supplementation of vitamin E and C resulted in significant reduction of preeclampsia in the treatment group [79]. However, supplementation in women with established preeclampsia did not yield any benefit [80]. A recent randomized trial failed to demonstrate any beneficial effects of vitamin C and E supplementation in preventing preeclampsia [81]. Poston et al. investigated the use of vitamin C and E supplementation to reduce OS, limit the injury of endothelial cells, and prevent or reduce disease severity of preeclampsia. In this placebo-controlled trial in a diverse group of high-risk women, antioxidant supplementation was not associated with a reduction in the preeclampsia risk. Instead, treatment was associated with a significantly higher incidence of complications, including low birth weight, gestational hypertension, and increased need for intravenous antihypertensive and magnesium sulphate therapy [82].

Although a causal relationship between OS and preeclampsia is well-established, trials have failed to detect any risk reduction for preeclampsia with antioxidant supplementation. Trials powered to detect any smaller, more subtle benefits of antioxidant therapy in preventing placental pathology must be conducted before the routine use of anti-
oxidant vitamins by nulliparous, pregnant women can be recommended.

**Recurrent Pregnancy Loss**

Abnormal placentation has been implicated in the pathogenesis of both preeclampsia and miscarriage [71]. Recurrent pregnancy loss is a condition in which three or more consecutive, spontaneous abortions occur [83]. It affects 0.5% to 3% of women of reproductive age. Recurrent pregnancy loss has been associated with several factors, including maternal age, genetic factors, endocrinologic factors, anatomic problems, and environmental toxins [83]. Moreover, the etiology of recurrent pregnancy loss may be linked to chromosomal abnormalities, uterine anatomic anomalies, immunologic disorders such as antiphospholipid antibody syndrome, clotting disorders, and sperm DNA fragmentation [6]. However, 50%-60% of recurrent pregnancy losses are considered idiopathic [84]. Oxidative stress may be implicated in this subgroup as placental oxidative stress can lead to recurrent abortions by impairing placental development and causing syncytiotrophoblast degeneration [85]. During pregnancy both extracellular and intracellular ROS production increases sharply, originating from the developing embryo [84]. Thus, the demand for enzymatic antioxidant defense is increased in embryos and oocytes and their tubal and follicular fluid microenvironments to successfully support a pregnancy and the heightened OS it produces.

The increase in peripheral white blood cell count consisting of polymorphonuclear leukocytes (PMNL) accounts for the normal and natural rise in OS seen with normal pregnancy [86]. Fait et al. compared the changes in peripheral PMNL counts during early pregnancy with the non-pregnant state and found that in an uncomplicated early pregnancy, peripheral PMNL and neutrophilia counts were elevated [86]. The priming of the PMNL is known to cause an increased release of ROS and OS, which occur in early pregnancy [86]. This conclusion is also supported by a study by Safronova et al. that explored the changes in regulation of oxidase activity of peripheral blood granulocytes in women with habitual abortion. Researchers found that in the early stages of pregnancy, the peripheral polymorphonuclear leukocyte count increases [84].

A successful pregnancy requires a successful embryo implantation and adequate uteroplacental circulation for materno-fetal exchange [84,87]. The sharp peak in the expression of OS markers in the trophoblast in normal pregnancy may result in damage to protein, lipids, and DNA, which may ultimately lead to cell death if this oxidative burst becomes excessive. Joanne et al. confirmed the contribution of placental oxidative stress to early pregnancy failure, demonstrating significant increases in both morphological and immunohistochemical evidence of syncytiotrophoblast stress and damage in miscarried placental tissues. In a miscarriage, disorganized placental blood flow may lead to hypoxia and reperfusion injury with a resultant increase in the oxygen tension within the early placenta [88].

Moreover, the increase in oxygen concentration seen during normal early pregnancy renders the body more vulnerable to ROS formation, particularly within the mitochondria where electron leakage from the enzymes of the respiratory chain occurs. This increase in oxygen concentration may also lead to acute stress in the syncytiotrophoblast, with loss of function and extensive degeneration [88]. The syncytiotrophoblast is susceptible to OS because of its location on the villous surface, which makes this tissue first to experience the increase in intervillous PO2. Also, the syncytiotrophoblast possesses much lower concentrations of the antioxidant enzymes than other villous tissues during early gestation [87].

The connection between recurrent pregnancy loss and OS is not only corroborated by the increase in ROS generation seen in early pregnancy but also can be related to increased levels of antioxidants needed to neutralize and scavenge excessive ROS present in women affected by habitual abortion. Wang et al. has reported that levels of plasma vitamin E and lipid peroxides are increased in pregnant women versus non-pregnant controls [89]. Lipid peroxidation is an oxidative process that normally occurs at low levels, and antioxidant function has the ability to control the amount of oxidative stress it induces. However, when there is a deterioration of the antioxidants’ capacity to neutralize ROS, peroxidative activity occurs at the expense of polyunsaturated fatty acids.

Simsek et al. evaluated the outcome of deficient antioxidant defense in women with habitual abortion and demonstrated elevated lipoperoxides and significantly decreased vitamin A, E, and beta carotene in this population compared with the control group. Their findings confirm that OS may be involved in the pathogenesis of recurrent pregnancy loss [90]. Sane et al. found that women undergoing induced or spontaneous abortions exhibited a maximum rise in serum lipid peroxide levels immediately before the onset of abortion and significantly depressed levels of serum lipid peroxidase after the abortion was complete [91]. Jenkins et al. studied changes in antioxidant levels by measuring SOD levels, which measure the amount of oxygen ion scavenger that may result in increased ROS production [92]. This study found that SOD levels were significantly lower in women whose pregnancies ended in miscarriage than in healthy pregnant women [92].

The glutathione and glutathione transferase family of enzymes has been investigated in patients who experience recurrent pregnancy loss [70, 71]. The glutathione peroxidase reductase antioxidant system is an ROS scavenger, preventing lipid peroxidation in cells and maintaining intracellular homeostasis and redox balance [84]. Studies have shown glutathione concentration and activity to be significantly higher in women with recurrent miscarriage compared with the glutathione concentration seen in women with normal pregnancies or in healthy, non-pregnant women [93]. Red blood cell GSH-Px activity was not seen to differ between pregnant women and the control group, but were seen to be significantly deficient in women that had a miscarriage [93].

The bioavailability of selenium is directly related to the activity of the GSH-Px system. GSH-Px catalyzes the reduction of hydrogen peroxide and hydroxylperoxides, acting as a free radical scavenger and preventing the lipid peroxidation of cell membranes and [93]. And because GSH-Px is an enzyme that is essential in cells to neutralize the effects of free
radicals, selenium concentrations may decrease in those patients at risk of recurrent miscarriage because selenium is incorporated into the active site of GSH-Px [94,95]. Al-Kunani et al. reported significantly lower concentrations of selenium in the hair samples of women with recurrent pregnancy loss compared with controls [96]. Although this study failed to confirm a difference in the overall blood plasma selenium concentrations in women who had a miscarriage compared with those with viable pregnancies, selenium levels were found to be significantly higher in non-pregnant women [96], confirming that pregnancy in general is accompanied by a state of increased OS.

In addition to the various female factors related to recurrent pregnancy loss, several male factors can contribute to OS in these patients. A recent study by Gil-Villa et al. assessed sperm factors possibly involved in early recurrent pregnancy loss by evaluating and comparing standard sperm parameters, lipid peroxidation of sperm plasma membranes, antioxidant capacity of seminal plasma, and sperm chromatin integrity in ejaculates from partners who have a history of recurrent pregnancy loss with those from a control group [97]. Reactive oxygen species in semen from sources such as seminal leukocytes and morphologically abnormal spermatozoa is harmful to the functionality and structure of the sperm. An increase in sperm DNA damage has been associated with increased risk of undergoing embryo loss and augmented time to reach pregnancy [98]. This study successfully showed that a larger number of individuals of the recurrent pregnancy loss group presented alterations in sperm concentration, motility, morphology, and thiobarbituric acid-reactive substance production and lower antioxidant capacity of seminal plasma than did the individuals in the control group [97]. These findings may justify the use of antioxidant therapy in the male partner in couples experiencing recurrent pregnancy loss [97].

Given the array of evidence implicating OS in the pathogenesis of recurrent abortion, many studies have focused on the role of antioxidant supplementation in women affected by recurrent pregnancy loss [99]. Poor dietary intake of vitamins has been associated with an increased risk of miscarriage [100]. For instance, there are a variety of non-enzymatic antioxidants, including vitamins C, E, and A, lycopene, selenium compounds, lipoic acid, and ubiquinones [101] that have the ability to scavenge ROS and ultimately prevent OS and cellular damage [102]. An observational study demonstrated an association between the risk of spontaneous early miscarriage and intake of green vegetables, fruit, and dairy products [103]. Some evidence suggests that reduced intake of micronutrients during pregnancy exposes women to nutritional deficiencies and may affect fetal growth [104]. Thus, adequate maternal nutrition, particularly vitamin intake, may be an important factor in preventing miscarriage [100]. Although the evidence in regards to exactly what vitamin combinations, type, and amount are optimal for a pregnant woman is insufficient, the use of any vitamin supplements in pregnancy needs to be carefully monitored and evaluated [100].

Vitamin C and E are two popular vitamins that may have a potential role in alleviating the effects of OS in women affected with miscarriages. Vitamin E’s principal function is to protect against OS-related damage and thereby serve as an antioxidant. In a normal pregnancy, vitamin E level naturally increases, while in an abnormal pregnancy, vitamin E concentrations are lower [102]. Moreover, vitamin C levels increase physiologically during pregnancy [102]. These occurrences suggest that perhaps vitamins C and E may play a role in compensating for the oxidative burst during early pregnancy, reducing the risk of pregnancy loss [102]. However, it is necessary to perform an accurate assessment of the appropriate type and dosage of vitamins that can be tolerated without causing deleterious side-effects to the mother and baby [100].

Homocysteine is a thiol-containing amino acid that is involved in the sulfurylation and methylation metabolic pathways and has been proposed to have pro-oxidant effects [84]. Normally, plasma homocysteine levels fall during a normal pregnancy. However, Del Banco et al. demonstrated significantly elevated homocysteine levels in women with a history of at least two consecutive miscarriages [105].

Quere et al. conducted a study evaluating the effect of vitamin supplementation on pregnancy outcome in 25 women with recurrent early pregnancy loss and hyperhomocysteinemia in the absence of any folate supplementation during pregnancy [106]. This study involved hyperhomocysteinemic patients. Folic acid supplements are believed to reduce a woman’s risk for having a baby with a neural-tube defect. The potential for folic acid to prevent elevated homocysteine levels and OS-induced miscarriage has been the focus of many investigations. Szymanski et al. found that women receiving folic acid supplements had better quality oocytes and a higher degree of mature oocytes compared with those who did not receive folic acid supplementation [107]. However, the results of a study by Gindler et al. failed to confirm that the consumption of folic acid decrease a woman’s risk for miscarriage [108]. Thus, the role of folic acid supplementation to prevent recurrent pregnancy loss is inconclusive and requires further analysis.

Antiphospholipid (aPL) antibody syndrome is a known autoimmune cause of recurrent pregnancy loss [84]. The pathophysiology of antibody formation is still not clear; however, OS has been proposed to have a role in the formation of these antibodies [84]. Omega-3 supplements have been used in prevention of recurrent miscarriage with antiphospholipid syndrome [109]. Del Bianco et al. studied the effects of omega-3 fatty acid supplementation in women with three or more miscarriages associated with antiphospholipid syndrome [105]. All of the subjects in this trial achieved successful pregnancy with no further miscarriages [105]. Although the results of this study are promising, the safety and efficacy of omega-3 supplementation must be confirmed by follow-up trials.

Melatonin, a hormone that acts as a powerful agent against ROS, has been hypothesized to have properties essential for successful pregnancy and prevention of spontaneous abortion [110]. Tamura et al. propose that deficient melatonin production in early pregnancy may be related to the development of spontaneous abortion as melatonin is a free-radical scavenger and antioxidant that is known to physiologically increase to compensate for the oxidative burst during normal pregnancy [110]. Therefore, the safety
and efficacy of melatonin supplementation to combat OS and prevent recurrent pregnancy loss should be further investigated.

**EXPERT COMMENTARY**

The purpose of this article was to discuss the role of antioxidant supplementation in the treatment of various gynecological and obstetric conditions known to contribute to female infertility and poor pregnancy outcome. The association between high levels of uncontrolled oxidative stress and polycystic ovarian syndrome, endometriosis, unexplained infertility, preeclampsia, and recurrent pregnancy loss has been well established by numerous studies that have measured various biomarkers of redox status. As high degrees of reactive oxygen species and low antioxidant status has been implicated in these diseases, treatment based on strategies to boost the exhausted antioxidant defense of the female reproductive microenvironment are intuitive. This approach seems especially plausible in light of the fact that oral antioxidant supplementation in males has been proven effective to treat male infertility and is widely employed in current clinical practice. However, reports regarding the safety and efficacy of oral antioxidant supplementation in the treatment of female infertility are conflicting. Additional studies using double-blinded, randomized, controlled trials are needed to further evaluate the potential use of antioxidants to treat female reproductive disease.

**FIVE YEAR VIEW**

There is a need for continued investigation of the efficacy and safety profiles of various oral antioxidant supplements before this modality of treatment can be relied upon to modulate the levels of oxidative stress that contribute to infertility and abnormal pregnancy in women with obstetrical and gynecologic disease. Observational studies and randomized control trials have identified several antioxidant therapies of interest which have demonstrated striking promise in the prevention and treatment of female reproductive disease. However, it is still unclear as to what types or combinations of therapy as well as the amounts and dosing that are optimal for women, particularly during pregnancy. It is necessary to conduct further studies to identify any possibility of deleterious side effects of antioxidants on mothers and their unborn baby. Despite the fact that oxidative stress is strongly implicated in the etiopathogenesis of preeclampsia, the literature fails to show strong evidence to support the efficacy of antioxidant supplementation in preventing or treating preeclampsia. Further investigation with randomized controlled trials powered to detect subtler effects may reveal any previously hidden benefits of antioxidant therapy for preeclampsia. In addition to analyzing the effect of antioxidant supplementation in women to improve fertility and pregnancy outcome, the benefits of antioxidant supplementation in male partners of couples with infertility and recurrent pregnancy loss should be studied.

**KEY ISSUES**

- Oxidative stress (OS) occurs when the generation of reactive oxygen species (ROS) and other radical species overrides the scavenging capacity of antioxidants, either due to excessive ROS production or an inadequate availability of antioxidants.
  - At controlled levels, OS facilitates the following physiological female reproductive functions: oocyte maturation, folliculogenesis, ovarian steroidogenesis, luteolysis, ovulation, cyclical endometrial changes, and menstruation.
  - At higher levels, OS is implicated in pathological processes of the female reproductive tract that contribute to infertility and poor pregnancy outcome, such as polycystic ovarian syndrome (PCOS), endometriosis, unexplained infertility, preeclampsia, and recurrent pregnancy loss.
- Antioxidant treatment of PCOS: N-acetyl cysteine may improve glucose control and peripheral insulin sensitivity in hyperinsulinemic patients. Oral hypoglycaemic agent rosiglitazone and anti-androgenic oral contraceptives have been shown to reduce parameters of OS in hyperinsulinemic patients.
- Endometriosis: The antioxidants catalase, RU486, curcumin, melatonin, and catechins may have anti-proliferative and anti-angiogenic effects capable of halting disease progression.
- Selenium supplementation of women with unexplained infertility has been shown to normalize patient’s RBC-Mg levels and result in clinical pregnancy after 8 months of treatment.
- Despite the well-established causal relationship between OS and preeclampsia, studies have failed to detect any risk reduction for preeclampsia with vitamin C and vitamin E supplementation.
- The antioxidants folic acid, melatonin, and omega-3 fatty acids (particularly in women with antiphospholid antibody syndrome) have been investigated for their use in preventing recurrent pregnancy loss. Further studies to confirm the safety and efficacy of these compounds are needed.

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Role of Oxidative Stress in Polycystic Ovary Syndrome

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Abstract: Polycystic ovary syndrome (PCOS) is a multifactorial disorder affecting many women of reproductive age, typically due to hyperandrogenemia, hyperinsulinemia, and enigmatic genetic factors. The complex nature of PCOS is reflected in the broad spectrum of the disorder’s clinical presentation, including metabolic and reproductive disorders. As a result, while the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine (ESHRE/ASRM) have agreed on a consensus definition of PCOS to help clinical investigators, the condition is recognized to have multiple clinical phenotypes.

Oxidative stress (OS) occurs when destructive reactive oxygen species (ROS) outbalance antioxidants, causing DNA damage and/or cell apoptosis. Moreover, reactive nitrogen species (RNS), such as nitrogen oxide (NO) with an unpaired electron also are highly reactive and toxic. In a quest to delineate the role of OS in the pathogenesis of PCOS, investigators have examined patients with the disorder for a wide array of OS biomarkers, including malondialdehyde (MDA), protein carbonyl, total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione (GSH).

Keywords: Polycystic ovary syndrome (PCOS), oxidative stress (OS), insulin resistance, hyperandrogenism, reactive oxygen species (ROS), nitric oxide synthase (NOS).

INTRODUCTION

PCOS is one of the most common endocrinological pathologies in women during their reproductive years exhibiting a wide spectrum of clinical manifestations. PCOS women commonly have features of hyperandrogenism and the primary cause of PCOS is probably multifactorial in origin [1]. Increased insulin resistance is viewed as a central feature of PCOS irrespective of the body mass index (BMI). The resulting hyperinsulinemia together with central obesity, which is frequently encountered in PCOS patients, are components of metabolic syndrome. Metabolic syndrome, which affects one in five people, increases the risk of developing cardiovascular disease and type II diabetes, and its prevalence increases with age. PCOS patients have been reported to have markers of cardiovascular and endothelial disorders in addition to the familiar features of hirsutisms, acne, and anovulatory infertility [2].

Oxidative stress is commonly referred as the imbalance between oxidants and antioxidants. When the imbalance favors oxidants, generation of excessive amounts of reactive oxygen species harm our body in various ways [3] through the generation of excessive amounts of reactive oxygen species. In other words, reproductive cells and tissues will remain stable only when antioxidant and oxidant status is in balance. Oxidative stress, which is generally known to be present in women with PCOS regardless of whether they are lean or have metabolic abnormalities, has been documented in infertile women [4]. The present review study provides an overview of current knowledge in PCOS and ROS’ roles in women during their reproductive years, exhibiting a wide spectrum of clinical manifestations in PCOS women, which have been investigated more actively in recent years.

DEFINITION AND DIAGNOSIS OF POLYCYSTIC OVARY SYNDROME

The definition of PCOS has been controversial and still remains unclear due to the syndrome’s heterotrophic nature. Following the first report on women with polycystic ovaries in 1935, the term “polycystic ovarian syndrome” was established as more clinicians noticed the correlations between hyperinsulinemia, androstenedione, testosterone levels, and PCOS [5]. However, a wide spectrum of clinical manifestations, including impaired glucose tolerance [6], prevalence of type II diabetes [7], increased risk of hypertension and dyslipidemia, and elevated endothelial dysfunction [8] further complicated the debate on defining PCOS. The presence of clinical or biochemical hyperandrogenism or polycystic ovaries with regular cycles was broadly interpreted as PCOS [9, 10]. As a result, there were no widely accepted diagnostic criteria available until the National Institute of Health (NIH) criteria were introduced in 1990.

In 1990, the NIH established diagnostic criteria that characterize PCOS as the combination of oligomenorrhea or amenorrhea and hyperandrogenemia in the absence of non-classical adrenal hyperplasia, hyperprolactinemia, and thyroid dysfunction [11]. These criteria, however, did not include ultrasound morphology of polycystic ovaries in the belief that broader clinical diagnostic criteria were in need for clinicians to accurately diagnose multi-etiological PCOS. In Europe, clinicians maintained that the ultrasound appearance of polycystic ovary was an essential criterion to diag-
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The boundaries should be set in diagnosing PCOS. As a result of the continued dialogue between the ESHRE and the ASRM, a consensus document was produced, commonly referred to as the Rotterdam 2003 criteria for defining PCOS.

The new definition of PCOS suggested that the diagnosis of PCOS must be based on the presence of two of the three following criteria: (i) oligo- and/or anovulation, (ii) clinical and/or biochemical signs of hyperandrogenism, and (iii) polycystic ovaries on ultrasonography and exclusion of related disorders [12, 13]. The ultrasound criteria for polycystic ovaries is defined as the presence of 12 or more follicles measuring 2 to 9 mm in diameter and/or an increased ovarian volume > 10 cm³ on transvaginal ultrasound scanning. PCOS is diagnosed even when only one polycystic ovary is present [14]. However, these criteria do not apply to women taking oral contraceptive pills since their use modifies ovarian morphology that slightly different biochemical findings were included in Rotterdam criteria (Table 1) [14]. While NIH criteria considered total testosterone, free testosterone, androstenedione, and DHEA as biochemical markers, the 2003 Rotterdam criteria now consider free androgen index, total testosterone, and DHEA as diagnostic biochemical markers. Moreover, the Rotterdam criteria recognize the role of genetics in PCOS and encourage clinicians to take family histories to identify PCOS individuals more effectively [12, 13]. Compared with the NIH definition, the new definition introduced two new phenotypes: (i) ovulatory women with polycystic ovaries and hyperandrogenism, and (ii) oligo-ovulatory women with polycystic ovaries without hyperandrogenism. This has stimulated more debate as to where the boundaries should be set in diagnosing PCOS [1].

Table 1. Comparison of Two Established Diagnostic Criteria of PCOS

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<td>(i) Oligomenorrhea or amenorrhea</td>
<td>(i) Oligo- and/or anovulation</td>
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<td>(ii) Hyperandrogenemia in the absence of related disorders</td>
<td>(ii) Clinical and/or biochemical signs of hyperandrogenism</td>
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<td>(iii) Polycystic ovaries on ultrasonography and exclusion of related disorders</td>
<td>Diagnostic markers: total testosterone, free testosterone, androstenedione and DHEA</td>
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<td>Both (i) and (ii) must be present</td>
<td>Two of three criteria must be satisfied</td>
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Hyperandrogenism (Hirsutism, Acne, and Male Pattern Alopecia)

Hypersecretion of androgens is the most widespread biochemical feature in PCOS women [19]. PCOS accounts for 70-80% of hyperandrogenism and is associated with elevated serum total or free testosterone concentrations [20]. Hyperandrogenism can manifest as hirsutism, acne, and male pattern alopecia. Whether hyperandrogenemia affects oxidant and antioxidant status in women with PCOS is unknown. However, in a human study, ROS generation was demonstrated to directly correlate with testosterone and androstenedione [21], suggesting that ROS induces OS, which may consequently contribute to hyperandrogenism in PCOS women. Plasma testosterone or androstenedione and ROS generation are associated, suggesting that OS may directly stimulate hyperandrogenism. In vitro studies have demonstrated that OS stimulates the androgen-producing ovarian steroidogenic enzymes, while antioxidants such as statins suppress these enzymes [22].

PCOS is present in 60-90% of women with hirsutism [16, 17, 23, 24] as increased androgen production leads to hirsutism and acne. Among women with PCOS, 35% have acne, and 6% express alopecia [17]. An inflammatory disorder of the hair follicle, acne is associated mainly with elevated levels of sebaceous secretion [25, 26]. Among women of mixed ethnicities with androgenic alopecia, 67% had polycystic ovaries compared with the 27% expressed in the BMI-, waist-hip-ratio- and age-matched control group [27]. Also, 21% of the women with androgenic alopecia demonstrated hirsutism, while this is true for only 4% of the BMI-, waist-hip-ratio- and age-matched control group.

Ovarian invasion by macrophages has been observed in PCOS women [28]. Moreover, mononuclear cells in the polycystic ovary activated by glucose can generate OS that could stimulate a local inflammatory response, which could in turn induce the generation of ovarian androgen in PCOS women [29]. More specifically, theca cells in the ovarian

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tissue overproduce androgens and insulin receptors and lead to hyperandrogenism [31]. Moreover, some researchers have investigated genetically programmed androgen secretion by the ovary during early childhood or puberty, which may contribute to pathophysiology in PCOS women [30].

**Acanthosis Nigricans**

Acanthosis nigricans, a disorder seen as dark and velvety skin with hyperpigmentation and papillomatosis, manifests itself normally in the axillae, skin flexures, and nape of the neck. Among women with PCOS, only 3% express acanthosis nigricans [17], which is associated with insulin resistance and, consequently, hyperinsulinemia [32].

**Insulin Resistance**

Increased oxidant status has been shown to correlate with insulin resistance. Insulin resistance can be found in 25-60% of women with PCOS [33]. The wide range may be due to varying diagnostic criteria, the heterogeneous nature of PCOS, and ethnic variations. Insulin resistance (IR) and hyperglycemia both can increase OS levels, although higher levels of total oxidant and antioxidant status have been demonstrated in non-obese PCOS patients without IR [34]. Hyperglycemia has been demonstrated to increase lipid peroxidation and lower antioxidant levels [35]. A significantly negative correlation between MDA levels, a marker of OS, and insulin sensitivity, as well as MDA levels and GSH (antioxidant) levels has been demonstrated [4]. This may imply that insulin resistance decreases antioxidant levels and increases lipid hydroperoxide (LPO).

Insulin resistance encourages oxidative stress because hyperglycemia and higher levels of free fatty acids lead to ROS production. An increase in ROS generation resulting from hyperglycemia has been observed in women with PCOS [36], and insulin infusion in obese individuals has been shown to inhibit ROS production [37]. Thus, insulin may defend against pro-inflammatory responses to hyperglycemia by acting as an anti-inflammatory agent.

Approximately 75% of obese PCOS women have IR and hyperinsulinemia [38]. However, insulin resistance independent of obesity can play a role in PCOS. Young, non-obese PCOS patients with high triglyceride levels as the only dyslipidemic feature have demonstrated high oxidative levels [2]. Furthermore, 20-40% of women with PCOS have impaired glucose tolerance [6], and women with PCOS exhibit higher levels of type II diabetes (T2DM) than non-PCOS controls (15% vs 2-3% in normal women) [7]. Insulin resistance and hyperinsulinemia also are features of metabolic syndrome, and women with PCOS exhibit an increased risk of hypertension, dyslipidemia, elevated plasminogen inhibitor type 1, elevated endothelin, endothelial dysfunction, and cardiovascular disease similar to the risks associated with metabolic syndrome [8].

**Reproductive Aberration (Irregular Menses, Infertility, Miscarriage)**

PCOS is a known cause of menstrual irregularity as well as infertility. Most commonly, irregular menstruation is as-
associated with anovulation. Between 30-40% of women with amenorrhea are found to have PCOS [39].

Among PCOS women, more than 60% manifest infertility (primary/secondary), and 19% experience amenorrhea [17]. Moreover, pregnancy in PCOS women is more likely to be complicated by gestational diabetes, preeclampsia, pregnancy hypertension, and preterm labor leading to miscarriage [40]. Obesity in PCOS further increases resistance to ovulation induction treatment since obesity is associated with a disturbed pattern of gonadotrophin-releasing hormone production resulting in chronic elevation of tonic LH level with negative consequences on follicular development in the ovary [40].

**Obesity**

Obesity is more common in women with PCOS, and it can lead to severe hyperandrogenism. According to Franks (1989), 35% of women with PCOS are obese [10]. Increasing visceral adipose tissue and/or its activity may contribute to androgenic modulation [41]. Androgen excess is a known contributor to visceral adiposity in women, which provides high metabolically active tissue that stimulates the ovaries and adrenal to proceed with androgenization [15]. Both androgens and IR seem to have a combined effect on upper body adipose distribution. Obesity contributes to PCOS, as it affects hyperandrogenism and IR. In fact, the most influential factor in endocrinologic and metabolic disturbances in women with PCOS has been shown to be an elevated BMI > 25 [42].

Central obesity is also related to increased oxidant status [4]. Obesity has been shown to play an important role in elevated oxidative stress, which contributes to IR [43]. PCOS patients who are obese express higher levels of insulin resistance than lean PCOS patients [8]. The study by González et al. (2006) shows that compared with lean controls, PCOS women express higher p47 phox levels independent of obesity. In oxidative stress, p47phox plays a role as part of enzymes that produce the superoxide radical. Increase in p47phox expression decreases insulin sensitivity. It also has been shown to be greater in PCOS women versus controls and in obese PCOS and non-PCOS women versus lean PCOS women and non-PCOS women [29]. Thus, increased obesity may determine ROS-induced OS in obese PCOS women. PCOS also affects insulin performance, as increase in abdominal fat is associated with insulin resistance [1]. Slightly reducing the body weight of anovulatory, obese women was demonstrated to restore ovulation and increase insulin sensitivity by 71% [44]. Weight loss also reduces testosterone concentration, improves menstrual function and conception rates, decreases the likelihood of miscarriage, and increases sex hormone-binding globulin (SHBG) concentration [45-48].

Aside from obese PCOS women, lean PCOS women can express increased levels of abdominal adiposity [21]. In a study of 16 women with PCOS and 15 women without PCOS, mononuclear cells produced elevated levels of ROS in response to hyperglycemia in PCOS women, independent of obesity [29].

**ETIOLOGY OF POLYCYSTIC OVARY SYNDROME**

**Pathogenesis of PCOS**

Women with PCOS manifest a wide spectrum of symptoms and clinical features, including hyperandrogenism, ovulatory disturbances and polycystic ovaries and metabolic syndromes (Fig. 1). The latter is linked to insulin resistance and obesity that are often associated with PCOS [6, 49, 50]. In other words, the heterogeneity of PCOS is reflected in the multiplicity of factors such as insulin resistance, hyperandrogenism, and dysfunctional gonadotrophin dynamics that must come into play to manifest the disorder, and no single mechanism accounts for all clinical and biochemical forms of this syndrome. Moreover, environmental factors such as diet or stress also can trigger underlying risk factors and cause the development of PCOS. The most commonly discussed causes of PCOS can be categorized into three mechanisms: (i) insulin resistance and hyperinsulinemia, (ii) hyperandrogenemia and (iii) genetic factors.

**(i) Insulin Resistance and Hyperinsulinemia**

Insulin resistance, in which an abnormally high amount of insulin (hyperinsulinemia) is required to initiate a cellular response, is the most commonly encountered clinical disorder in both obese and non-obese PCOS women [51]. An oral glucose tolerance test is recommended for PCOS patients with BMI greater than 27 kg/m² [14] because of the high risk for developing impaired glucose tolerance and diabetes in obese PCOS women (31% of obese PCOS patients vs. 10.3% of lean PCOS patients and 7.5% of obese PCOS patients vs. 1.5% of lean PCOS patients, respectively) [6]. Women with IR display increased fasting insulin level compared with controls of similar age and body weight. As a result, clinical and molecular research has focused on insulin receptor and post-receptor defects [19]. Some studies have correlated severity of hyperinsulinemia to the degree of clinical manifestation.

Insulin signaling, mediated through a protein tyrosine kinase receptor, has been investigated in PCOS patients. Dunai et al. (1997) reported excessive serine phosphorylation, which inhibits insulin receptor tyrosine kinase activity, of insulin receptors in insulin-resistant PCOS patients. Moreover, adverse roles of serine phosphorylation in insulin signaling were further supported by the mechanism of tumor necrosis factor (TNF)-α-mediated insulin resistance in obese women [53] and P450c17 enzyme activity leading to hyperandrogenism in PCOS women [54]. Hyperinsulinemia also potentiates the effects of LH on theca interstitial cells, resulting in increased androgen production [19] while arresting the follicular maturation process [55, 56].

**(ii) Hyperandrogenemia**

The ovary is the primary source of hyperandrogenism in PCOS, driven by increased levels of LH hormone as ovarian dysfunction causes LH insensitivity [57]. The increase in basal LH level is the result of a disrupted hypothalamic-pituitary-gonadal axis [58]. Moreover, hyperandrogenemia impairs progesterone’s ability to slow down the gonadotropin-releasing hormone (GnRH) pulse [58]. As a result, elevated GnRH pulses further increase LH level and reduce
FSH, which converts excess androgen into estrogens via aromatase activity in normal women [15]. The elevated LH level arrests follicular cells and stimulates theca-cell-mediated androgen synthesis. Consequently, the increased androgenic environment in the ovary impairs follicular maturation [8].

Some studies have shown hyperandrogenemia and hypoestrogenemia in PCOS-like conditions as the result of ovarian steroidogenic enzyme deficiencies such as 3β-hydroxysteroid dehydrogenase type II and aromatase [59]. In other words, follicles that are unable to change their surroundings from androgen-dominant to estrogen-dominant environments will not acquire normal follicular growth and manifest as a polycystic ovary, a characteristic feature of PCOS. Adrenal steroidogenesis dysfunction also has been implicated in establishing a state of hyperandrogenemia in PCOS. When adrenal steroidogenesis dysfunction results in reduced cortisol production, adrenocorticotropic hormone (ACTH) production is increased to maintain normal serum cortisol level [8]. Increased ACTH production consequently stimulates adrenal androgen excess [8]. Thus, hyperandrogenism in PCOS women is caused by synergic aberration in steroidogenesis of both ovary and adrenal glands.

As stated above, hyperinsulinemia drives increased androgen production by theca cells [60]. Studies have shown that bilateral oophorectomy, the surgical removal of both ovaries [61, 62], the administration of GnRH-agonists to mimic an increased GnRH pulse [63], or antiandrogenic compounds [64] did not alter hyperinsulinemia and IR in PCOS women. Evidence supports disordered insulin action as a predecessor to development of hyperandrogenemia in PCOS patients.

(iii) Genetic Factors

Given that the incidence of PCOS is 6-8% in the general population [65], 35% of premenopausal mothers and 40% of sisters of PCOS women [65] suggests a probable role for genetics in PCOS. However, no conclusive role for any gene has been defined. This may be due to the limited selection of candidate genes, PCOS’s heterogeneous nature, or lack of knowledge of disease pathophysiology and the role of environmental and lifestyle factors such as diet and obesity in modifying gene expressions [66]. Moreover, lack of universal male patterns and reliable markers for PCOS in women further challenge investigations of the syndrome’s genetic origin. The proposed male phenotypes, such as increased serum dehydroepiandrosterone sulfate concentrations in brothers of PCOS women and insulin resistance in fathers and brothers of PCOS women still require further investigation for their practical uses [67]. However, more than 100 candidate gene approaches have selected genes based on their hypothetical roles in PCOS and target four general areas: (i) steroid biosynthesis and action; (ii) gonadotrophin synthesis and action; (iii) weight and energy regulation; and (iv) insulin secretion and action, as well as several areas added recently such as cardiovascular disease via inflammation, hypercoagulation, and blood pressure [66]. Of those, genes involved in steroidogenic abnormalities and insulin metabolism aberrations have been investigated the most due to their importance in PCOS’ clinical manifestations.

Hyperandrogenemia in PCOS women is due partially to intrinsic defects in metabolic pathways. Because hyperandrogenism is prevalent among PCOS patients, genes involved in steroidogenesis such as cytochrome P450 17α-hydroxylase/17,20-desmolase (CYP17) and the aromatase gene (CYP19) have been investigated. Upregulation of 3α-hydroxysteroid dehydrogenase and 17α-hydroxylase/17,20-lyase activities in PCOS women [68] are reflected in increased mRNA expression and an enhanced promoter region of CYP17 genes of the theca cells in young girls compared with controls [69]. On the other hand, a functional mutation of the CYP19 aromatase gene leads to excess circulating androgens in PCOS women [70-72]. However, family studies have not yet shown a correlation between CYP19 and PCOS [73], and more evidence is needed to confirm this hypothesis.

Genes involved in insulin signal transduction have been investigated. Variable number tandem repeat (VNTR) polymorphism in the promoter region of the insulin gene at 11p15.5 has shown quite conflicting results. While Waterworth et al. (1997) [74] found strong correlations between class III variable number tandem repeats of the insulin gene allele and PCOS, Urbanek et al. (1999) [75] did not find evidence to link the class III allele and PCOS. The insulin receptor gene is another probable candidate gene since it seems to be silenced in molecular studies [19]. However, defective insulin receptor function is observed in the presence of serine phosphorylation instead of tyrosine phosphorylation in insulin receptors [52], suggesting more studies are required on downstream targets of the insulin receptor gene [19].

Hormonal Markers in PCOS Patients

Hormonal markers in PCOS women are viewed as a way to evaluate steroidogenesis. The most commonly encountered markers include, but are not limited to LH, FSH, estrogen, sex hormone-binding globulins (SHBG), insulin-like growth factor -1 (IGF-1), total/free testosterone, androstenedione, dehydroepiandrosterone (DHEA) and DHEA metabolite DHEAS, anti-Mullerian hormone (AMH), and 17-hydroxyprogesterone [8, 19, 60, 76].

Testosterone production and high insulin level in PCOS women directly down-regulate SHBG synthesis by the liver, which makes a low SHBG level a good indicator of insulin resistance [77]. SHBG has strong binding affinity to testosterone and dihydrotestosterone thus controlling androgen bioavailability in serum [78]. Reduced SHBG results in increased levels of bioavailable testosterone. Since serum-bound testosterone (T) is the most frequent androgen measured to diagnose hyperandrogenemia, the reduction in the proportion bound to SHBG makes the assessment somewhat unreliable [76]. As a result, the free androgen index (FAI=T/SHBG * 100%) or the association constant for testosterone binding to SHBG and albumin are utilized to account for these metabolic changes [79]. Free T also may be measured directly via equilibrium dialysis [76]. Although other androgens such as androstenedione (A4) or total testosterone may also be utilized to diagnose hyperandrogenemia, no studies have indicated their superiority as surrogate markers. For example, Knochenhauer et al. (1998) showed that only 2 out of 11 (18%) PCOS women had abnormally higher
thyroxine (T4) level, which is blunted by high testosterone level.

Insulin binds to IGF-1 receptors on theca cells with significantly higher affinities than IGF-1 [81]. Hepatic IGF-1 binding protein secretion also is inhibited in PCOS women, leading to excessive free IGF-1, which is suspected to play a role in the abnormal androgenesis of theca cells along with high LH [82]. IGF-1 and insulin further increase mRNA of P450c17, leading to increased androgen biosynthesis in ovary and adrenal glands [8]. The use of insulin-sensitizing agents such as metformin has been demonstrated not only to reduce circulating insulin concentration but also to reduce ovarian androgen biosynthesis [83].

DHEA secreted from the adrenal zona reticularis is another actively investigated hormonal marker in PCOS women. However, DHEA has several shortcomings as a surrogate marker due to its diurnal variation, intra-subject variation and low serum concentration [84]. On the other hand, DHEAS, DHEA’s sulfate ester, is not subject to these variations, making it a more preferred marker to assess increased adrenal androgen production [85]. In clinical studies, approximately 20-70% of PCOS women manifest excess DHEAS serum levels [86-88]. However, DHEAS levels decrease with age [88], and levels are controlled by the activity of DHEA sulfotransferase [89]. Moreover, ethnicity also may affect circulating DHEAS levels with lower circulating levels of DHEAS is reported in Mexican American group compared with Caucasian American controls [90]. Consequently, in PCOS patients with high DHEAS measurements, only 10% will actually have hyperandrogenaemia [76]. Thus, DHEAS measurements should be interpreted with caution [76].

AMH is secreted from the Sertoli cells of the fetal testis to inhibit female Mullerian ducts development in a male embryo. It also is produced by the granulosa cells of small antral and pre-antral follicles to disrupt FSH’s aromatase induction in the ovary [91], compromising normal ovulation in PCOS women. Studies have shown that AMH levels are significantly higher in PCOS women [92] and confirmed that granulosa cells release more AMH when cultured in vitro [93]. Moreover, AMH level was positively correlated with antral follicle counts [94], suggesting that serum AMH measurements may serve as an alternative diagnostic tool when ultrasonography is not an option inpatients younger than 35 (1). While AMH’s role in folliculogenesis is generally established, its association with circulating androgens is more controversial. Pigny et al. (2003) [95] found a correlation between AMH and testosterone and androstenedione only in PCOS women, while Piltonen et al. (2005) [96] reported AMH levels in both PCOS women and controls were correlated with both testosterone and androstenedione.

In conclusion, no hormonal marker can be used as the sole criterion to diagnose PCOS. Hormonal assays may serve as supplementary diagnostic tools for clinicians and scientists.

INTRODUCTION TO OXIDATIVE STRESS

Unstable and highly reactive, free radicals achieve stability by stealing electrons from nucleic acids, proteins, lipids, carbohydrates, and other nearby molecules [97], thus inducing cellular damage. The two major forms of free radicals are ROS and RNS. Free electrons typically form reactive oxygen species during oxygen reduction as a by-product of natural metabolic pathways [98]. Most of the mitochondrial generation of ROS occurs at complexes I (where NADH dehydrogenase acts), and III (where the ubiquinol to ubiquisemiquinone to ubiquinone conversion occurs) of the electron transport chain (ETC) [99].

Of inspired oxygen, 98% is reduced during lipolysis and chemical energy generation, and 2% is incompletely reduced, leading to three major forms of ROS [97]. The three major forms of reactive oxygen species are the superoxide radical [O2·−], hydrogen peroxide [H2O2], and hydroxyl [HO·]. Superoxide is formed through electron leakage at the electron transport train. At complex IV, molecular oxygen normally is converted to water, but it may gain an extra electron as they are being passed down the ETC during ATP generation [100]. Hydrogen peroxide is formed from either superoxide dismutation or oxidase enzymes. The most reactive form is the hydroxyl ion, as it has three extra electrons. Through alteration of purines and pyrimidines it can cause strand breaks and damage DNA. When the balance between antioxidants and oxidants does not exist, modification of key transcription factors can occur, which can alter gene expression (Fig. 2). The superoxide radical can be converted to hydrogen peroxide by mitochondrial superoxide dismutase 2, preceding further modification by GSH peroxidase to form water. Thus, the presence of antioxidants is vital to maintaining redox homeostasis. Decreased amounts of antioxidants to counteract the production of ROS can lead to cell damage [99].

ROLE OF ROS IN PCOS

ROS are free radicals with oxygen centers. An unpaired electron in the outermost shell is an extremely unstable configuration, and free radicals quickly react with other molecules or radicals to achieve the stable configuration of pairs of electrons in their outermost shells [101]. Several basic cellular processes lead to the production of ROS within a cell. Cellular respiration involves the reduction of molecular oxygen (O2) to water in the electron transport chain. This reduction occurs through a series of reactions: (i) O2 + e− → O2−, (ii) O2− + 2H2O → 2H2O2, (iii) O2− + H2O2 → OH− + OH + O2. As mentioned earlier, the superoxide anion radical (O2·−), hydrogen peroxide (H2O2), and the hydroxyl radical (HO·) are three major species of ROS [97].

Role of MDA in PCOS

Unsaturated fatty acid peroxidation is a radical chain reaction initiated by the abstraction of a hydrogen atom from a methylene group of the fatty acid chain. The carbon radical formed by this reaction tends to be stabilized by molecular rearrangement, leading to conjugated double bonds. By reaction with oxygen, a reactive peroxy radical is generated that can abstract a hydrogen atom from lipids [102].

Products of lipid peroxidation reactions have been widely employed as biomarkers for OS. MDA, produced during the decomposition of polyunsaturated fatty acids, is one of the stable end products of lipid peroxidation that can serve as a
good biomarker [102]. Several methods are available for quantification of lipid hydroperoxides and secondary lipid peroxidation products. MDA is most commonly measured by a thiobarbituric acid-reactive substances (TBARS) assay with a simple spectrophotometric method. The amount of MDA corresponds to the chromogen found from MDA and thiobarbituric acid (TBA) with a maximum absorption at 532-535 nm. While the assay for MDA is non-specific, HPLC is a more accurate tool for MDA estimation.

Fig. (2). Oxidative stress occurs when the balance between highly reactive radicals (oxidants) and antioxidants tips towards the oxidants; it negatively contributes to reproductive processes.

Kușcu et al. (2009) compared PCOS patients (n=31, mean age 23.8 years and mean BMI 21.8) with healthy controls. Blood MDA level, not specified as measured from serum or erythrocyte, was found to be significantly higher in the PCOS group (0.12±0.03 vs 0.10±0.03, p=0.01). This study demonstrated that PCOS subjects had significantly elevated concentration of plasma MDA independent of obesity. PCOS patients in this study were further divided into two subgroups in terms of insulin resistance, IR- and IR+. The results showed that MDA level is significantly higher in young, non-obese PCOS patients even in the absence of IR when compared with controls (0.125±0.03 vs 0.101±0.03, p=0.03) [2].

Sabuncu et al. (2001) compared PCOS patients (n=27, mean BMI 31.4 and mean age 26.7 years) with BMI- and age-matched controls. They demonstrated that higher levels of erythrocyte MDA were seen in PCOS patients (mean=70.9 µmol/mol Hb) compared with controls (p=0.009). Significantly higher levels of MDA in PCOS patients compared with controls also were also found by Palacio et al. (2006) [103].

Zhang et al. (2008) demonstrated that serum MDA levels in PCOS patients (n=30) were significantly higher than those of controls (12.31±2.51 vs 6.93±1.66 µmol/L, P<0.05) [104]. A negative point of this study was that some of the important patient characteristics, such as BMI and age, were not recorded.

However, Karadeniz et al. (2008) [105] found MDA levels in PCOS patients (n=58) were similar to those of controls (5.38±2.47 vs 4.47±2.06, p>0.05) [105]. Furthermore, MDA levels were found to be similar in a PCOS patient group where the homeostatic model assessment (HOMA)-IR was below and above the cutoff value of 1.75. This observation suggests that the presence of insulin resistance in PCOS patients has no effect on MDA levels. In addition, Dursun et al. studied PCOS patients (n=23, mean BMI 23.0 and mean age 24.4 years) and found serum MDA levels in PCOS patients were similar to those of BMI- and smoking status-matched controls (3.60±1.22 vs 3.53±1.0 µmol/l) [106].

Role of Protein Carbonyl

Protein oxidation status often is assessed with a colorimetric assay that measures protein carbonyl (PC) content, after reacting the serum with dinitrophenylhydrazine. Fenkci et al. (2007) demonstrated that the PC level was significantly higher in PCOS patients with normal BMI compared with controls (18.01±0.80 vs 14.19±0.40 nmol/L, p=0.001). This observation of higher protein oxidation suggested that free radicals damage proteins in PCOS patients [107]. Furthermore, protein carbonyls were shown to have a positive correlation with fasting insulin, suggesting a strong association between insulin resistance and protein oxidation in PCOS [107].

Role of NOS in PCOS

RNS are free radicals with nitrogen centers. The two major examples of RNS are nitric oxide (NO) and nitrogen dioxide (NO₂). NO is specifically synthesized by NOS during the conversion of L-arginine to L-citrulline [97]. Under normal physiological process, NO acts in a variety of tissues to regulate normal cell functions, but excess of NO can be toxic [101]. NO, with an unpaired electron, is highly reactive and can damage proteins, carbohydrates, nucleotides and lipids. RNS have been associated with asthma, ischemic/reperfusion injury, septic shock, and atherosclerosis [108].

Role of NO

Measuring plasma concentration of NO³⁻ and NO₂⁻ assesses NO concentration. The sum of NO³⁻ and NO₂⁻ is assumed as the best index of total NO. NO contents are assessed by a two-step process consisting of the conversion of nitrate to nitrite first, followed by spectrophotometric detection of total nitrite at 540 nm [109].

Nácul et al. (2007) reported that NO levels in PCOS patients (n=31, mean age 22.4±7.1 years and mean BMI 26.7±10.1) were similar to that of age- and BMI-matched controls (NO mean value 11.5 vs 10.2 µmol/L, p>0.05). Moreover, a significantly negative correlation was observed
between NO and fasting insulin levels (r=-0.39, p=0.03) and HOMA (r=-0.41, p=0.02) (84). These data suggested that NO was related to the presence of insulin resistance in PCOS patients, although further studies are needed to clarify the role of NO in PCOS.

**ROLE OF ANTIOXIDANTS IN PCOS**

Antioxidants scavenge excess ROS to counteract potential for significant cell damage by excess ROS. Antioxidants help create a balance between beneficial oxidant generation (frequently act as cell signaling molecules) and damaging oxidative stress. There are two categories of antioxidants: enzymatic and non-enzymatic. Enzymatic antioxidants include SOD, catalase, and GPx. Non-enzymatic antioxidants include GSH, α-tocopherol (vitamin E), β-carotene, ascorbate (vitamine C), taurine, L-carnitine, coenzyme Q10, etc (97). There are three SOD isoforms in eukaryotes: manganese (vitamine C), copper/zinc SOD (Cu/Zn-SOD), and extracellular SOD (EC-SOD)1.

Antioxidants that prevent or limit the damaging effects of oxygen radicals have been reported to have important roles in the female reproductive system and in the pathogenesis of female infertility.2 Changes in antioxidant concentrations in serum and peritoneal fluid have been studied in idiopathic infertility, tubal infertility, and endometriosis patients [111, 112]. Results indicate that investigation of antioxidant concentrations in PCOS patients is promising. Various studies have measured antioxidant markers to correlate OS and PCOS and the diverse clinical manifestations of metabolic syndrome including diabetes, obesity, and cardiovascular diseases.

**Role of TAC in PCOS**

Total antioxidant capacity is the ability of serum to quench free radical production, protecting the cell structure from molecular damage. Various detection assays for TAC have been described, one of which is the spectrophotometric assay in which long-lived 2,2'-azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS) radical cation is measured. ABTS radical is formed by the incubation of ABTS with a peroxidase (metmyoglobin) and hydrogen peroxide. The principle of the assay is to measure the ability of aqueous and lipid antioxidants to inhibit the oxidation of ABTS to ABTS+ [113]. The capacity of the antioxidants to prevent ABTS oxidation was compared with that of standard Trolox, a brand name for 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a water-soluble derivative of vitamin E. This assay measures the combined antioxidant capacity of all its components including vitamins, proteins, lipids, glutathione, uric acid, etc. [113].

Another method to measure TAC is through the production of hydroxyl radical via Fenton reaction. It is initiated by the hydroxyl radical, and the brown-colored dianisidinyl radical cations are produced in the reaction medium of the assay [114]. Antioxidant capacity of the added sample against these colored potent free-radical reactions is measured as a whole to represent TAC. The results also were expressed as millimoles of Trolox equivalent per liter [34].

Fenkci et al. (2003) demonstrated that TAC was significantly lower in PCOS patients (n=30 mean age 25.80±0.63 years and mean BMI 24.3±1.1) compared with the age-, BMI-, and smoking-status-matched controls (1.15±0.01 vs 1.30±0.02 mmol/L, p=0.001) [115]. This observation suggested that the oxidative status imbalance in PCOS women might contribute to their increased risk of cardiovascular diseases. Moreover, there was a negative correlation between fasting insulin level and TAC, suggesting that that IR may have a detrimental effect on antioxidant defense system in PCOS.

However, Verit et al. (2008) reported that TAC levels were significantly higher in PCOS patients (n=63 mean age 24.4±4.1 years and mean BMI 21.2±1.8) compared with age- and BMI-matched controls (1.8±0.5 vs 1.1±0.2 mmol Trolox Eq/L, p<0.0001). This study demonstrated that TAC was increased in non-obese, normoinsulinemic PCOS patients (fasting insulin 10.7±5.0 μIU/mL, no significant difference compared with controls). High levels of antioxidants in PCOS are thus suggested to have detrimental effects. This result was inconsistent with other studies in the literature. Although the complete mechanism of this elevation is unknown, it is proposed that TAC was increased as to compensate for the increase in total oxidative stress (19.1±7.6 vs 12.3±4.8 μmol H2O2 Eq/L, p<0.0001) [34].

Although results of studies about antioxidant levels are conflicting, it is possible to conclude that an imbalance between oxidants and antioxidants occurs in PCOS. Further studies of oxidative stress defenses in PCOS are needed to clarify the association between antioxidants and PCOS.

**Role of SOD in PCOS**

SOD induces the conversion of superoxide to H2O2, a toxic substance that is converted to water by GPx. High SOD levels may explain the absence of endothelial dysfunction markers. Generation of an adequate antioxidant response against such an intrinsic oxidative load may provide proper functioning of vascular system.

Kuşçu et al. (2009) demonstrated that SOD levels were significantly higher in a PCOS group compared with a control group (8.0±0.7 vs 7.28±0.8, p=0.001). In this study the PCOS patients were further divided into two subgroups: IR- and IR+. SOD levels were significantly higher in both subgroups compared with the control (7.99±0.7 vs 8.22±0.8 vs 7.28±0.8, p=0.009 and 0.03, respectively). This elevation may have been due to the body’s defense mechanisms. Subjects used in this study were relatively young (mean age

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1 Mn-SOD, which contains a manganese prosthetic group, resides in the mitochondria. It is thought to protect mitochondrial membranes, proteins, and DNA from O2·- generated as a result of the electron transport chain. The Cu/Zn-SOD, which contains copper and zinc prosthetic groups, often resides in cytosol. EC-SOD, is secreted and binds to the elements of the extracellular matrix. All forms of SODs are thought to reduce O2·- to form O2 via the oxidation of the prosthetic group [110].

2 The production of H2O2 within cells may lead to the production of HO and subsequent cellular damage. Thus, it is important to remove H2O2. Catalase functions to rapidly transform H2O2 to water and oxygen via the redox reactions achieved by its manganese or heme group. Catalase resides mainly in peroxisomes, mitochondria and the cytosol [110].
Sabuncu et al. (2001) demonstrated elevated SOD levels (mean value 94.62 MU/mol Hb) in a group of PCOS patients with mean BMI 31.4 (p<0.05). They proposed that the increase in SOD levels might be due to a compensatory response to OS.

Zhang et al. (2008) demonstrated that the serum SOD level in PCOS patients (n=30) was significantly lower than that in the control group (67.31±12.46 vs 113.81±13.003 μU/mL, P<0.05) [104]. However, the study did not comment as to why SOD level was lower in this selected PCOS group.

Role of GPx

Sabuncu et al. (2001) demonstrated that GPx did not differ between a PCOS group and a healthy control group (2.88±0.52 vs 2.98±0.54 MU/mol Hb). In an environment with increased H_2O_2, an increase in GPx is to be expected. However, the fact that GPx activity did not increase in PCOS women might result from the low amount of GSH, which is the substrate of GPx [4].

Role of GSH

GSH was often determined by adding 5,5'-dithiobis(2-nitro-benzoic acid), which is a disulfide chromogen that is readily reduced by sulfhydryl compounds, to an intensely yellow compound. Reduced chromogen absorbance is measured at 412 nm and is directly proportional to GSH concentration [116, 117].

Sabuncu et al. (2001) demonstrated that GSH was significantly lower in the PCOS patient group than in the control group (0.39±0.07 vs 0.44±0.07 mol/mol Hb, p=0.03). Low levels of GSH may have been partly related to IR. Increased ROS and peroxides may also have led to GSH depletion.

In accordance with the findings of Sabuncu et al. (2001), Dincer et al. (2005) also found GSH levels to be significantly lower in women with PCOS than in the control group (5.03±0.96 vs 5.59±0.82 μmol/gHb, p<0.05) [118]. They proposed that GSH depletion might have resulted from increased production of ROS in PCOS patients.

CONCLUSION

In this review we documented the burgeoning interest in the relationship between OS and PCOS, evidenced by a rapidly increasing body of literature. The discussion has included multiple biomarkers of both ROS and antioxidants in various PCOS patient groups. Cumulative studies to date do not yield a definitive conclusion regarding the association between OS and PCOS. Measurement of biomarkers of OS also is known to be a controversial issue. Units of measurement in published studies are not consistent. Standardized measurement units of each biomarker should be used in the future to facilitate comparison across studies. Additional studies are recommended to examine the association and mechanism of OS on PCOS.

KEY POINTS

- PCOS is the most common female endocrinological abnormality, affecting 4-8% of women in their reproductive years.
- Clinical PCOS is diagnosed in women based on presence of at least two of the following criteria a) oligo- or anovulation, b) biochemical and/or clinical features of hyperandrogenism, c) polycystic ovary appearance on ultrasound scanning.
- The condition is multifactorial, but insulin resistance appears to be a central feature that explains many of the manifestations of the syndrome and the increased risk of developing type II diabetes.
- Components of metabolic syndrome, particularly hyperinsulinemia and central obesity (visceral adiposity), are frequently encountered in PCOS.
- Risk markers for cardiovascular disease, endothelial dysfunction, and dyslipidemia are increased in PCOS.
- Oxidative stress seems to be involved in altered steroidogenesis in the ovaries, thus contributing to increased androgen production, disturbed follicular development, and, ultimately, infertility.

EXPERT COMMENTARY

There is mounting evidence to substantiate the etiological relationship between PCOS and metabolic syndrome. However, epidemiological research thus far has failed to demonstrate that the markers of cardiovascular disease, endothelial dysfunction, and dyslipidemia in PCOS are associated with increased mortality. The role of oxidative stress in the pathogenesis of PCOS is not fully understood, and the evidence is conflicting. The current evidence merely points towards an association between the oxidative microenvironment of the ovarian tissue and ovarian steroidogenesis and follicular development. Whether oxidative stress is the cause or the result of the metabolic disturbances encountered in PCOS remains to be elucidated. However, a strong relationship among hyperinsulinemia, hyperlipaemia, and oxidative stress is recognized.

FIVE-YEAR VIEW

Research is underway to determine whether reducing visceral adiposity in PCOS patient is associated with reduced markers of cardiovascular risk, improved insulin resistance, and the amelioration of the clinical symptoms of PCOS. Health economic constraints mean that issues associated with PCOS should be addressed in a radical way to modify the associated health risks. A prominent example of this is the increased adoption of systems in which the availability of fertility treatment is restricted for overweight PCOS patients because of poor treatment outcome. In the next few years clinical trials will determine the role of exercise, diet, and other lifestyle modifications, as well as pharmacological intervention, on improving fertility outcomes and reducing health risks in these patients.
REFERENCES


Role of Oxidative Stress in Polycystic Ovary Syndrome


Polycystic Ovary Syndrome in Adolescents

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Abstract: Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women, typically presenting with menstrual irregularities and signs of androgen excess. Approximately 4-10% of reproductive aged women have PCOS. The fact that PCOS often presents in adolescence and the chance of long-term adverse health consequences indicate the need for early diagnosis and intervention. Menstrual dysfunction and hyperandrogenism are key features of the diagnosis of PCOS in reproductive aged women. Diagnosis can be challenging, however, in adolescent girls. The etiology of PCOS is complex and incompletely understood. Therapy should focus on alleviating symptoms and preventing adverse health consequences.

Keywords: Polycystic ovary syndrome, polycystic ovaries, adolescence, diagnosis, management.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women, and typically presents with signs of androgen excess and menstrual irregularities. Approximately 4-10% of reproductive aged women have PCOS. The prevalence in adolescent girls may be higher when compared to other cohorts; as high as 9% in girls with regular cycles, 28% with irregular cycles, and 45% with oligomenorrhea. The incidence varies with the definition of PCOS being used.

PCOS is a major risk factor for a number of chronic health problems including type 2 diabetes mellitus (DM type 2), cardiovascular disease, infertility, and endometrial carcinoma. Early diagnosis and treatment are mandatory to improve quality of life and prevent progression towards serious health problems. This review will address the pathophysiologic mechanisms and current guidelines in the diagnosis and management of PCOS in adolescence.

ETIOLOGY

The etiology of PCOS is complex and incompletely understood. It is clear from twin studies that a significant genetic component contributes to the pathogenesis of PCOS. Environmental factors, principally dietary, are also likely to be involved in the modulation of the phenotypic expression of PCOS through the effects of obesity. Strong data suggest that PCOS can be inherited. Studies on monozygotic and dizygotic twin sisters from the Netherlands twin registry showed a high correlation of heritability between monozygotic and dizygotic twins [4].

The fact that PCOS often presents in adolescence suggests that the underlying predisposition to the typical ovarian abnormalities originates before puberty. Franks et al. proposed that the ovary is genetically predisposed to hypersecretion of androgens, perhaps as early as intrauterine life, but certainly during the activation of the hypothalamic-pituitary-ovarian axis that occurs transiently in infancy and in a sustained manner at puberty. This predisposition to hyperandrogenism was shown to be associated with luteinizing hormone (LH) abnormalities and amplification of the so-called physiologic insulin resistance of puberty. Higher than normal concentrations of LH and insulin further enhance ovarian androgen production and may contribute to the mechanism of anovulation.

Many genes have been investigated as possible susceptibility loci but the effect of any one gene is likely small. One candidate gene is a locus on chromosome 19 (p13.2), which is close to the insulin receptor gene. Its function remains uncertain [5, 6]. Large family-based case-control studies are needed.

The clinical and biochemical abnormalities seen in PCOS are amplified by obesity. In a series of 350 women with PCOS, an increase in body mass index (BMI) seemed to negatively affect metabolic indices [7]. Many studies report that calorie restriction and lifestyle modification with as little
as a 5% reduction in body weight in obese women with PCOS improve metabolic indices and infertility [8, 9].

In utero androgen exposure has also been proposed to influence the phenotypic expression of PCOS. In fact, in nonhuman primates, fetal exposure to high levels of androgen during early intrauterine development is associated with defects in insulin secretion and action in adult life. Prenatally androgenized female rhesus monkeys exhibit glucose regulatory deficits similar to those seen in adult women with PCOS [10]. However, a recent prospective human study failed to find a relationship between prenatal androgen exposure and PCOS in adolescence [11].

PATHOGENESIS

Altered Gonadotropins Secretion

One of the well-described features of PCOS is an increase in LH levels and a relative decrease in follicle stimulating hormone (FSH) levels [12]. The relative decrease in FSH could be the chief cause of PCOS-associated anovulation. The pulsatile secretion of LH from the pituitary gland is increased in amplitude and frequency in PCOS [13]. In addition, the pituitary in women with PCOS exhibits a greater response to gonadotropin releasing hormone (GnRH) than the pituitary in typical women [13,14]. This eventually leads to an abnormal circulating LH- to- FSH ratio in some women with PCOS. Overall, these data suggest the presence of a defect in the hypothalamic-pituitary axis in PCOS.

Obesity, Insulin Resistance and Androgen Excess

Obesity is commonly associated with PCOS. More than 50% of women with PCOS are obese, which is defined as a BMI >30 kg/m² [15]. In many cases, obesity is believed to cause the hormonal abnormalities that lead to PCOS, as discussed below. However, the fact that PCOS can be diagnosed in women of normal weight indicates that obesity is not the only cause of PCOS.

The link between PCOS and obesity is often insulin resistance (IR) [16]. IR can be defined as a state in which a greater than normal amount of insulin is required to facilitate glucose transport into cells. Early in the IR disease process, pancreatic beta cells increase insulin secretion; compensatory hyperinsulinemia maintains plasma glucose levels in the normal range. If the ability of the pancreas to secrete insulin declines or the degree of IR becomes greater than can be compensated for by hyperinsulinemia, impaired glucose tolerance (IGT) or frank Type 2 diabetes develops, both of which are manifested by abnormally elevated plasma glucose levels [17]. Impaired fasting glucose is a relatively late abnormality that develops in patients with IR. Therefore, so fasting glucose is not recommended as a screening test for diabetes in the PCOS population.

Androgen excess in women with IR is believed to be the result of insulin’s stimulatory effect on androgen production by ovarian theca cells [18]. Approximately 70% of women with PCOS have elevated serum androgen levels and 25% have high concentrations of dehydroepiandrosterone sulphate (DHEAS) [19]. This has led investigators to suggest that uncontrolled steroidogenesis may be the primary abnormality in this disorder [20]. These increased circulating androgens are believed to be associated with LH abnormalities. In Rhesus monkeys, positive feedback driven by increased levels of LH increase androgen secretion by theca cells. [The result is often PCOS with anovulation, signs of androgen excess and polycystic ovaries.] The central role of elevated insulin in many cases of PCOS is evidenced by studies that show that lowering insulin levels in women with IR using oral insulin sensitizers often results in spontaneous ovulation [21].

Compensatory hyperinsulinemia may contribute to anovulation directly by interfering with follicular development, causing premature follicular atresia and follicular arrest, and indirectly, by diminishing gonadotropins’ effects on the follicles [22]. On the other hand, increased insulin binds to insulin growth factor-I receptors (IGF-I), enhancing the theca cell production of androgen in response to LH stimulation [22].

Insulin may increase the cytochrome P450 17α activity, a key enzyme in the biosynthesis of ovarian and adrenal androgens. In ovarian theca cells, the 17 α-hydroxylase activity converts progesterone to 17 α-hydroxyprogesterone, whereas its 17, 20 lyase activity converts 17 α-hydroxyprogesterone to androstenedione. Androstenedione is then converted to testosterone by the enzyme 17β-reductase. Thus, the increase in P450c17 α activity is accompanied by an increase in the serum testosterone concentration. In this regard, this abnormality may be responsible for exaggerating the 17α-hydroxyprogesterone response to stimulation by GnRH analogs [23].

Excess insulin has a major impact at the hepatic level. Hyperinsulinemia inhibits the synthesis of sex hormone binding globulin (SHBG), increasing free androgens and consequently peripheral androgen action. At the same time, hyperinsulinemia inhibits the hepatic secretion of the insulin growth factor binding protein (IGFBP 1), leading to increased bioactivity of IGF-I and IGF-II. Eventually, this will augment ovarian androgen production from theca cells by stimulating IGF-I receptors [22].

DIAGNOSIS

PCOS is a syndrome, not a disease, and it may have several different etiologies with a final common pathway. Even the definition and diagnosis are still controversial.

PCOS was first described by Stein and Leventhal in 1935 [24]. They made their diagnosis of PCOS when patients with amenorrhea and infertility were seen to have enlarged ovaries at surgery.

In 1990, a National Institutes of Health (NIH) conference led to diagnostic criteria based on a majority opinion of conference participants [25]. The criteria included: hyperandrogenism and/or hyperandrogenemia, chronic anovulation, and exclusion of other similar disorders. The NIH criteria did not comment on ovarian morphology. At a PCOS consensus workshop in Rotterdam, both the European Society of Reproduction and Embryology (ESHRE) and American Society of Reproductive Medicine (ASRM) revised the diagnostic criteria for PCOS. Their revised criteria state that PCOS re-
mains a diagnosis of exclusion, and diagnosis is complete in women found to have at least two of the following conditions: 1) ovulation dysfunction as evidenced by oligo or amenorrhea, 2) hyperandrogenism, determined either clinically or biochemically, and 3) polycystic ovaries demonstrated ultrasonographically [26].

However, the Androgen Excess and PCOS Society (AE - PCOS Society) disputes this definition. They claim that PCOS is a hyperandrogenic disorder, meaning that hyperandrogenism, either clinical or biochemical, is the cornerstone of the diagnosis. Diagnosing a woman with PCOS implies that there is an increased risk for many health conditions [27] associated with the hyperandrogenism. The AE - PCOS Society suggests the following criteria for diagnosis: 1) hyperandrogenism: either hirsutism or hyperandrogenemia, 2) ovarian dysfunction: oligo-ovulation and/or polycystic ovaries and 3) exclusion of other causes of androgen excess or related conditions [28] (Table 1).

More stringent criteria have been proposed to diagnose PCOS in adolescence. These include four out of the five criteria shown in (Table 2) [30].

**Table 1. Definition of PCOS by Different Organizations**

<table>
<thead>
<tr>
<th>NIH Criteria (Both 1 and 2)*</th>
<th>Rotterdam Criteria (2 out of the following 3)*</th>
<th>Androgen Excess and PCOS Society (Both 1 and 2)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - Chronic anovulation.</td>
<td>1 - Chronic anovulation.</td>
<td>1 - Hyperandrogenism (Clinical or biochemical)</td>
</tr>
<tr>
<td>2 - Hyperandrogenism (Clinical or biochemical)</td>
<td>2 - Hyperandrogenism (Clinical or biochemical)</td>
<td>2 - Ovarian dysfunction (Oligo-anovulation and/or polycystic ovaries)</td>
</tr>
</tbody>
</table>

*After exclusion of other related disorders.

Adoption of any of the above criteria in diagnosing PCOS is challenging in adolescent girls. For two to three years after menarche, adolescents exhibit a transient phase of anovulation [29], during which the menstrual cycle can be irregular. The difficulty of diagnosing PCOS in adolescence is not only due to the physiologic anovulation, but also to transient hyperactivity of the hypothalamic-pituitary-gonadal axis leading to increased androgen production [29] and relative insulin resistance related to growth hormone levels.

More stringent criteria have been proposed to diagnose PCOS in adolescents. These include four out of the five criteria shown in (Table 2) [30].

**CLINICAL FEATURES**

PCOS presents as a spectrum of clinical disorders with at the extreme hyperandrogenism, ovulatory dysfunction, obesity, and insulin resistance.

**Hyperandrogenism**

Clinical signs of androgen excess are the most constant and prominent components of PCOS. Approximately 72% of women with PCOS will exhibit hirsutism, defined as the presence of excessive thick, pigmented (terminal) hair in androgen-dependent body areas such as the lip, chin, sideburn area, neck, abdomen, and inner thighs [31]. As stated by DeUgrate et al., degrees of hirsutism vary greatly in different ethnic populations (e.g. it is rare in Asian women [32]), so the threshold of defining hirsutism should be set based on the population [33].

Another sign of androgen excess is acne, which will be found in 15-25% of women with PCOS. However, it is unclear whether the prevalence of acne is significantly increased in PCOS over that observed in the general population [34]. The present recommendation regarding acne and alopecia is to consider them as unreliable clinical signs of hyperandrogenism, particularly if they are the only diagnostic features [27].

Approximately 70% of women with PCOS will be found to have mildly elevated serum androgen levels, and 20-30% will have mildly elevated dehydroepiandrosterone sulfate (DHEAS) levels [19]. Marked elevations of total testosterone, DHEAS, or 17 hydroxyprogesterone may indicate underlying pathology that is mimicking PCOS such as an ovarian or adrenal tumor (especially if these elevations are associated with symptoms and signs of rapid onset virilization), or non-classical congenital adrenal hyperplasia [35].

Serum analysis may fail to identify biochemical hyperandrogenism in 20-40% of patients [36]. Even semi-quantitative measurements, such as the Ferriman-Gallwey score for hirsutism [37], may underestimate clinical hyperandrogenism [33].

Most commercial assays for total testosterone are not designed or validated for use in women [38], raising concerns about their diagnostic value. Moreover, the range that is regarded as healthy for women by commercial laboratories is very broad and may fail to diagnose hyperandrogenemia even in some women with severe hirsutism [39]. An alternative explanation for the disparity between normal serum androgens and the presence of clinically significant hirsutism is the fact that some individuals may have different hair follicle sensitivity to androgens (idiopathic hirsutism). This patient category will still benefit from suppression of their normal levels of androgens, however. Another issue to be considered is that women with asymptomatic polycystic ovaries who have normal baseline serum androgens on repeated testing may have occult androgen excess [40].
Although controversial, many investigators believe that failure to detect biochemical or clinical hyperandrogenism should not exclude diagnosis of PCOS in the presence of other clinical signs [41]. This is supported by the Rotterdam criteria, which allow the diagnosis of PCOS in patients without androgen excess.

**Laboratory Criteria for Androgen Excess**

Biochemically, hyperandrogenemia is most commonly assessed by measuring serum total testosterone (T) and sex hormone binding globulin (SHBG), followed by calculation of the free fraction by the free androgen index (Testosterone/SHBG). The mass action equation [38, 42] is used to calculate free testosterone, which is widely available, but the results are highly unreliable and should be interpreted carefully [38, 43]. The concentrations of other serum androgens, such as androstenedione or adrenal androgen dehydroepiandrosterone sulfate (DHEAS), are often high in women with PCOS, but their measurement is of little value in the average clinical setting.

**Ovulation Dysfunction**

Ovulation dysfunction is the most common cause of female infertility and is diagnosed in 70-80% of infertile women with PCOS [27]. The most frequent sign of ovulation dysfunction is irregular menses. The irregularity may be in amount, duration, or frequency. It occurs in 75% of PCOS patients, and is often one of the earliest symptoms [7]. However, using menstrual irregularity to diagnose PCOS in adolescence is difficult because irregular cycles may be normal in the first few years after menarche [44].

Anovulation in adult women with PCOS is attributed to the disturbance of folliculogenesis that characterizes the syndrome. The follicular defect in PCOS consists of accelerated early follicular growth and interference with the selection of the dominant follicle [45]. Accelerated early follicular growth is mainly due to androgen excess and increased insulin that induces LH receptor expression and premature luteinization of granulosa cells. Excess amounts of insulin and LH further enhance ovarian androgen production [3].

**Ultrasonographic Criteria for Polycystic Ovaries**

Significant advances in ultrasonography made in the last decade, especially coupled with use of the transvaginal approach, have made evaluation of the ovaries more accurate and detailed. Polycystic ovaries are defined by the Rotterdam Criteria as “the presence of at least 1 ovary, using a transvaginal probe, featuring 12 or more follicles having a mean diameter 2 to 9 mm, irrespective of location, and/or a total ovarian volume > 10 ml.” This definition is applied to the follicular phase ovary (one that lacks follicles larger than 10 mm in diameter) [46].

Imaging for polycystic ovaries in virginal adolescent girls, if desired, can be done by transabdominal ultrasound. The ovarian volume may be the only parameter that should be measured, because the follicle criteria are much less reliable by the abdominal route, especially in obese individuals [47]. The transperineal or transrectal route may be an alternative when abdominal ultrasonography is not conclusive. A combination of abdominal and three-dimensional transrectal ultrasound revealed accurate and reliable results in adolescent girls in one study [48].

Measurement of serum anti-Mullerian hormone (AMH) is emerging as a potential surrogate for ultrasonography; values correlated closely to the antral follicle count in pilot investigations [49]. This assay may facilitate the diagnosis of PCOS when transvaginal ultrasonography is not appropriate.

**Other Phenotypes**

Although obesity and IR are common in women with PCOS, their presence is not necessary to make the diagnosis. Their presence, however, increases the risk of metabolic syndrome and other health consequences [3].

**LONG TERM RISKS OF PCOS**

**Pregnancy, Infertility, and Miscarriage**

Girls with PCOS and their parents may worry about their future fertility. PCOS is the most common cause of anovulatory infertility so it is important to reassure adolescent girls that if they do have difficulty conceiving in the future, they can be treated with medications and/or assisted reproductive technologies. However, obese patients should be warned of the pregnancy complications associated with obesity. Pregnancy complications (gestational diabetes, preeclampsia, and macrosomia) are significantly increased in morbidly obese individuals [50-52]. In addition, the risk of first trimester miscarriage is reported to be significantly higher for patients with PCOS—possibly as high as 30% [53].

**Diabetes Mellitus**

Women with PCOS are at increased risk for glucose intolerance, manifesting as either impaired glucose tolerance (IGT) or Type 2 diabetes mellitus (DM) [54]. This increased risk is related to the association of PCOS with obesity and IR; both are risk factors for glucose intolerance. The incidence of IGT and type 2 DM was reported as 10% and 5%, respectively, in a cohort of adolescents with PCOS [55].

Women with PCOS and baseline normal glucose tolerance have a 16% conversion rate per year to DM type 2 [56], whereas by the age of 30 years, 30-50% of obese women with PCOS develop IGT or overt DM type 2. This is a 3-7 fold greater risk than that for an age-comparable population [17].

The mechanisms underlying the association between PCOS and glucose metabolism impairment are still unknown. During fetal life, growth restriction, low birth weight, and/or small size for gestational age followed by catch-up weight gain during infancy may lead to hyperinsulinemia, IR, obesity, PCOS, and DM type 2 in later life [57, 58].
Evidence suggests that insulin resistance may play a major pathophysiological role in the development of glucose intolerance [59]. There is also a subset of women with PCOS who are not obese, but have IR because of some molecular abnormality, such as an insulin receptor defect [60]. Because of the strong association of PCOS and DM type 2, effective DM screening of PCOS patients is imperative.

**Metabolic Syndrome and Cardiovascular Risks**

Metabolic syndrome (MtS) is a cluster of cardiovascular risk factors, including diabetes or prediabetes, central (abdominal) obesity, atherogenic dyslipidemia, and hypertension [61]. Adult women with PCOS were found to have an 11-fold increased risk of MtS when compared to that of healthy controls [61]. Thirty seven percent of adolescent girls with PCOS have been shown to have MtS, compared with 5% of the general population [62].

The World Health Organization (WHO) defines the MtS as the presence of IGT or IR, with at least two of the following: hypertension, dyslipidemia, obesity and microalbuminuria [63]. Many revisions have been made to that definition [64,65], although most are difficult to use in everyday practice or clinical studies. The most commonly used definition for diagnosing MtS is the updated Adult treatment Panel III criteria [66,67] (Table 3).

**Table 3. Updated Adult Treatment Panel III for diagnosis of Metabolic Syndrome* **

*Three or more criteria required for the diagnosis (HDL-C: High Density Lipoprotein Cholesterol).

- Fasting glucose ≥6.1 mmol/L [110 mg/dL]
- HDL-C <1.3 mmol/L [50 mg/dL]
- Triglycerides ≥1.7 mmol/L [150 mg/dL]
- Obesity BMI > 30 kg/m²
- Waist circumference ≥ 88 cm
- Hypertension ≥130/85 mm Hg

PCOS and metabolic syndrome are related conditions. Adult women with MtS are at a greater risk of developing cardiovascular disease; women with PCOS also appear to carry an increased risk in their postmenopausal life. Women with MtS may also experience reproductive disturbances more commonly than their counterparts from the general population [68].

Gynecologic symptoms may be the earliest manifestation of MtS in young women. Early presentation affords a clinician the opportunity for diagnosis, counseling, and treatment to alter the risk profile for the development of MtS and cardiovascular disease later in life.

**Endometrial Cancer**

Endometrial hyperplasia is a consequence of unopposed estrogen stimulation of the endometrium. Adolescents and women with PCOS are at increased risk because chronic anovulation results in this dysfunction. Untreated endometrial hyperplasia can lead to adenocarcinoma. Recent meta-analysis suggests that women with PCOS have a more than 2-fold risk of developing cancer of the endometrium compared to the general population [69].

**DIFFERENTIAL DIAGNOSIS**

Some disorders may mimic PCOS (Table 4). These disorders must be ruled out before making the diagnosis of PCOS [27, 46, 70].

**Table 4. Differential Diagnosis of PCOS**

- Congenital Adrenal Hyperplasia
- Androgen-secreting neoplasm
- Cushing Syndrome
- Thyroid Disease
- Prolactin-secreting tumor

**Congenital Adrenal Hyperplasia**

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders resulting from mutations in the genes coding for steroidogenic enzymes. In these disorders, a block in cortisol biosynthesis leads to loss of negative feedback inhibition, increased adrenocorticotropic hormone (ACTH) secretion, and subsequent excessive adrenal androgen secretion [71]. The most common form of CAH is 21-hydroxylase deficiency, which accounts for 90 to 95% of patients with CAH [71]. 11β-hydroxylase deficiency and 3β-hydroxysteroid deficiency represent 5 to 10% of CAH [72,73]. “Late onset” non-classical congenital adrenal hyperplasia can present in adulthood.

**Androgen-Secreting Tumors**

Androgen-secreting tumors, although rare, may initially mimic the hyperandrogenism and menstrual dysfunction seen in PCOS. However, they tend to present with rapidly progressive hyperandrogenism and early development of frank virilization. Sertoli Lydig cell tumors are the most common virilizing ovarian tumor and account for 0.5% of all ovarian neoplasms [74]. Androgen-secreting adrenal neoplasms are less common than ovarian neoplasms [35]. They usually present with a mixed picture of Cushing syndrome and virilization [75].

**Cushing Syndrome**

Cushing Syndrome, caused by excess cortisol, is characterized by moon face, buffalo hump, obesity, hypertension, and dyslipidemia. The most common cause of Cushing phenotypes is exogenous glucocorticoids. Endogenous Cushing syndrome may be dependent on ACTH secretion or may be due to autonomous cortisol secretion by the adrenal gland [76]. Excess ACTH secretion results in androgen secretion and then hirsutism in a slowly progressive fashion. If a patient with Cushing syndrome presents with rapid hair growth, an adrenal tumor is more likely [77]. Although Cushing syndrome is a rare etiology of hirsutism, excess facial and body hair are present in approximately 81% of patients with Cushing’s [78].
**SUGGESTED EVALUATION OF ADOLESCENTS WITH FEATURES OF PCOS**

**History**

A detailed menstrual history should be obtained. Approximately one half of menstrual cycles during the first 2 years after menarche are anovulatory [79], and bleeding patterns may be slightly irregular. By five years after menarche, 80% of cycles are ovulatory. During the history, the patient should be questioned about chronic illness, weight changes, unwanted hair growth, drug use, and a family history of endocrine malfunction, including dysfunctional bleeding, infertility, diabetes, and MtS. Depression is common in adolescents with hirsutism and obesity.

**Physical**

A focused physical examination is essential in the evaluation of adolescents suspected of having PCOS. The clinician should document the blood pressure, BMI, and waist circumference to determine body fat distribution. A BMI > 30 defines obesity and waist circumference greater than 35 inches is considered abnormal and suggests MtS [70]. Stigmata of hyperandrogenism include acne, hirsutism, and (rarely) androgenic alopecia. Acanthosis nigricans, a velvety hyperpigmentation usually seen around the neck, correlates with insulin resistance.

The gynecologic examination should include inspection of the external genitalia for clitorimegaly. If bimanual examination to exclude adnexal masses can not be tolerated, ultrasound can be performed if necessary. In the context of fairly normal testosterone levels, examination of the ovaries for tumor is not imperative.

**LABORATORY**

**Testosterone**

The current criteria for the diagnosis of PCOS permit hirsutism to serve as a surrogate marker for biochemical evidence of androgen excess. However, total testosterone must be measured to exclude the presence of androgen-secreting tumors. A total testosterone level greater than 90 ng/dl usually indicates the presence of androgen excess [80] while levels of serum testosterone greater than 200 ng/dl are strongly suggestive of virilizing tumors [81].

Although free testosterone is the best single indicator of androgen excess, there is no standardization of the test, and measured free testosterone levels are unreliable [42]. For research purposes, utilization of the free androgen index (Testosterone/Sex Hormone Binding Globulin × 100) is recommended. Values greater than 5 are diagnostic of androgen excess [41].

**Dehydroepiandrosterone Sulfate (DHEAS)**

DHEAS is exclusively secreted by the adrenal glands, making it a good marker for adrenal androgen production. It may be elevated in anovulation [82] with approximately 20-30% of women with PCOS having elevated DHEAS levels [19]. DHEAS values above 700 ng/dl suggest adrenal neoplasim [83]. If serum testosterone is not exceedingly elevated, DHEAS measurements are not necessary in the workup of hirsutism or PCOS.

**17 Hydroxy Progesterone**

17 hydroxyprogesterone (17-OH-P) is a byproduct of cortisol synthesis in congenital adrenal hyperplasia. A morning serum level of 17-OH-P during the follicular phase of the cycle that is less than 3 ng/dl can be used to exclude non-classical CAH. In a patient with irregular cycles, it is often helpful to draw a serum progesterone level simultaneously, to establish that the patient is not in the luteal phase. If the 17-OH-P level is elevated (greater than 2 ng/ml), a corticotropin stimulation test can help establish the diagnosis. Intravenous injection of 250 ug of synthetic corticotropin, followed by measuring 17-OH-P after 60 minutes, is used. A level above 10 ng/dL is diagnostic for non-classic adrenal hyperplasia [84].

**24 Hour Urine Free Cortisol**

Adolescents with clinical evidence of Cushing syndrome (moon face, buffalo hump, obesity, hypertension, and dyslipidemia) should undergo a 24 hour urine free cortisol test, which has a sensitivity of 95 -100% and a specificity of 98% [85] in the diagnosis of Cushing Syndrome. The 24 hour urine free cortisol test has virtually no false negative results. False-positive results may be attributable to conditions such as depression, chronic active alcoholism, and glucocorticoid resistance [86]. The dexamethasone stimulation test and midnight salivary test may be helpful if the 24 urinary free cortisol result is suspected of being falsely positive.

**Prolactin**

It is not uncommon to detect mild elevations of prolactin (30-100 ug/L) in women with PCOS. If serum prolactin is found to be elevated, then thyroid function should be assessed. Magnetic resonance imaging (MRI) of the pituitary should be obtained to exclude pituitary tumor if prolactin levels exceed 100 ug/L.

**Thyroid Stimulating Hormone**

Hypothyroidism and hyperthyroidism should be excluded before making the diagnosis of PCOS. Subclinical thyroid dysfunction may be the cause of anovulation and menstrual irregularities. Thyroid stimulating hormone (TSH) is the screening test of choice, particularly in patients with irregular cycles [83].

**Oral Glucose Tolerance Test (OGTT)**

The 75 gram glucose, two hour OGTT has been recommended by many organizations for both initial and periodic screening for IGT and DM in women with PCOS [26, 70, 87, 88] (Table 5).

Recently, both the American Diabetes Association (ADA) and the endocrine society encouraged clinicians to use glycosylated hemoglobin testing when screening for and diagnosing Type 2 diabetes mellitus in the general population [50,89]. No recent published data addressing the use of glycosylated hemoglobin in adults or adolescents with PCOS.
are available. Currently, the OGTT is the gold standard for screening for IGT and diabetes in patients with PCOS.

Table 5. American Diabetic Association Criteria for Hyperglycemia

<table>
<thead>
<tr>
<th></th>
<th>2 Hour GTT</th>
<th>Fasting Glucose</th>
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<tbody>
<tr>
<td>Normal</td>
<td>&lt; 140 mg/dl</td>
<td>&lt; 100 mg/dl*</td>
</tr>
<tr>
<td>Impaired</td>
<td>140-199 mg/dl</td>
<td>100-125 mg/dl**</td>
</tr>
<tr>
<td>Diabetes</td>
<td>≥ 200 mg/dl</td>
<td>≥ 126 mg/dl</td>
</tr>
</tbody>
</table>

* WHO defines normal fasting glucose < 110 mg%  
** WHO defines impaired fasting glucose from 110 mg% to 125 mg%

Fasting Lipid and Lipoprotein Levels

Patients with PCOS should be screened for cardiovascular risk using fasting lipids and lipoprotein levels. A high density lipoprotein cholesterol (HDL) level less than or equal to 50 mg/dl and a triglyceride level greater than or equal to 150 mg/dl are considered abnormal [90].

IMAGING STUDIES

Ultrasoundography

Ultrasoundography is a useful tool in evaluating both the ovaries and the adrenal glands [91], and ovarian characteristics are included in some diagnostic criteria for PCOS. Ultrasoundography is imperative if serum androgen levels are in tumor range. If a mass is found, computed tomography (CT) and MRI should be used as they are more accurate in distinguishing between benign and malignant neoplasms [92].

CT Scan and MRI

CT and MRI are indicated if there is a strong suspicion of adrenal neoplasm. CT can diagnose an adrenal nodule of less than 5 mm [75]. MRI provides a clear discrimination of tumor invasion into the blood vessels. Recent CT and MRI techniques allow accurate differentiation between adenomas and malignant nodules of the adrenal gland [93].

TREATMENT

Treatment of the adolescent with PCOS should be started as soon as the diagnosis has been made. Early intervention will help not only to alleviate the distressing symptoms and progression of hirsutism but also to prevent long-term health consequences retard mental health consultation may be needed in adolescents with marked emotional distress.

Treatment of Hirsutism

The majority of adolescent girls with PCOS seek medical advice due to cosmetic issues related to hirsutism. Many of these patients may have considerable emotional distress and psychological morbidity with symptoms of anxiety and depression mental health referral should be considered in girls who exhibit significant distress.

NON-SYSTEMIC TREATMENTS

Many women are familiar with different hair removal methods like shaving, waxing, and using depilatory creams. They are all easy, safe, and inexpensive. However mild skin irritation, either due to local trauma or chemical reaction, may occur [94]. Women using these methods can be reassured that they do not increase future hair growth, but they will not prevent progression of hirsutism related to hyperandrogenemia. Combining one of these local measures with systemic treatment may help achieve rapid response and adequate patient satisfaction.

Laser Hair Removal

Laser hair removal reduced the number of hairs by 50% over six months in a systematic review of 11 trials, but the long-term efficacy of these treatments is not well established [95]. Laser targets the pigmented hair at the base of the hair follicle, and is most effective in light-skinned individuals. Most patients experience 2 to 6 months of growth delay after a single treatment, while some have permanent resolution of excess hair growth after multiple sessions. Despite the lack of evidence of efficacy, laser hair removal is frequently asked for by patients who choose permanent hair removal therapy once their androgens are under control [96].

Electrolysis

Electrolysis is a procedure by which a fine needle is introduced into the hair shaft and an electric current is applied to destroy the hair follicle. In comparison to laser treatment, electrolysis is less expensive, but laser therapy has been shown to be more effective, less painful, and time-saving [97].

Topical Creams

Eflornithine is a topical cream used in many countries for the treatment of hirsutism. It does not remove the hair, but decreases the rate of growth by inhibiting ornithine decarboxylase, an important enzyme for hair growth [98]. Eflornithine will limit the rate of hair growth after 6-8 weeks of therapy [99].

SYSTEMIC TREATMENTS

First Line Therapy

Combined Oral Contraceptives

Combined oral contraceptive pills (COCs) are the first line treatment for the majority of patients with hirsutism who do not wish to become pregnant. COCs decrease androgen levels by several mechanisms. First, they inhibit gonadotropin secretion from the pituitary, leading to decreased androgen secretion by the ovary. Second, they increase SHBG levels, which decrease free circulating androgens. Theoretically, pills with the least androgenic progestins may be of most therapeutic benefit; however, all COCs suppress ovarian androgens and raise SHBG levels and therefore can be used for treatment [100]. The contraceptive ring provides a similar combination of estrogen and progestin and can be used interchangeably with COCs for girls who are willing to use a vaginal route of administration.

Oral contraceptive pills that contain antiandrogenic progestins (cyproterone acetate and drospirenone) have theoreti-
cal benefits over other oral contraceptives in the treatment of hirsutism. In women treated with drospirenone over 12 cycles, hirsutism scores improved from the sixth cycle of treatment more significantly than those after treatment with placebo [101]. The effects of other oral contraceptives on hirsutism have not been studied.

Women being treated for hirsutism may have relative contraindications to COC use, including hypertension. The usual exclusion criteria for oral contraceptives apply in this population [102].

**Antiandrogens**

Antiandrogens are another group of agents used as a first line therapy for hirsutism. However, the teratogenic potential of these drugs means that they should be used in conjunction with adequate contraception in women of reproductive age. Spironolactone, an aldosterone antagonist, competes with testosterone and dihydrotestosterone at the androgen receptor. Although it is primarily used as a diuretic, a dose of 50-200 mg/day will reduce facial hair growth in the majority of patients after 6 cycles of treatment [103]. Concurrent use of spironolactone with oral contraceptive pills has been shown to significantly improve hirsutism and reduce serum androgen levels [104]. For patients with hirsutism that is refractory to oral contraceptives after 6 months, adding spironolactone may be effective.

Flutamide is an anti androgen used for the treatment of prostate cancer. It is more effective in treating hirsutism than spironolactone [105]. However, a recent study concluded that although flutamide is very effective in treating hirsutism, it is associated with frequent side effects and low long-term compliance [117]. Hepatic cell damage, the major complication of flutamide, may be fatal [106]. Consequently, flutamide is not approved by the FDA for treatment of hirsutism.

Cyproterone acetate is an anti androgen as well as a progestin. In one systematic review, cyproterone acetate (2mg) was more effective than placebo, but not better than any other anti androgen [107] in the treatment of hirsutism. It is also available in an oral contraceptive pill, as cyproterone acetate (2mg) with 35 ug ethinyl estradiol, which has been shown to be well tolerated. This drug is not currently available in the United States.

Fenasteride, also an anti androgen, inhibits only the type 2 isoenzyme of 5 α-reductase. It is anticipated that the effect of fenasteride may be partial. Whether it is equally effective or less effective than spironolactone is controversial [108, 109]. The FDA has not approved fenasteride for treatment of hirsutism.

**Insulin Sensitizing Agents**

Hyperinsulinemia has been shown to increase ovarian androgen production [110] and decrease SHBG production [111]. Consequently, reducing insulin levels with insulin sensitizing agents such as metformin should lower total and free androgen levels. However, the effects on hirsutism are not clearly better than if oral contraceptives are used; some studies have shown insulin sensitizing agents to improve hirsutism and others have not [112-115]. One systematic review and one meta-analysis of 8 trials collectively found no significant difference in hirsutism scores between COCs and metformin [116, 117]. On the other hand, antiandrogen drugs (spironolactone, cyproterone acetate, and flutamide) have been found to significantly reduce hirsutism scores when compared to metformin in a recent meta-analysis [116].

Troglitazone was withdrawn from the market due to hepatotoxicity, and rosiglitazone (thiazolidinediones) may increase the risk of cardiovascular events in patients at risk of or having diabetes mellitus [118]. Metformin is a category B drug that can be used for patients with PCOS desiring pregnancy, but its effects on hirsutism may not be significant.

**Second Line Therapies**

Glucocorticoids and gonadotropin releasing hormone agonists (GnRHa) can be used as a second line therapy for treatment of hirsutism in patients with PCOS. They may be useful in patients with severe hirsutism who do not respond to first line therapy [119].

**Glucocorticoids**

Glucocorticoids are known to decrease adrenal androgen secretion. They produce long-term suppression of the adrenal glands [120]. Unfortunately, even in women with non-classical congenital adrenal hyperplasia, the results of treatment of hirsutism with glucocorticoids are disappointing. Cyproterone acetate has been shown to be more effective than hydrocortisone after one year of use in hirsute women with late onset adrenal hyperplasia [121]. Moreover, in a study comparing cyproterone acetate COCs to dexamethasone, which has a longer half-life than hydrocortisone, the hirsutism scores were significantly lower in the former group (66% versus 31%) [122]. Of note, glucocorticoids are effective in maintaining ovulation and improving fertility outcomes for patients with non-classic adrenal hyperplasia [123].

**Gonadotropin Releasing Hormone Agonist (GnRHa)**

GnRH is a hypothalamic hormone released in a pulsatile manner that regulates the production of FSH and LH from the anterior pituitary in a permissive fashion. Exogenous use of a GnRHa in a non-pulsatile manner results in suppression of gonadotropin secretion (FSH and LH) [124]. GnRHa therapy has been tried in uncontrolled and controlled trials [120, 125, 126]. Although weak evidence suggests that GnRHa therapy is more effective than placebo or no therapy for hirsutism, it appears to have no therapeutic advantage when compared with other available agents such as COCs and anti androgens. Furthermore, GnRH agonists are expensive and may have undesirable side effects. The current recommendation suggests that use of a GnRHa for most women with hirsutism should be avoided [96].

**TREATMENT OF MENSTRUAL IRREGULARITY**

**Oligomenorrhea and Amenorrhea**

Menstrual irregularity should be treated in adolescents with PCOS to prevent prolonged unopposed estrogen stimu-
lation of the endometrium. Although the risk of endometrial hyperplasia has not been studied in adolescent girls with PCOS, the risk of endometrial hyperplasia is significantly correlated with an inter-menstrual interval longer than 3 months and/or endometrial thickness greater than 7 mm in women with PCOS [127]. In addition, anovulatory bleeding can be heavy and prolonged, leading to iron deficiency anemia as well as difficulties managing the menstrual flow.

In adolescents with amenorrhea or oligomenorrhea, progestins have been widely used to induce withdrawal bleeding. Micronized progesterone (100-200 mg daily), medroxyprogesterone acetate (5mg/day) or norethindrone acetate (2.5 or 5 mg/day) for 5-10 days is sufficient to induce withdrawal bleeding. However, 12 days of progestin therapy every one to three months is necessary to fully oppose the effects of continuous endogenous estrogen. Progestin-induced withdrawal bleeds should be considered if spontaneous menses occur with a frequency of less than 8 weeks [128]. Continuous progestin treatment with depot medroxyprogesterone acetate or the levonorgestrel-containing intrauterine device (IUD) can be considered for some adolescents, particularly those who also need a birth control method.

**Treatment of Mild to Moderate Anovulatory Bleeding**

Progestins have an important role in the treatment of the anovulatory dysfunctional uterine bleeding (DUB) often seen in adolescents with PCOS. For moderate DUB, cyclic progestins can be administered for 12 days every month to stabilize the endometrium and prevent the action of unopposed estrogen. Low dose oral contraceptives are another alternative, especially when progestins fail to control the bleeding. Treatment should be continued for 3-6 months [129] or until regular ovulation is established. Concurrent oral iron intake is encouraged if the hemoglobin level is low or bleeding is excessive.

**Severe Anovulatory Bleeding**

Hospitalization must be offered in cases of severe DUB. Immediate resuscitation with intravenous fluids or even blood transfusion may be necessary to restore hemodynamic stability. Intravenous conjugated estrogen doses in a dose of 25 mg every 4 hours until bleeding resolves (the maximum is four doses) provides excellent control of heavy bleeding in most cases [130]. To prevent recurrence of anovulatory hemorrhage, a combination of 2.5 mg conjugated estrogen and 10 mg medroxyprogesterone acetate for 20-25 days, or oral contraceptive pills, should follow intravenous therapy [129]. Consideration should be given to menstrual suppression with continuous oral contraceptive pills (without placebo) until severe anemia is resolved. In teens with severe uterine bleeding leading to anemia requiring transfusion or with bleeding that occurs at the time of the first menstrual period, workup for coagulopathy is indicated.

COCs induce regular menstrual periods with a high degree of reliability. In the setting of severe DUB, after establishing hemodynamic balance, COCs containing 35-50 μg ethinyl estradiol may be taken every 6 hours. After 8 doses in the first 48 hours, the dose can be tapered over 3 days to one pill daily. Then the patient should begin a new package containing the same amount of estrogen for 21 days taking one pill daily. At the end of that time, withdrawal bleeding can be allowed, or continuous suppression with pills can be chosen.

**TREATMENT OF OBESITY**

Weight loss counseling is an important component of any treatment provided to obese women with PCOS [131]. A loss of 5-10% of body weight is enough to greatly improve hirsutism, reduce testosterone, increase sex hormone binding globulin, resume ovulation and effectively modulate insulin resistance in a majority of patients [8,132-137]. In light of the health risks associated with PCOS, including diabetes, hypertension, heart disease (hypercholesterolemia), and endometrial cancer (unopposed estrogen), patients should be advised and helped to achieve sustained weight loss.

Regular exercise and behavioral modification programs are essential for acute and long-term weight management. Different exercise regimens have been advocated from thirty minutes three times a week, to ten minutes multiple times a day, to one hour of exercise most days of the week. The best program is one that the patient will actually follow [138]. Restricting calories should accompany increased energy expenditure. With an average weight loss of 1b Clark et al. [133] found a 92% ovulation rate and 33% to 45% spontaneous pregnancy rate after applying calorie restriction and exercise to a group of women with PCOS. Unfortunately, lifestyle modification and weight loss programs require prolonged patient motivation and are therefore difficult to achieve beyond the research setting. Advice alone is typically ineffective at promoting sustained weight loss.

Insulin sensitizing agents have been reported to suppress the appetite and enhance weight loss. Metformin appears to have the most utility among insulin sensitizers. A position statement of the American Association of Clinical Endocrinologists recommends the use of metformin in most women with PCOS, particularly if they are overweight or obese [87]. In a recent meta-analysis of 5 trials [139] in obese children and adolescents, metformin reduced BMI by 1.42 kg/m² (95% CI: 0.83-2.02) and insulin resistance scores by 2.01 (95% CI 0.75-3.26) compared with placebo. Another study found metformin significantly reduced waist circumference and testosterone levels when used in a dose of 1500 mg/day over 6 months when compared to lifestyle modification, oral contraceptives, and placebo [140]. However, no significant change in BMI was found between the groups. Lifestyle modification combined with metformin in obese patients with PCOS may improve long-term outcomes regarding obesity and metabolic consequences [131] than either treatment alone. This usually requires prolonged treatment, since weight is often regained after drug therapy is discontinued [141].

Although controversial, anti-obesity drugs (Orlistat and Sibutramine) have been proven to be relatively safe and effective in treating obesity in the general population. Sibutramine is not approved for use in adolescents. Studies on adolescents and Orlistat are inconclusive. Gastrointestinal side effects often limit compliance in teenagers [142].
Bariatric surgery may be the last resort for patients unable to lose weight with other strategies. The current recommendation is to offer bariatric surgery for any adult patient with a BMI of 40 or greater, or for those with BMI of 35 or greater who have a serious coexisting medical problem worsened by obesity [143]. The advantages of bariatric surgery include weight loss, improved diabetes, hypertension, and dyslipidemia, and resumption of normal cycle rhythm and fertility [144-146]. A recent randomized control trial reported that 84% of adolescents with BMI >35 kg/m2 who underwent bariatric surgery lost more than 50% of their weight versus 12% in the control group. Furthermore, MtS was eliminated in 100% of participants in the bariatric surgery group compared to 22% in the lifestyle group after 24 months follow up [147]. However, current data are not sufficient to support bariatric surgery for adolescents with PCOS.

Effective strategies, including the use of medications and surgery, for sustained weight loss in obese adolescents with PCOS should be evaluated by large long-term randomized control trials [131].

**Insulin Resistance and Metabolic Sequelae**

IR is a significant component of the etiology of PCOS and plays an important role in the risk of type 2 diabetes mellitus and MtS. Many studies on the treatment of PCOS address therapies that target IR [148, 149] Insulin sensitizers have been used in women with PCOS, aiming to reduce insulin resistance and improve lipid profiles. Some studies report positive effects of metformin on insulin sensitivity and lipid parameters in women with PCOS [150-152]. Although the Food and Drug Administration does not approve the use of metformin for PCOS, many clinical practitioners support the use of metformin as a protective measure against the metabolic and cardiovascular effects of insulin resistance [153] and for the prevention of diabetes.

The Endocrine Society guidelines recommend diet control and moderate physical activity for 30 minutes 5 days a week to prevent diabetes before drug therapy is initiated, even in patients with risk factors for MtS [154]. The ASRM/ESHRE consensus also reserves drug therapy for patients who have PCOS with impaired glucose tolerance [155].

**EXPERT COMMENTARY**

Polycystic ovary syndrome is a heterogeneous endocrinopathy that affects girls and women during their reproductive years. The exact etiology of PCOS is still unknown. However, hyperinsulinemia and hyperandrogenism are the main pathologic features responsible for most of the consequences of the syndrome. In adolescents, the irregular cycles of PCOS may be hard to distinguish from physiologic anovulation. However, persistence for more than 2 years after menarche is a strong predictor of long-term ovulatory dysfunction. Menstrual irregularity in the context of obesity, hirsutism, and acanthosis nigricans is likely to represent PCOS that will be longstanding. Polycystic ovaries may or may not be present in women and girls with PCOS. Ovarian size may be used as a surrogate for polycystic ovaries when abdominal ultrasonography is being used. Although obesity, acne, and insulin resistance are important phenotypes of PCOS, they are not required for the diagnosis.

Diagnosis of the syndrome in adolescence provides the opportunity to alleviate distressing symptoms and prevent or delay long-term health consequences. Metabolic syndrome, Type 2 DM, cardiovascular disease, infertility, and endometrial cancer are all potential risks in untreated women.

The diagnosis of PCOS is clinical and can be made only after ruling out other causes of hyperandrogenism. While the Rotterdam criteria can be used to make the diagnosis, PCOS presents along a continuum, and making a strict diagnosis may not be critical except in a research environment. Time will often help differentiate an adolescent who is destined to struggle with PCOS from one who is temporarily suffering with irregular menses and acne.

Treatment should focus on prevention of medical complications and alleviation of symptoms. Lifestyle modification and weight loss are the mainstay of treatment in obese individuals with PCOS. Oral contraceptives will regulate menstruation and decrease the chance of endometrial cancer, and may slow the progression of hirsutism. Metformin is indicated in the context of IGT or diabetes. Its use in insulin resistance is controversial, although studies have shown promise in metformin’s ability to prevent overt diabetes. More research in adolescent PCOS is needed to guide recommendations on diagnostic criteria and appropriate pharmaceutical and surgical management.

**FIVE YEAR VIEW**

**Causes of PCOS**

PCOS is a multifactorial syndrome, involving genetic, endocrinologic, metabolic and environmental factors. Further research on the basic pathophysiology of PCOS and the roles of the different etiologic components will aid in the understanding of this condition, and help clinicians in their management of adolescents with PCOS.

**PREVENTION OF PCOS**

Rates of obesity in the US increased in the 1980s and 1990s and have stabilized in the past decade [156]. On a public health level, efforts at improving the rates of overweight and obesity in the population, if effective, could have the greatest effect on the incidence of PCOS in adolescents. National programs like first lady Michele Obama’s Let’s Move campaign (http://www.letsmove.gov/) are good first steps toward systematically addressing the obesity epidemic with prevention. Research on effective treatments for childhood and adolescent obesity and programs to support successful weight loss for individuals are sorely needed.

**DIAGNOSTIC CRITERIA**

Controversies exist in the diagnosis of PCOS in adolescents. The Rotterdam criteria (Table 1) or Sultan’s criteria specifically for adolescents (Table 2) are useful but may not identify all the adolescents who could benefit from symptomatic treatment and anticipatory guidance. Population-based studies to identify diagnostic criteria that would predict
which adolescents will continue to struggle with PCOS as adults would be most useful.

Management of PCOS

Most recommendations for management of PCOS are based on expert opinion. Evidence-based recommendations that encompass the optimal management for symptom control and the prevention of sequelae would allow a more scientific approach to patient care. For example, although fourth generation COCs containing drospirenone have the theoretical advantage of direct anti-androgen effects, they have not been compared with COCs containing other progestins in the treatment of PCOS symptoms. Optimum management of hirsutism is another area for research.

COCs do not treat metabolic features of PCOS including obesity, insulin resistance, metabolic syndrome and diabetes mellitus. Metformin is recommended for impaired glucose tolerance and frank diabetes, although its use in insulin resistance requires further evaluation.

Prevention of Long-Term Sequelae of PCOS

The Oral Glucose Tolerance Test is currently the gold standard test for diabetes screening in women with PCOS, and needs to be repeated at undetermined intervals. Glycosylated hemoglobin was recently recommended for screening and diagnosis of diabetes in general population. We are currently evaluating the accuracy of glycosylated hemoglobin compared to the oral glucose tolerance test for diabetes detection in women with PCOS.

KEY POINTS

1. PCOS is a complex metabolic derangement involving insulin resistance, hyperandrogenism, and anovulation. Genetic, metabolic, endocrinologic and environmental factors all play a role. Obesity contributes to the development of PCOS in most but not all patients.

2. PCOS is a clinical diagnosis made after eliminating other causes of androgen excess and anovulation (Fig. 1). Several organizations have attempted to create strict definitions of PCOS (see Table 1), which are most useful in the research setting. The varied definitions of PCOS influence reports of the incidence of this condition.

3. Patients diagnosed with PCOS should be screened for glucose intolerance and dyslipidemia. Diabetes screening should be repeated periodically. Fasting glucose is usually normal in the IGT and diabetes associated with PCOS. Serum glucose testing two hours after a 75 gram glucose challenge is the standard for testing.

![Flow Chart showing the evaluation of adolescents with PCOS to exclude other conditions.](image-url)

*24 hour urinary cortisol is required when Cushing syndrome is suspected. DHEAS screening is optional if total T is normal.*
Polycystic Ovary Syndrome in Adolescents

4. Long-term consequences of untreated PCOS include an increased risk for diabetes, hypertension, heart disease, infertility and endometrial cancer.

5. Weight loss is the mainstay of treatment for obese women with PCOS. As little as a 5% weight reduction can reverse many of the metabolic disturbances of PCOS and diminish lifetime risks.

6. Other than weight loss, treatment for PCOS is symptom-specific.
   a. Anovulatory cycles pose a short-term risk for excess bleeding and iron deficiency anemia, and a long-term risk for endometrial cancer. Treatment with estrogen-progestin oral contraceptives or long-term progestins can diminish this risk.
   b. Hirsutism can be treated with temporary measures such as plucking or shaving, or permanent methods like laser or electrolysis. Progression of hirsutism can be slowed by decreasing free androgen exposure of the hair follicle via COCs, systemic anti androgens like spironolactone, or topical eflornithine.
   c. Insulin resistance is best treated with weight loss. While insulin sensitizing agents like metformin may prevent diabetes, their use in adolescents is not yet the standard of care.
   d. Dyslipidemia should be identified and treated.

7. Patients with PCOS should be counseled about their short- and long-term risks. Overweight teens should be encouraged to lose weight through calorie restriction and exercise. Symptoms can be distressing and should be addressed with sensitivity and empathy. Obese adolescents are often victimized; referral for counseling should be considered in girls with PCOS who are experiencing emotional distress.

REFERENCES

Papers of special note have been highlighted as:

- of interest
- of considerable interest


Polycystic Ovary Syndrome in Adolescents


•Study showed the effects of metformin-diet combination on obese women.


• Study showed possible side effects of rosiglitazone on general population.


Important article reviewing different treatment options for obese women with PCOS.


Advanced Management Options for Endometriosis

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Abstract: Endometriosis is a benign disease affecting 10% of reproductive age females. Approximately 35% of women with this condition are infertile. The exact cause of the disease is still unknown, but advances in human and animal research have further elaborated its pathogenesis. Conventional, non-fertility related treatment for endometriosis focuses on chronic pelvic pain, which is the most common manifestation of this disease. While traditional methods of treatment have not proven to be completely effective, both medical and surgical therapies still hold some promise in controlling pain.

Empiric medical therapy is still the most common mode for initiating treatment. Controversies exist regarding fertility outcomes after surgical treatment. Assisted reproduction holds promise in patients with advanced endometriosis. New treatment options have arisen in response to advances in research targeting the pathogenesis of the disease. Most of the newer therapies are still experimental, but results in animal models show promise, which has served as an impetus for conducting human trials. This article will focus on these new treatment options for endometriosis while also briefly describing the pathogenesis, diagnosis and controversies of existing treatment modalities.

Keywords: Endometriosis, infertility, angiogenesis, matrix metalloproteinase, SPRM, cytokine, IVF.

INTRODUCTION

Infertility is a growing problem worldwide, which not only affects individual health issues but also has a significant social and economical impact [1]. About 10% of reproductive age females (15 to 45 years) are infertile based on 2002 data from NCHS (National Center for Health Statistics) [2]. The prevalence of female infertility has increased in recent years due to multiple factors including age, delayed childbearing due to career interests, an increased incidence of sexually transmitted infections, and endometriosis. This review will highlight endometriosis, focusing on its pathogenesis, diagnosis and treatment options.

ENDOMETRIOSIS AND INFERTILITY

Endometriosis is associated with both infertility and subfertility. Some studies suggest that monthly fecundity rates (MFR) range from 2-10% in patients with endometriosis compared with 30% in healthy controls [3]. Various factors may cause infertility in patients with endometriosis. Severe endometriosis may induce adhesion formation in the fallopian tubes, thereby mechanically impairing tubal function. More recently, evidence suggests that there is an association between endometriosis and ovulatory dysfunction [4, 5]. Poor oocyte quality, abnormal folliculogenesis [6], poor implantation [7] and luteal phase defects have been associated with the presence of endometriosis [8]. Furthermore, in-vitro fertilization studies (IVF) have suggested that endometriosis is a contributing factor to abnormal sperm parameters [9, 10].

PATHOGENESIS OF ENDOMETRIOSIS

The pathogenesis for endometriosis is somewhat unclear, though many hypotheses have been proposed. Sampson’s theory of retrograde menstruation suggests that during menses, endometrial tissue simultaneously flows in both anterograde as well as retrograde directions, thereby seeding the pelvis through the fallopian tubes [11, 12]. While intuitive, this theory fails to explain the documented presence of endometriosis in distant sites not contiguous with the pelvis. It also does not explain why some women develop pelvic implants in response to retrograde menstruation while others do not. Theories such as celomic metaplasia [13, 14] and lymphatic and vascular dissemination have been proposed to explain the shortcomings in Sampson’s theory. Recent research has focused on a potential genetic basis for the disease with an emphasis on tissue factors and the possible role of stem cells in the pathogenesis of endometriosis [15, 16].

The basic pathogenesis of endometriosis at the cellular level is quite complicated. It is thought to be an immunoinflammatory state [17, 18]. The peritoneal fluid milieu has been demonstrated to be altered due to multiple factors arising from this immunoinflammatory state. Cytokines (TNF-α, IL-6, IL-18, TGF-β) [5, 17] and oxidative stress have been implicated in the causation and progression of this benign disease [19]. Cytokines, which are mediators of inflammation, are released by macrophages, which assemble in the peritoneal environment to defend against foreign endometrial tissue and thus create the state of inflammation in the peritoneal cells and fluid [17]. Micro array analyses of peritoneal fluid have demonstrated that the expression of cytokines in patients with endometriosis is altered overall and that the level of expression varies according to the different stages of the disease [20].

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Pain is the most common symptom, and it has been correlated in some studies with disease stage (i.e., pain increases as the disease becomes more widespread) and with cytokine expression. Although the etiology of endometriotic pain is still not clear, recent evidence has focused on the role of prostaglandins and proinflammatory cytokines released by the endometriotic implants into the peritoneal cavity [21].

The ultimate fate of the pelvic peritoneum is healing by scarring. This process can distort the tubal anatomy indirectly, affecting the tubes’ ability to receive an ovum and transport it to the uterus [22]. These adhesions at specific places have also been related to pelvic pain during menstruation (progressive dysmenorrhea) and also during intercourse (deep dyspareunia), which is the classical presenting symptom [23-25].

Peritoneal adhesions are not the only etiology of infertility. It has been established by in vitro studies that the cytokines and oxidative stress may affect the oocyte, early embryo development and sperm qualities by either altering the morphology or assaulting the nuclear DNA [10, 26].

Apart from cytokines, endometriotic implants in the peritoneal fluid have been shown to possess abnormal proteolytic and fibrinolytic properties, which may assist their implantation. Plasminogen activator and plasminogen activator inhibitor have also been studied in the pathogenesis of endometriosis. A state of hypofibrinolysis has been hypothesized which may aid the persistence of endometrial implants. This may be an indirect result of altered polymorphism in the genes expressing plasminogen activator inhibitor-1 (PAI-1) [27, 28]. Newer theories focus on their roles not only in endometriosis but also in subfertile populations [29].

The role of matrix metalloproteinase (MMPs) has also been extensively studied as a contributor to the pathogenesis of endometriosis. These proteolytic enzymes erode the extracellular matrix and help the endometriotic implants to grow into peritoneal cells. Under normal circumstances, their action is kept in check by tissue inhibitors of metalloproteinases (TIMP). The TIMP-MMP balance may be disturbed in endometriosis, leading to uninhibited MMP expression [30]. The expression of both TIMP and MMP in endometriosis implants is higher than in normal endometrial tissue [31]. It has also been found that matrix degrading MMP-1 is expressed along with its regulator cytokine IL-1 alpha in stroma of endometrial implants [32, 33].

Oxidative stress has a key role in the pathogenesis of the disease. This is a state where the balance between pro-oxidants and anti-oxidants in any cell or tissue is perturbed in favor of the pro-oxidants which are mainly free oxygen radicals. This abundance of free radicals can damage cells, mainly by affecting membrane stability and accelerating apoptosis [5, 18, 31, 34-36]. Oxidative stress not only affects the disease directly but also plays synergistic roles in modifying cytokine actions and assisting MMP activity in the peritoneal cells. Reactive oxygen species have been demonstrated to activate latent MMPs to their active forms and also to increase adhesion formation in the peritoneal cavity [37].

Angiogenesis is a key factor in the survival of ectopic endometrial issue in the pelvis. Neovascularisation from the effects of vascular growth factors, tissue factors [16], and other cytokines allow the implants to remain viable and help maintain the ectopic endometrium. Studies have shown that angiogenesis in cancer and endometriosis behave similarly and that surgery for endometriosis reduces expression of vascular growth factors in the peritoneal fluid [38-40].

**DIAGNOSIS**

Diagnosing endometriosis based solely on the clinical presentation can be difficult, given the non-specific nature of many of its symptoms and the common occurrence of pelvic pain in women without endometriosis. Therefore, laboratory studies, imaging studies and diagnostic laparoscopy are often necessary [41]. Previous studies have suggested that there is a significant delay in the diagnosis for many women, with some studies showing a mean delay of 11.7 years in the United States [42]. Failure to identify a noninvasive tool or a serum marker makes clinical examination, imaging and laparoscopy the most reliable diagnostic tools. A study performed to verify the use of the concomitant serum levels of CA 125, CA 19-9 and interleukin – 6 with CA 125 alone concluded that the former did not add significant information in diagnosing either early or advanced stages of endometriosis [43].

Imaging has emerged as an additional tool in the diagnosis of endometriosis. Transvaginal ultrasonography (TVUS) is usually the first imaging modality to be used for further evaluation of a patient with chronic pelvic pain with suspected endometriosis. TVUS is sensitive in detecting ovarian endometriomas and other endometriotic plaques located near the vagina [44]. However, it can not detect pelvic adhesions or superficial peritoneal foci. A combination of clinical and vaginal probe ultrasound findings may improve the accuracy of diagnosis [45]. Abaro et al. and Goncalves et al. state that TVUS may be more sensitive in detecting rectovaginal deep endometriosis [46, 47].

MRI is now being used more commonly to diagnose endometriotic lesions. Small nodules may be recognized as hyperintense lesions on T1-weighted sequences, and plaque lesions have a similar appearance, with a variable signal on T2-weighted sequences [48]. Even though MRI is superior to TVS in detecting small peritoneal lesions, it is recommended in patients with chronic pelvic pain with suspected adhesions with endometriosis. TVUS seems superior in detecting rectovaginal disease [49, 50].

**MANAGEMENT**

Endometriosis is a chronic disease marked by acute flare ups. The condition is generally managed with medical therapy. Surgical treatment is reserved for more problematic cases. Medical management encompasses the use of traditional treatments: combined oral contraceptives, GnRH analogues, and progesterone, although the most common medication used for pain relief is NSAIDs (non steroidal anti-inflammatory drugs). We will discuss options of surgical treatment, traditional medical treatment and newer treatment in this section.
SURGICAL MANAGEMENT

Laparoscopy is still considered the gold standard in diagnosing endometriosis [41, 51, 52]. Recent reviews have demonstrated that in selected groups of patients, laparoscopic surgery improves pelvic pain, irrespective of the nature of surgery [53, 54]. Various surgical treatments have been utilized to treat chronic pelvic pain in patients with endometriosis including lysis of adhesions, laser ablation of implants, and aspiration and excision of endometriomas [55, 56]. Whether these ablative surgeries compromise ovarian reserve is not currently known. Multiple authors have concluded that surgery for endometriosis does not negatively alter IVF outcomes [57, 58].

Minimally invasive surgery for endometriosis might improve pain in most patients, but the overall debate on whether surgery is essential to improve fertility outcomes in patients with endometriosis still prevails. Littman et al. demonstrated that of 29 patients with IVF failure, 22 conceived after laparoscopic surgery. Fifteen of these pregnancies were spontaneous and the other 7 were initiated by IVF [59]. Adamson et al. while studying the factors affecting reproductive outcomes after surgery in patients with endometriosis stressed the importance of the combination of surgical skills and proper patient selection for IVF is required to achieve success [60].

MEDICAL MANAGEMENT

Medical management is largely empirical and based mostly on clinical findings. Management can be broadly divided into traditional and newer treatment groups.

TRADITIONAL

NSAIDs

The most commonly used medication for pain in endometriosis is the NSAID. But its role and efficacy are doubtful, and the side effect profile is extensive [61].

Oral Contraceptives

With hormone therapy, the goal is to create a pseudo-pregnancy or a pseudo-menopause state in order to ablate the active endometriotic deposits. Oral contraceptives may be used cyclically or continuously. Combined oral contraceptive (those containing a combination of an estrogen and a progestin) decidualizes endometriotic implants, thus reducing their activity [56], possibly by inducing apoptosis [62]. Contraceptives containing androgenic progestogens are normally preferred, but those containing desogestrel may be used with acceptable results [63, 64]. With cyclical OC therapy, dysmenorrhea can recur during the pill-free period. In that case, continuous usage might be more beneficial [65]. The use of combined oral contraceptives did not alter recurrence rates in post surgical patients with endometriosis [66].

Gonadotrophin Releasing Hormone Agonists (GnRH)

This is the most commonly used medication to treat endometriosis [67]. It acts by down-regulating the gonadotropin receptors, abolishing the pulsatility in natural secretion [56]. These drugs include leuprolide, goserelin, nafarelin, buserelin, and histrelin. The main side effect is the hypoestrogenic state that ensues after an initial flare effect. This hypoestrogenic state can manifest as post menopausal vascular symptoms and also by bone mineral density loss [68]. These effects can be counteracted by using “add back therapy” either with norethindrone or combined oral contraceptives [63, 69]. Disease recurrence is also common after GnRH therapy is stopped [70].

Danazol

This 17-ethinyl-testosterone derivative has shown to have similar efficacy when compared with GnRH agonists in relieving pain. It has fallen out of favor secondary to its androgenic side effects, which include weight gain, hirsutism, altered lipid profile and abnormal liver enzymes [71].

Progestins

Progesterone treatment creates a pseudo pregnant state. The exact mechanism of action is unknown but it is believed that it not only counteracts estrogenic effects on the endometrial cells but also affects MMP activity and prevents angiogenesis as seen in animal studies [72]. Murine studies have also demonstrated the repressive effect of progesterone on estrogen-induced stroma cell derived factor in endometrial implants, which were unaffected by selective progesterone receptor modulators (Asoprisnil) or anti progestogens (RU486) [73].

Various types of progestins are used for treatment including medroxyprogesterone acetate (20-30 mg/day), Depo Medroxyprogesterone acetate (DMPA/ Depo-Provera, Pfizer) and Depo SubQ -104–Provera (Pfizer). These agents have comparable efficacy with GnRH analogues but have lesser hypoestrogenic effects on the bone. A recent study conducted in Japan found that a highly selective progesterone receptor analogue called Dienogest (DNP) had anti-proliferative and inhibitory effects on cytokines. It has been shown to be as effective as intranasal buserelin in relieving pain [74].

The Levonorgestrel (LNG) IUD system may have therapeutic implications in patients with endometriosis. One animal study found that LNG microspheres created a pseudo pregnancy state in the uterus of a rabbit model [75].

Comparative Studies

GnRH agonists have been compared with Danazol in randomized studies, and the results showed little if any significant difference in pain control [76]. When compared with COCs in a small randomized trial, goserelin was better able to alleviate dysmenorrhea [77] but the combination of triptorelin and COC had a better efficacy at 1 year follow up [78]. Human studies have compared the effects of LNG IUD with GnRH agonist therapy on endometrial thickness as evaluated by ultrasonography. This study demonstrated smaller uterine changes in the LNG arm but similar effects on endometrial thickness [79].
NEWER TREATMENT METHODS

Advancements in animal and human research exploring the pathogenesis of endometriosis have opened up newer avenues of treatment. Medications targeting the disease pathology are being developed. Some of these agents under investigation include aromatase inhibitors, matrix metalloproteinase (MMP) inhibitors, anti-angiogenic agents, anti-TNF-α, selective estrogen receptor modulators (SERMs), and selective progesterone receptor modulators (SPRM) (Asoprisnil, Schering and TAP Pharmaceuticals).

Aromatase Inhibitors

Normal endometrial tissue does not express high levels of aromatase activity whereas endometrial implants do, secondary to the inflammatory effects of prostaglandins (PGE) and estradiol. Letrozole and Anastrozole are the two most commonly used 3rd generation aromatase inhibitors. They act by inhibiting aromatase, the enzyme that converts androstenedione to estrone and testosterone to estradiol by binding competitively with the heme moiety of the cytochrome P450 subunit. This mainly reduces excess estrogen and prostaglandin (PGE) production in peripheral tissues, thus inhibiting the endometrial implants’ ability to proliferate [80]. A mouse model study demonstrated that aromatase inhibitors did not prevent implants from becoming established in the mouse peritoneum but significantly reduced their growth when administered on day 1 of the menstrual cycle. Letrozole also increased apoptosis when started on day 28. They also reduced vascular endothelial growth factor (VEGF) and prostaglandin (PGE) production in the peritoneal fluid [81].

Several studies have shown that aromatase inhibitors may act singly or in combination with combined oral contraceptives or with GnRH agonists in alleviating pain [82, 83]. The advantage of combining aromatase inhibitors with a GnRH agonist is that they can prevent the sudden growth of ovarian cysts due to excess FSH secreted secondary to low estrogen levels [84]. This combination therapy, however, can profoundly reduce bone mineral density (BMD) immediately following treatment, which can take 2 years to rebuild after discontinuing treatment.

Matrix Metalloproteinase Inhibitors

As discussed in the pathogenesis section, a balance between MMP and TIMP is essential to maintain homeostasis in peritoneal fluid. In patients with endometriosis, the strict regulation of MMPs is lost, and TIMP expression declines, as shown in animal studies [85]. As a result, ectopic endometrial tissue can implant more readily within the pelvic peritoneum [31].

The role of melatonin as an antioxidant has been documented in animal studies in relation to MMPs. It has been shown to reduce the activity of pro-MMP-9 in the peritoneal cavity [85]. This may be attributed to its anti-oxidant properties by which it reduces protein oxidation and lipid peroxidation. It can also augment antioxidants such as superoxide dismutase and catalase and decrease malondialdehyde levels, as seen in murine studies [86]. The role of melatonin in the treatment for endometriosis is still experimental and needs further research.

Anti-Angiogenic Agents

The process of angiogenesis helps keep ectopic endometrial implants biologically active. These implants express vascular endothelial growth factor-A (VEGF –A) in peritoneal fluid, which initiates endothelial migration, proliferation and neovascularisation. This in turn stimulates production of estrogen and PGE2. VEGF in turn stimulates COX-2 production, thus maintaining PGE levels and leading to a viscus cycle of persistent vascular growth [87].

Most anti-angiogenic therapies are currently being used in patients with advanced cancer. Bevacizumab, a humanized anti-VEGF antibody (Avastin, Genentech), has been approved by FDA [88], but no human studies have been performed using such agents for endometriosis. Many animal studies have documented that use of a competitive inhibitor, or antibodies to VEGF, may retard implant survival and growth by preventing new vessel growth [89, 90]. The only drawback of these studies was that they could not demonstrate whether the therapy had effect on existing mature vessels. Human studies have yet to be conducted. Other angiogenesis inhibiting factors, namely TNP-470 [90], endostatin [91], and angiexin [90], are still experimental agents.

Recently, vascular-disruptive agents (VDAs) have been studied in cancer therapy. They have some advantages over existing angiogenic inhibitors regarding their effect on pre-existing vasculature and mature vessels. They can be used in acute treatment and also in advanced disease [92]. VDAs can be used in advanced endometriosis as they are able to specifically target the new and existing vessels supplying the implants better than other anti-angiogenic agents, but no human studies have been reported to date.

Recent animal research also has targeted tissue factor factor in the endometrium of endometriotic implants. A novel agent called ICON (Immuno Conjugate), designed from Factor VII-a, has been used to target the tissue factor in the endometrium. ICON binds with the tissue factor and recruits NK cells to destroy the entire endometriotic lesion [16, 93, 94].

Anti-TNF-α

TNF-α plays an important role in the pathogenesis of endometriosis. Targeting this cytokine may help by disrupt the signaling pathways leading to decreased angiogenesis and reduced MMP activity. This type of therapy may also help prevent oocyte and sperm damage.

Animal models mainly the baboon and rat have been used to demonstrate the effects of monoclonal antibodies on endometriotic implants. In one study, the size of endometriotic lesions decreased, and the efficacy was comparable to that of GnRH agonists [95]. Randomized trials in humans with anti- TNF-α have not been conducted. Results from a single case report were not encouraging after long-term use of Etaanarcept and Leflumide. There was no reduction of lesion size although the patient was able to conceived with the help of IVF after 8 years of treatment [96]. Hence, the therapeutic role of anti-TNF-α agents in established endometriosis has yet to be proven.
Selective Estrogen Receptor Modulator (SERM)

Estrogen receptor beta (ERB) binds to SERMS 200 times more than that of estrogen receptor alpha (ERA). These ligands (ERB-L) or SERMs have anti-inflammatory properties as shown in a rat model. They reduce the size of approximately 75% of implants without inhibiting ovulation. Current human trials are ongoing, and preliminary results suggest that SERMs do not change the hormone profile. This early phase II data is encouraging [97].

Selective Progesterone Receptor Modulator (SPRM)

These are progesterone receptor ligands with agonist, antagonist, and mixed agonist-antagonist activity in various target tissues. Asoprisnil (Schering and TAC Pharmaceuticals) is the first SPRM manufactured for therapeutic use. It has tissue specificity and minimal anti-estrogenic side effects. These agents are also known to reduce PGE2 and COX-2 synthesis in implants of guinea pigs [98]. Hence, these agents may also reduce pain in patients with endometriosis. Their role in endometriosis is selective inhibition of endometrium without side effects and reduction of endometrial bleeding secondary to their direct effect on endothelial vasculature [99]. Recently, it was found that SPRM can cause endometrial hyperplasia after 3 to 4 months of use [100]. The efficacy and side effect profile should be established by further human studies.

CONCLUSION

Endometriosis remains an area of great research interest. New clues to its pathogenesis are leading scientists to explore new therapies. In the absence of a non-invasive blood marker, laparoscopy is still the gold standard for diagnosis. Surgical treatment can help improve pain but controversies exist regarding its relation to reproductive outcomes in patients undergoing IVF. In properly selected patients, surgery might prove to be beneficial. Researchers have explored the possible use of a new generation of medical therapies, including aromatase inhibitors, MMP inhibitors, SPRMs, anti-TNF-α agents, and anti-angiogenesis factors. Most of these agents have been proven useful in animal studies and in studies relating to cancer therapy, but major human trials are still required to establish their efficacy in endometriosis.

REFERENCES


[46] Abrao MS, Podgaec S, Dias JA, Jr., et al. Endometriosis lesions that compromise the rectum deeper than the inner muscularis layer have more than 40% of the circumference of the rectum affected by the disease. J Minin Invasive Gynecol 2008; 15: 280-5.


Advanced Management Options for Endometriosis


Prevention and Management of Ovarian Hyperstimulation Syndrome

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Abstract: Ovarian hyperstimulation is an iatrogenic syndrome that presents as the most serious complication of ovarian induction. This syndrome is characterized by bilateral multiple cysts and third space fluid distribution. While mild forms have no consequences to the patient, severe forms may result in mortality and severe morbidity. A new classification of the syndrome is proposed based on the severity. The classification should guide the fertility specialist in determining the plan of management. OHSS is most prevalent in patients with polycystic ovarian syndrome. Prediction depends on the clinical acumen and careful monitoring using ultrasonography and serum estradiol measurements. The most popular method for prevention is coasting. The role of dopamine agonists has become part of the standard management. The management of OHSS could be achieved on an outpatient basis in many circumstances. Correction of the circulatory volume and the prevention of thromboembolism are the two basics that should be achieved by all clinicians involved in the management of the patient.

Keywords: OHSS, ascites, pleural effusion, thromboembolism, genetics, dopamine agonist.

INTRODUCTION

Ovarian hyperstimulation syndrome (OHSS) is the most serious iatrogenic complication of ovulation induction [1-20]. OHSS is characterized by bilateral, multiple follicular and theca-lutein ovarian cysts (Fig. 1) and an acute shift in body fluid distribution resulting in ascites (Fig. 2) and pleural effusion (3) (Fig. 3). Induction of ovulation by gonadotropins is one of the major advances in the treatment of infertility in the second half of the 20th century. Some degree of ovarian hyperstimulation occurs in all women who respond to ovulation induction but this should be distinguished from the clinical entity of ovarian hyperstimulation syndrome. Whilst mild OHSS is of no clinical relevance, severe OHSS, characterized by massive ovarian enlargement, ascites, pleural effusion, oliguria, hemoconcentration and thromboembolic phenomena, is a life-threatening complication. This manuscript is based on our previous publications and reviews [1-3].

OHSS CLASSIFICATIONS

OHSS may be moderate or severe, early or late in onset (Fig. 4), and spontaneous or iatrogenic in etiology. Early OHSS presents 3 to 7 days after the ovulatory dose of HCG, whereas late OHSS presents 12 to 17 days after HCG. Early OHSS relates to “excessive” preovulatory response to stimulation. Late OHSS develops only in connection with pregnancy, is more likely to be severe and has a low correlation with pre-ovulatory events. Most cases of OHSS are iatrogenic following gonadotropin stimulation. Rarely, OHSS occurs spontaneously as a result of mutations in the follicle stimulating hormone (FSH) receptor leading to its stimulation by chorionic gonadotropin, which is abundant in early pregnancy.

There has been no unanimity in classifying OHSS, and divergent classifications have made comparisons between studies difficult [2]. Aboulghar and Mansour [6] (2003) reviewed the classifications used for OHSS over the last four decades (Table 1). The most recent classification was introduced in 1999 by Rizk and Aboulghar (1999) [7]. They classified the syndrome into only two categories, moderate and severe, with the intent of categorizing patients into more defined clinical groups that correlate with the syndrome’s


Fig. (1), contd…..

prognosis. Their classifications can be correlated with the treatment protocol and prognosis.

Fig. (3). Right pleural effusion in OHSS. Reproduced with permission from: Rizk B, Rizk CB, Nawar MG, Garcia-Velasco JA, Sallam HN. Ultrasonography in the prediction and management of ovarian hyperstimulation syndrome In: Rizk B, Ed. Ultrasonography in reproductive medicine and infertility Cambridge, UK: Cambridge University Press 2010; Chapter 36: 299-312.

The new classification omits mild OHSS, used by most previous authors, as this level of OHSS occurs in the majority of cases of ovarian stimulation and does not require special treatment. Most cases of OHSS present as moderate OHSS. In addition to the presence of ascites on ultrasound, the patient’s complaints usually are limited to mild abdominal pain and distension and hematological and biochemical profiles are normal.

MODERATE OHSS

- Abdominal discomfort, pain, nausea, abdominal distension, ultrasonic evidence of ascites and enlarged ovaries, normal hematological and biological profiles. Treatable on an outpatient basis with extreme vigilance.

SEVERE OHSS

- Grade A: Dyspnea, oliguria, nausea, vomiting, diarrhea, abdominal pain, clinical evidence of ascites plus marked distension of abdomen or hydrothorax, large ovaries and marked ascites on ultrasound, normal biochemical profiles. Can be treated as inpatient or outpatient depending on physician’s experience, patient compliance, and medical facility.

- Grade B: All symptoms of grade A, plus massive tension ascites, markedly enlarged ovaries, severe dyspnea, and marked oliguria; biochemical changes, including increased hematocrit, elevated serum creatinine, and liver dysfunction. Treated as a hospital inpatient hospital under expert supervision.

- Grade C: OHSS complicated by respiratory distress syndrome, renal shut-down or venous thrombosis. Considered critical and should be treated in an intensive care setting.

Fig. (4). Classification of ovarian hyperstimulation syndrome early and late. Reproduced with permission from: Rizk B, Rizk CB, Nawar MG, Garcia-Velasco JA, Sallam HN. Ultrasonography in the prediction and management of ovarian hyperstimulation syndrome In: Rizk B, Ed. Ultrasonography in reproductive medicine and infertility Cambridge, UK: Cambridge University Press 2010; Chapter 36: 299-312.
COMPLICATIONS OF OHSS

Vascular Complications

Cerebrovascular complications are by far the most serious complications of OHSS. The first cases of severe thromboembolic phenomena following human menopausal gonadotropin and human chorionic gonadotropin (hMG/hCG) treatment caused the death of one patient (carotid embolism) and a limb amputation in the other [2, 3].

Hemoconcentration and increased hematocrit value in the presence of normal coagulation parameters were found in 9 of 25 patients [2, 3]. Increased levels of factor V, platelets, fibrinogen, profibrinolyis, fibrinolytic inhibitors, and thromboplastin generation were observed [2, 3]. Thrombophlebitis occurred in 2 of 25 patients and deep venous thrombosis has been reported in at least one case of severe OHSS [2, 3].

Venous compression due to enlarged ovaries and ascites, together with immobility and a transient change in coagulation were thought to be the main etiological factors in thromboembolism. Internal jugular vein thrombosis occurred >6 weeks after ovulation, and a subclavian vein thrombosis occurred 7 weeks after egg collection for in vitro fertilization (IVF). Both of these cases suggest a generalized effect on the coagulation system that may persist for several weeks.

Liver Dysfunction

Hepatic function abnormalities have been recognized increasingly as a complication in severe OHSS that may persist for >2 months. Liver biopsies typically demonstrate significant morphological abnormalities only at the ultrastructural level [2, 3]. Electron microscopy revealed paracrystalline inclusions in the majority of the liver cell mitochondria. There were prominent membrane-bound bodies containing


<table>
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<tr>
<th>Study</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
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<tbody>
<tr>
<td>Rabau et al. (1967)</td>
<td>Grade 1: estrogen &gt;150 µg and pregnanediol &gt;10 mg 24 h</td>
<td>Grade 3: grade 2 + confirmed palpable cysts and distended abdomen</td>
<td>Grade 5: grade 4 + ascites and possibly hydrothorax</td>
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<tr>
<td></td>
<td>Grade 2: enlarged ovaries and possibly palpable cysts Grade 1 and 2 were not included under the title of mild OHSS</td>
<td>Grade 4: grade 3 + vomiting and possibly diarrhea</td>
<td>Grade 6: grade 5 + changes in blood volume, viscosity and coagulation, time</td>
</tr>
<tr>
<td>Schenker and Weinstein (1978)</td>
<td>Grade 1: estrogen &gt;150 (ig/24 h and pregnanediol &gt;10 mg 24 h</td>
<td>Grade 3: grade 2 + abdominal distension</td>
<td>Grade 5: grade 4 + large ovarian cysts, ascites and/or hydrothorax</td>
</tr>
<tr>
<td></td>
<td>Grade 2: grade 1 + enlarged ovaries, sometimes small cysts</td>
<td>Grade 4: grade 3 + nausea, vomiting and/or diarrhea</td>
<td>Grade 6: marked haemoconcentration + increased blood viscosity and possibly coagulation abnormalities</td>
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<tr>
<td>Golan et al. (1989)</td>
<td>Grade 1: abdominal distension and discomfort</td>
<td>Grade 3: grade 2 + ultrasound evidence of ascites</td>
<td>Grade 4: grade 3 + clinical evidence of ascites and/or hydrothorax and breathing difficulties</td>
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<td></td>
<td>Grade 2: grade 1 + nausea, vomiting and/or diarrhea, enlarged ovaries 5-12 cm</td>
<td></td>
<td>Grade 5: grade 4 + haemoconcentration, increased blood viscosity, coagulation abnormality and diminished renal perfusion</td>
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<td>Navot et al. (1992)</td>
<td>Severe OHSS: variable enlarged ovary; massive ascites ± hydrothorax; Hct &gt;45%; WBC &gt;15 000; oliguria; creatinine 1.0-1.5; creatinine clearance &gt;50 ml/min; liver dysfunction; anasarca</td>
<td></td>
<td>Critical OHSS: variable enlarged ovary; tense ascites ± hydrothorax; Hct &gt;55%; WBC 22=25 000; oliguria; creatinine 5=1.6; creatinine clearance &lt;50 ml/min; renal failure; thromboembolic phenomena; ARDS</td>
</tr>
<tr>
<td>Rizk and Aboulghar (1999)</td>
<td>Discomfort, pain, nausea, distension, ultrasonic evidence of ascites and enlarged ovaries, normal haematological and biological profiles</td>
<td>Grade A: Dyspnoea, oliguria, nausea, vomiting, diarrhea, abdominal pain, clinical evidence of ascites, marked distension of abdomen or hydro-thorax, US showing large ovaries and marked ascites, normal biochemical profile</td>
<td>Grade B: Grade A plus massive tension ascites, markedly enlarged ovaries, severe dyspnoea and marked oliguria, increased haematocrit, elevated serum creatinine and liver dysfunction</td>
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<td></td>
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<td>Grade C: Complications as respiratory distress syndrome, renal shut-down or venous thrombosis</td>
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granular matrix material and interpreted as microbodies or peroxisomes. The cisternae of the smooth endoplasmic reticulum were dilated, and focal microvillus proliferation was present in the region of the canaliculi. No significant changes in the Golgi apparatus or glycogen granules were noted. These changes may be related to the increased estrogen production induced by hMG. Similar hepatic changes have been found after the administration of oral contraceptives or anabolic steroids. The ultrastructural changes may be a compensatory morphological change in response to the increase in demand on the liver enzymes rather than true pathological alterations.

Respiratory Complications

Respiratory distress, secondary to ascitic fluid accumulation, is common in severe OHSS and is usually relieved by aspiration of ascitic fluid. Adult respiratory distress syndrome was reported in a patient with severe OHSS. A rare case of pleural effusion as the sole presentation of OHSS and a similar case after IVF have been described [2, 3].

Gastrointestinal Complications

With the widespread use of ovulation induction for assisted conception, it is mandatory that general practitioners become aware that gastrointestinal symptoms could be the initial presentation of ovarian hyperstimulation. One such case presented with a cerebrovascular accident due to such symptoms being overlooked [2, 3].

Adnexal Torsion

Adnexal torsion after superovulation is caused primarily by ovarian enlargement due to the development of multiple follicular or luteal cysts that make this complication more common in cases of OHSS [2, 3]. OHSS may worsen during the pregnancy with the continuing increase in ovarian size eventually leading to torsion. In a series of 154 patients hospitalized for severe OHSS, torsion was noted in 16% of the pregnant patients compared with 2.3% of non-pregnant patients. Laparoscopic unwinding of twisted ischemic hemorrhagic adnexum after IVF was successfully accomplished [2, 3].

PATHOPHYSIOLOGY OF OHSS

Over the years many substances involved in the regulation of vascular permeability have been implicated as a cause of OHSS [11], and several of them are still under investigation. The list of potential mediators includes estradiol; histamines; prostaglandins; the ovarian rennin-angiotensin system; interleukin (IL)0-6, IL-2 and IL-8; angiogenin; endothelin-1; insulin; and the ovarian kinin-kallikrein system. Rizk et al. (1997) and Pellicer et al. (1999) investigated the role of vascular endothelial growth factor (VEGF) as a mediator for capillary permeability and fluid leakage [8] (Figs. 5-7). VEGF production is dependent on human chorionic gonadotropin stimulation that is administered to trigger ovulation or during the early phase of pregnancy.

PREDICTION OF OHSS

Predicting the risk of OHSS is the cornerstone of prevention. Prediction is based on identifying the characteristics of patients who are high responders as well as the use of ultrasonography and estradiol assessment (Table 2). Rizk et al. (2010) studied the role of ultrasonography before, during, and after ovarian stimulation as the key to early prediction and successful prevention (Figs. 8-11) [3]. Rizk and Aboulghar emphasized the role of estradiol measurement in the detection of high responders who are at risk for severe OHSS [7].

Rizk (2009) investigated the role of FSH receptor (FSHR) mutations and polymorphisms in the development of OHSS [16]. Mutations in FSH receptors could be either activated, thus resulting in OHSS, or inactivating, thus resulting in sterility. To date, 744 single nucleotide polymorphisms have been identified in the FSH receptor gene. Genetic studies of FSH receptor mutations have increased the expectations that OHSS could be predicted based on the FSH recep-

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Fig. (5). Pathophysiology of ovarian hyperstimulation syndrome. Reproduced with permission from: Rizk B, Rizk CB, Nawar MG, Garcia-Velasco JA, Sallam HN. Ultrasonography in the prediction and management of ovarian hyperstimulation syndrome In: Rizk B Ed. Ultrasonography in reproductive medicine and infertility Cambridge, UK: Cambridge University Press 2010; Chapter 36: 299-312.
tor genotype. The potential association of the S<sup>680</sup> allele with poor responders to ovarian stimulation for IVF led to the hypothesis that the N<sup>680</sup> allele could be associated with the hyper-responders, i.e., patients at risk of iatrogenic OHSS. In an elegant study, no statistically significant differences between the IVF control population and the OHSS patients in allelic or genotypic frequencies were found. However, a significant enrichment in allele 680 was observed as the severity of OHSS increased (P = 0.034). The results of this study also suggested that the genotype in position 680 of the FSH receptor cannot predict which patient will develop OHSS but could be a predictor of severity of OHSS symptoms in women who develop the syndrome.

Bone morphogenetic protein-15 (BMP-15) is an important oocyte-derived growth factor which is essential for normal folliculogenesis and female fertility of mammals. BMP-15 is a member of the transforming growth factor β (TGFβ) superfamily. Within the ovary, BMP-15 mRNA is found exclusively in the oocyte. In the human, BMP-15 is detected in the oocytes of primordial follicles and progressively expressed by oocytes in growing follicles through folliculogenesis [16]. High BMP-15 in follicular fluid is also associated with high quality oocytes and subsequent embryonic development. A genetic association study of ovarian stimulation outcome in 307 unrelated women with normal ovarian function who underwent ovarian stimulation using recombinant FSH was performed. Four single nucleotide polymorphisms located at the BMP-15 gene were analyzed in order to investigate the role of this gene in relation to ovarian stimulation outcome. The results support the hypothesis that BMP-15 alleles predict over-response to recombinant FSH and ovarian hyperstimulation syndrome in humans [16].

Table 2. Prediction of OHSS

<table>
<thead>
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<th>History and Physical</th>
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<tbody>
<tr>
<td>1. OHSS in a previous cycle</td>
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<tr>
<td>2. Polycystic ovarian syndrome (PCOS)</td>
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<tr>
<td>3. Young age</td>
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<tr>
<td>4. Low body mass index</td>
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<tr>
<td>5. Hyperinsulinism</td>
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<td>6. Allergies</td>
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<tr>
<th>During Ovarian Stimulation</th>
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</thead>
<tbody>
<tr>
<td>1. High serum estradiol, rapid slope of E2 and absolute value</td>
</tr>
<tr>
<td>2. Ultrasoundography</td>
</tr>
<tr>
<td>a. Baseline PCO pattern</td>
</tr>
<tr>
<td>b. PCO pattern of response to GnRH before gonadotropins</td>
</tr>
<tr>
<td>c. Large number of follicles &gt; 20, on each ovary</td>
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<tr>
<td>3. Doppler low intraovarian vascular resistance</td>
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<table>
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<tr>
<th>Outcome of ART Cycles</th>
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<tbody>
<tr>
<td>1. Conception cycles</td>
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<tr>
<td>2. Multiple pregnancy</td>
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PREVENTION OF OVARIAN HYPERSTIMULATION SYNDROME

Rizk in 1993 [17] suggested a ‘Ten Commandments’ for the prevention of OHSS. These consisted of identifying patients at risk, use of treatment of other than gonadotropins for PCOS patients (such as metformin and ovarian diathermy), and use of low doses and GnRH antagonists when gonadotropins were necessary (Table 3). A second ‘Ten Commandments’ addressed the secondary prevention of
OHSS and included withholding or delaying hCG, follicular aspiration, switching to IVF with cryopreservation of all embryos, and progesterone for luteal phase support (Table 4) [10]. Other measures unique to prevention of OHSS are: use of GnRH antagonists instead of agonists to prevent premature LH surge, decrease in the dose of hCG, use of LH or GnRH agonist in place of hCG for triggering ovulation, administration of albumin, use of glucocorticoids, and administration of dopaminergic drugs.

Fig. (9). Ascites in moderate ovarian hyperstimulation syndrome in early pregnancy. Reproduced with permission from: Rizk B, Rizk CB, Nawar MG, Garcia-Velasco JA, Sallam HN. Ultrasonography in the prediction and management of ovarian hyperstimulation syndrome In: Rizk B, Ed. Ultrasonography in reproductive medicine and infertility Cambridge, UK: Cambridge University Press 2010; Chapter 36: 299-312.

Fig. (10). Twins associated with OHSS. Reproduced with permission from: Rizk B, Rizk CB, Nawar MG, Garcia-Velasco JA, Sallam HN. Ultrasonography in the prediction and management of ovarian hyperstimulation syndrome In: Rizk B, Ed. Ultrasonography in reproductive medicine and infertility Cambridge, UK: Cambridge University Press 2010; Chapter 36: 299-312.

Fig. (11). Coasting for prevention of ovarian hyperstimulation syndrome. Reproduced with permission from: Rizk B, Rizk CB, Nawar MG, Garcia-Velasco JA, Sallam HN. Ultrasonography in the prediction and management of ovarian hyperstimulation syndrome In: Rizk B, Ed. Ultrasonography in reproductive medicine and infertility Cambridge, UK: Cambridge University Press 2010; Chapter 36: 299-312.

Table 3. Primary Prevention of OHSS

<table>
<thead>
<tr>
<th>The Ten Commandments</th>
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</thead>
<tbody>
<tr>
<td>1. Prediction of OHSS from history, exam and ultrasound</td>
</tr>
<tr>
<td>2. Laparoscopic ovarian drilling in PCOS patients</td>
</tr>
<tr>
<td>3. Metformin in PCOS patients</td>
</tr>
<tr>
<td>4. Octreotide in PCOS patients</td>
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<tr>
<td>5. Low-dose gonadotropins in PCOS patients</td>
</tr>
<tr>
<td>6. GnRH antagonist protocol</td>
</tr>
<tr>
<td>7. Recombinant LH to trigger ovulation</td>
</tr>
<tr>
<td>8. GnRH agonist to trigger ovulation</td>
</tr>
<tr>
<td>9. In vitro maturation of oocytes</td>
</tr>
<tr>
<td>10. Replacement of only one embryo</td>
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</table>


GnRH Antagonist as an Alternative to the Long Agonist Protocol

In a Cochrane review, the efficacy of GnRH antagonist was compared to the long agonist protocol in assisted conception [18]. In comparison with the long GnRH agonist protocol, there was no statistically significant reduction in the occurrence of severe OHSS (RR=0.50; OR=0.79; 95% CI, 0.22-1.18); however, there were significantly fewer pregnancies with GnRH antagonist (OR=0.79; 95%CI, 0.63-0.99). In a review that compared the two GnRH antagonists Cetrorelix and Ganirelix to the long protocol, a difference was observed [19]. A significant reduction of OHSS was observed in Cetrorelix studies (OR=0.2; 95%CI, 0.10-0.54), but no reduction was observed for Ganirelix, (OR=1.13; 95% CI, 0.24-5.31). The pregnancy rate in the Cetrorelix studies was not significantly different from that found in the long
GnRH agonist protocol, (OR=0.91; 95% CI, 0.68-1.22). The pregnancy rate in the Ganirelix protocols was significantly lower compared with that in the long GnRH agonist protocol (OR=0.76; 95% CI, 0.59-0.98). The final word has not been said in relation to the development of OHSS in GnRH antagonist cycles. Further studies will clarify this situation.

### Table 4. Secondary Prevention of OHSS

<table>
<thead>
<tr>
<th>The Ten Commandments</th>
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<tbody>
<tr>
<td>1. Withholding HCG +/- continuation of GnRH-a/GnRH antagonist</td>
</tr>
<tr>
<td>2. Coasting or delaying hCG: currently most popular method</td>
</tr>
<tr>
<td>3. Use of GnRH-a to trigger ovulation</td>
</tr>
<tr>
<td>4. Follicular aspiration</td>
</tr>
<tr>
<td>5. Progesterone for luteal phase</td>
</tr>
<tr>
<td>6. Cryopreservation and replacement of frozen-thawed embryos at a subsequent cycle</td>
</tr>
<tr>
<td>7. Dopamine agonist</td>
</tr>
<tr>
<td>8. Albumin, administration at time of retrieval</td>
</tr>
<tr>
<td>9. Glucocorticoid administration</td>
</tr>
<tr>
<td>10. Aromatase inhibitors</td>
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</tbody>
</table>


### Cancelling Cycles to Avoid OHSS

Withholding hCG used to be the most common method used to prevent OHSS in patients at high risk for developing the syndrome [11]. The estradiol criteria for withholding hCG to prevent OHSS ranged from 800 pg/ml to 4000 pg/ml [11]. Mostly all authorities recommend withholding hCG when the estradiol level exceeds 4500 pg/ml. Equal or more important than estradiol levels as criteria for canceling cycles are the number of 8-10 mm follicles that may have acquired LH receptors.

### Decrease in HCG Dosage

Several clinical trials have been published in the literature regarding the impact of the dose of hCG on the occurrence of OHSS [22, 23]. Tsoumpou et al. elegantly reviewed the studies in the IVF agonist and antagonist cycles. No definitive conclusions could be made because of the differences in study design. Only one of the four trials regarding HCG dose using GnRH agonist was a randomized controlled and one of the two trials using GnRH antagonist was a randomized controlled trial.

### Coasting -- Delaying hCG Administration

“Coasting,” also known as a “controlled drift period” is the postponement of hCG administration to allow serum estradiol levels to drop below a certain threshold.

Coasting is the most popular method among physicians in the United States or Europe to prevent OHSS in patients undergoing IVF. Withholding gonadotropins and delaying the administration of HCG are techniques that have been employed in ovulation induction since the late 1980s and early 1990s. Shortly afterwards, coasting was used to prevent severe OHSS in IVF cycles. Many studies and critical reviews have evaluated the effect of coasting on OHSS.

### Advantages of Coasting

Coasting offers three potential advantages. The first is that the cycle is rescued and not canceled. Secondly, the embryos that are generated during the treatment cycle will be transferred, eliminating the need for cryopreservation. Thirdly, coasting avoids the need for gonadotropins or other medications or any supplementary procedures.

### How Coasting Works

The association between OHSS and high estradiol levels is very well established. This certainly does not mean that high estradiol levels per se result in the manifestations of increased permeability associated with OHSS.

Coasting may diminish the functional granulosa cell cohort, resulting in a gradual decline in circulating estradiol levels and, more importantly, reduction of the chemical mediators that augment capillary permeability and fluid retention. VEGF concentration in follicular fluid may depend on the quality and number of granulosa cells. Coasting acts through down-regulation of VEGF gene expression and protein secretion. The fact that medium and small follicles are more sensitive to undergoing atretic changes is of crucial relevance in both steroid and vasoactive mediator secretion.

### When to Initiate Coasting

Three factors should be considered in deciding to initiate coasting. The first is plasma estradiol concentration, which reflects the total functional granulose cell population; second is the number of ovarian follicles, which predicts the potential for further granulose cell population and estradiol rise; and third is the diameter of the leading follicles.

Most publications addressing coasting reflect that an estradiol concentration of 3000 pg/ml was the value most commonly chosen by clinicians. This relatively low threshold for coasting has been shown to reduce the incidence of OHSS effectively without compromising the cycle outcome. High cut-off levels of around 6000 pg/ml are associated with a higher incidence of OHSS and the need for longer periods of coasting.

After withholding gonadotropins, serum estradiol exhibits a subsequent increase for one or more days. When coasting was initiated at a plasma estradiol value of over 3000 pg/ml, the plasma estradiol increased to over 6000 pg/ml during the coasting period [20].

### Timing of hCG and Ending Coasting

Administration of hCG when the estradiol level drops below 3000 pg/ml has been termed to be effective in lowering the risk of OHSS.

### Duration of Coasting

The number of recorded days of coasting has varied between 1 and 11. The effect of coasting duration has remained
Prevention and Management of Ovarian Hyperstimulation Syndrome

How Successful is Coasting in Eliminating OHSS?

From an evidence-based medicine point of view, only limited data in terms of prospective randomized trials are available. Ethical considerations make it difficult to subject a test population to randomization in which one of the arms would not be coasted and thereby at risk for severe OHSS. Therefore these studies used a control group of another modality of OHSS prevention or a group of normo-responders.

GnRH Agonist as an Alternative to hCG to Trigger Ovulation

Alternatives to hCG to trigger ovulation include GnRH agonists, native GnRH, and recombinant LH. Although the incidence of OHSS is lowered, the ongoing pregnancies in IVF cycles are also reduced when ovulation has been triggered by a GnRH agonist or recombinant LH rather than urinary or recombinant hCG [7].

Intravenous Albumin

Experience in subjects with different forms of third space fluid accumulation has shown that albumin is efficacious in preventing and correcting hemodynamic instability. However, a series of publications for and against the efficacy of albumin in preventing OHSS have been published with contradictory results. A multifactorial role of albumin in prevention of OHSS has been proposed. First, it acts to sequester vasoactive substances released from the corpora lutea. Albumin also serves to sequester any additional substances that may have been synthesized as a result of OHSS. Finally, the oncotic properties of albumin serve to maintain intravascular volume and prevent the ensuing effects of hypovolemia, ascites, and hemoconcentration. Aboulghar et al. published, a Cochrane database review and found albumin to be effective in preventing OHSS [24]. However, a new Cochrane review soon to be published, includes new studies and found that there was no advantage in the use of albumin (Aboulghar, personal communication).

Hydroxyethyl Starch Solution

Hydroxyethyl starch (HES) is a synthetic colloid, glyco-
gen-like polysacharride derived from amylopectin. It has been used as an effective volume expander and is available in several molecular weights with different chemical properties. Several small studies suggested a beneficial effect of HES in decreasing OHSS and indicate that HES should be further investigated [25].

Glucocorticoid Administration

The pathophysiology of OHSS suggests the involvement of an inflammatory mechanism during the development of the fluid leakage that is associated with the syndrome. Therefore, investigators hypothesized that glucocorticoids possibly could prevent OHSS in patients at high risk. Rizk [2] and others found no protective effect of intravenous glucocorticoids.

Ovarian Electrocautery

Laparoscopic ovarian electrocautery may be effective in preventing OHSS in selected patients. Although the mechanisms of action are not clear, electrocautery appears to manipulate the intraovarian endocrine environment through rupture of androgen-rich cysts, destruction of androgen-producing stroma, or disruption of the thickened ovarian capsule. The recommendation is that electrocautery should be reserved for women who previously have experienced cancellation of at least cycle due to risk of OHSS.

Dopamine Agonists in OHSS Prevention

VEGF secreted by the hyperstimulated ovary acting via the VEGF receptor 2 (VEGFR-2) is a major cause of OHSS [26-28]. Dopamine receptor 2 (Dp-r2) agonists, used in the treatment of human hyperprolactinemia, inhibit VEGFR-2-dependent vascular permeability (VP) and angiogenesis when administered at high doses in animals. The dopamine agonist bromocriptine and, more recently, the Dp-r2 agonist cabergoline (Dostinex, Pharmacia & Upjohn, Bridgewater, N.J.) 0.5 mg daily for 8 days beginning the day of hCG administration (Fig. 13) have successfully prevented OHSS symptoms [26].

To test whether VEGFR-2-dependent vascular permeability and angiogenesis could be segregated in a dose-dependent fashion with cabergoline (Cb2), a well-established OHSS rat model supplemented with prolactin was used. A low dose (100 µg/kg) of Cb2 reversed VEGFR-2-dependent vascular permeability without affecting luteal angiogenesis through partial inhibition of ovarian VEGFR-2 phosphorylation levels. No luteolytic effects (no increase in serum pro-
gesterone concentrations or luteal apoptosis) were observed. Ch2 administration also did not affect VEGF/VEGFR-2 ovarian mRNA concentration.

In a recent prospective, double-blind study, more than 20 oocytes were retrieved from 54 donors in whom 20-30 follicles > 12 mm developed. Immediately after hCG administration, patients were divided into two groups by computer randomization. The study group (n=29) received 0.5 mg oral Ch2 daily for eight days; the control group (n=25) received one placebo tablet daily for eight days. Subjects were monitored every 48 hours from the day of hCG (day 0) up to day 8. Hemoconcentration and the presence and volume of ascitic fluid were significantly reduced in the study group. OHSS developed in 25% of women in the study group compared with 65% in the control group.

The frequency of OHSS was reduced by half in the dopamine agonist, quinagolide group compared with placebo [28]. Implantation rates and ongoing pregnancy rates did not differ. Interestingly, patients who did not become pregnant in the study cycle experienced more clinical benefits from treatment than did those women who achieved pregnancy. This leads to speculation that other vascular permeability parameters may reduce the effect of dopamine agonists in women who do become pregnant.

**TREATMENT OF OVARIAN HYPERSTIMULATION SYNDROME**

The clinical course of OHSS depends on its severity, the presence of complications, and whether the woman is pregnant [27, 28]. Clinical management involves dealing with electrolytic imbalance, neurohormonal and hemodynamic changes, pulmonary manifestation, liver dysfunction, hypoglobulinaemia, febrile morbidity, thromboembolic phenomena, neurological manifestations, and adnexal torsion (Fig. 14) [7-10]. The general approach should be adapted to the severity level. Specific approaches such as paracentesis and pleural puncture should be performed carefully when indicated.

**Successful medical management of OHSS requires familiarity with the disease. Many problems occur due to a lack of understanding by clinicians of the differences between OHSS and other medical syndromes that present with similar symptoms. A better understanding of the underlying pathophysiological mechanisms will help in refining the management of the disease.**

**Outpatient Management for Moderate OHSS**

Based on the classification of Rizk and Aboulghar [7], moderate OHSS should be followed with regular telephone calls at least daily and twice weekly office visits. Office assessment includes pelvic ultrasound, a complete blood count, liver function tests, and a coagulation profile. The patient should be instructed to report to the hospital if she develops dyspnea, diminished urine volume, or any unusual symptoms such as leg swelling, dizziness, numbness, or neurological problems.

**Outpatient Management for Severe OHSS**

Whether severe OHSS should be managed on an outpatient basis depends on the classification and definition of severity, the physician’s level of knowledge and experience with the disease, and the patient’s willingness to comply with treatment. OHSS grade A is treatable on an outpatient basis by aspiration of ascitic fluid, administration of intravenous fluids, and evaluation of all biochemical parameters.

**Inpatient Management of Severe OHSS**

Patients with severe OHSS grade B and C are admitted to the hospital for treatment. Indications for hospitalization are shown in Table 5. Hospitalization should be considered if...
one or more of these symptoms or signs are present [29]. Great caution is required in all grades of severe OHSS because complications can develop suddenly.

Table 5. Indications for Hospitalization of Patients with Severe OHSS

| 1. Severe abdominal pain or peritoneal signs |
| 2. Intractable nausea and vomiting that prevents ingestion of food and adequate fluids |
| 3. Severe oliguria or anuria |
| 4. Tense ascites |
| 5. Dyspnea or tachyspnea |
| 6. Hypotension (relative to baseline), dizziness, or syncope |
| 7. Severe electrolyte imbalance (hypernatremia, hyperkalemia) |

Inpatient Clinical and Biochemical Monitoring

The patient’s general condition must be assessed regularly, including documentation of vital signs, daily weight and girth measurement. Strict fluid balance recording is needed, particularly of urine output.

Biochemical monitoring should include serum and electrolytes, renal and liver function tests, a coagulation profile, and blood count. Serum and urinary osmolarity and urinary electrolyte estimation may be required as the disease progresses in severity. Respiratory compromise and/or significant deterioration of renal function require evaluation of blood gases and acid-base balance. The frequency of these evaluations depends on the severity of the condition.

Ultrasonographic examination provides accurate assessment of ovarian size and the presence or absence of ascites, as well as pleural or pericardial effusions. Ultrasound also is helpful in the diagnosis of intra- or extraterine pregnancy as well as multiple or heterotrophic pregnancy. β-hCG assay will help to diagnose pregnancy as early as possible, and a chest X-ray will provide information on the presence of hydrothorax.

Invasive hemodynamic monitoring (central venous pressure and pulmonary artery pressures) may be needed under certain circumstances in which volume expanders are being employed.

MEDICAL TREATMENT
Circulatory Volume Correction

The main line of treatment is correction of the circulatory volume and the electrolyte imbalance. Every effort should be directed towards restoring a normal intravascular volume and preserving adequate renal function. Volume replacement should begin with intravenous crystalloid fluids at 125-150 ml/h. Normal saline and lactated Ringer have been successfully used. Plasma colloid expanders may be used if necessary. One concern with using plasma expanders is that the beneficial effect is transitory before their redistribution into the extravascular space, further exacerbating ascites formation. Albumin has been utilized at 200 ml of 25% albumin solution. Dextran, mannitol, fresh frozen plasma, and hydroxyethylstarch (HES) have also been used. HES have the advantage of being non-biologic in origin and a high molecular weight (200-1000 kDA vs. 69 kDA for albumin). HES 6% and 10% have been used successfully, but larger prospective randomized controlled studies are needed [10].

Electrolyte Replacement

Appropriate solutions will correct electrolyte imbalances. If hypokalemia is significant, a cation exchange resin may be needed. Sodium and water restriction have been reported to be successful by some, but others found no change in the patient’s weight, abdominal circumference, or peripheral edema when sodium and water were restricted. With these inconclusive reports, salt and water restriction are not widely advocated [10].

Anticoagulant Therapy

Anticoagulant therapy is indicated if there is clinical evidence of thromboembolic complications or laboratory evidence of hypercoagulability [30,31]. Venous thrombosis is the most common serious complication of OHSS, and preventative heparin treatment should be used whenever there is a thromboembolic risk. In cases of severe OHSS, the following situations are recognized as indicating an increased risk of thromboembolism: immobilization, compression of pelvic vessels by large ovaries or ascites, pregnancy coagulation abnormalities, and hyperestrogenemia. Prevention using mobilization and antithrombosis stockings is insufficient as thrombosis may occur at all localizations and may be systemic in nature.

Anticoagulant Prophylaxis

Prophylaxis with heparin remains debatable on the basis that no randomized studies proving its efficacy in preventing thromboembolic complications during severe OHSS have been published. In fact, in some clinical scenarios, thromboembolism still occurs despite giving heparin [12]. Despite these reservations, Rizk [10] recommends giving heparin or enoxaparin (Lovenox, Sanofi-Aventis, Bridgewater, N.J.) for patients with severe OHSS. The incidence of deep vein thrombosis (DVT) is markedly increased in patients with Factor V Leiden mutation, one of the thrombophilias [32]. The mutation occurs in 4% of Northern European women. Patients should be questioned about a history of personal or familial thrombosis, and those with a positive history should be tested for Factor V Leiden mutation. Patients with Factor V Leiden mutation who develop OHSS should be placed on prophylactic heparin. Others risk factors for DVT in OHSS include protein C and S deficiency and antithrombin III deficiency.

Duration of Anticoagulation

The optimal duration of anticoagulant administration is undetermined. Some investigators have reported late thrombosis up to 20 weeks post-transfer, and many investigators favor maintaining heparin therapy for many weeks [4]. The severity of OHSS must be separated from the risk of throm-
boembolism because intrinsic coagulopathy may trigger the problem even in moderate cases. However, those who have followed a more liberal policy for prophylaxis have had to deal with operating on ruptured ectopic pregnancies in anticoagulated patients. Therefore, thromboembolism will remain a more difficult complication to prevent and may complicate the outcome of OHSS.

**Antibiotic Treatment**

Infections are not uncommon in the setting of treatment of OHSS because of frequent catheterizations, venipuncture, transvaginal aspiration of ascitic fluid, and pleural drainage. Furthermore, hypoglobulinemia is present in severe cases. Preoperative antibiotic prophylaxis is highly recommended. Some authors suggest the administration of immunoglobulins. However, this intervention still awaits further evaluation.

**Diuretics**

Diuretic therapy without prior volume expansion may prove detrimental, by further contracting the intravascular volume, thereby worsening hypotension and its sequelae. Diuretics will increase blood viscosity and increase the risk of venous thrombosis. Diuretic use should be restricted to the management of pulmonary edema.

**Dopamine**

Dopamine used in oliguric patients with severe OHSS results in significant improvement in renal function [34,35]. Dopamine produces its renal effect by increasing renal blood flow and glomerular filtration. Dopamine therapy should be given cautiously and under strict observation.

In one report, intravenous dopamine 4.32 mg/kg per 24 hours, was administered to 7 patients hospitalized with severe OHSS following gonadotropin stimulation for IVF or gamete intra-fallopian transfer (GIFT), beginning within 10 hours of admission [34]. Additional treatment consisted of bed rest, restriction of fluid intake to 500 ml/day, and daily monitoring of urine output, abdominal girth, and weight. Biochemical and hematological clotting factors were measured daily in the pregnant women. Serum hCG was measured every 2 days, and patients were given a protein- and salt-rich diet to increase the oncotic and osmotic blood pressure. Dopamine treatment was continued until complete resolution of ascites. In the 5 patients who were pregnant, dopamine treatment was required for 9 to 22 days. The duration of treatment was related to the magnitude of the increase of hCG. The longest duration (18-22 days) was needed in patients with triplets and the shortest (9-10 days) in patients with a singleton pregnancy; for patients with twins, treatment duration was intermediate (14 days). In the 2 non-pregnant women, dopamine was required for only 7 days.

In another report, a 750-mg tablet of docarpamine (Dostinex) was taken orally every 8 hours by 27 patients who were hospitalized because of OHSS and refractory to therapy with intravenous albumin [35]. Clinical symptoms associated with ascites were gradually improved; no major adverse effects occurred.

**Aspiration of Ascitic Fluid and Pleural Effusion in Severe OHSS**

Ascites is the hallmark of OHSS, and symptoms resulting from ascites are the most common reason for hospitalization. Aspiration is not indicated in every patient. Paracentesis by the transabdominal or transvaginal route is indicated for severe abdominal pain, pulmonary compromise as demonstrated by pulse oximetry, or tachypnea and renal compromise as demonstrated by oliguria and increased creatinine concentration [36,37].

**Abdominal Paracentesis**

Paracentesis is followed by increased urinary output shortly after the procedure, with a concomitant decrease in the patient’s weight, leg edema, and abdominal circumference. Creatinine clearance rate is increased following the procedure. Paracentesis offers temporary relief of respiratory and abdominal distress, but, since the fluid tends to recur, some patients need repeated paracentesis and drainage of effusions before spontaneous improvement occurs. The amount of fluid aspirated can range between 200 and 4000 ml. The risk of injury to ovaries is minimized by ultrasonographic guidance. Monitoring of plasma proteins is essential, and human albumin should be infused whenever necessary. Percutaneous placement of a pigtail catheter is a safe and effective treatment modality for severe OHSS that may represent an alternative to multiple vaginal or abdominal paracentesis [10].

**Transvaginal Ultrasound-Guided Aspiration**

Transvaginal ultrasound-guided aspiration is an effective and safe procedure. Injury to the ovary is easily avoided by puncture under ultrasonic visualization. No anesthesia is required for the procedure, and better drainage of the ascitic fluid is accomplished because the pouch of Douglas is the most dependent part.

**Autotransfusion of Ascitic Fluid**

Transvaginal aspiration of ascitic fluid and autotransfusion of the aspirated fluid has been used in treatment of severe OHSS. The procedure is simple, safe, and straightforward and shows a striking physiological success in correcting the maldistribution of fluid and proteins without the use of heterogenous biological material. However, autotransfusion is not recommended because of the possible reinjection of cytokines into the circulation.

**Pleurocentesis and Treatment of Pulmonary Complications**

Evaluation and treatment of patients with severe OHSS complaining of dyspnea includes physical examination, chest ultrasound and X-ray, and arterial blood gases. An accurate evaluation of any pulmonary complications that may result in hypoxia. When a pulmonary embolus is suspected, a CT scan or ventilation perfusion scan should be performed. Pulmonary compromise should be treated with oxygen supplementation. Thoracocentesis may be necessary for patients with significant hydrothorax. However, a dramatic improvement in the clinical status may occur after paracentesis.
Adult Respiratory Distress Syndrome (ARDS)

ARDS is encountered after fluid overload. The importance of a strict fluid input/output balance in patients with moderate complications of OHSS is stressed. Optimum management may require admission to an intensive care unit. ARDS subsides after 3 to 6 days with fluid restriction, forced diuresis, and dopamine therapy.

Pericardiocentesis

Pericardial effusion rarely occurs; if it does, drainage by specialists may be necessary [31].

SURGICAL TREATMENT

Anesthesia Considerations in OHSS Patients

Although surgery is needed only infrequently, when it is required there are several important considerations for the anesthesiologist [10]. Careful positioning of patients during surgery is important as the Trendelenberg position may further compromise the residual pulmonary functional capacity. Establishment of access lines may be necessary in patients with contracted vascular volume. Drainage of pleural effusions may assist in improving pulmonary status.

Surgery for Ruptured Cysts

Laparotomy, in general, should be avoided in OHSS. If deemed necessary, in cases of hemorrhage hemorrhagic ovarian cysts, it should be performed by an experienced gynecologist and only hemostatic measures undertaken so as to preserve the ovaries.

Ovarian Torsion

Ovarian torsion is an infrequent complication of ovulation induction, which, if unrecognized and untreated, results in the loss of one or both ovaries [38]. Presenting symptoms are severe unilateral adnexal pain, in a patient with enlarged ovaries due to ovulation stimulation or with multiple pregnancy. Sonography with Doppler flow analysis can be diagnostic, but a finding of apparently normal blood flow does not rule out ovarian torsion [38]. Although the adnexa usually appear dark, hemorrhagic, and ischemic, they can be saved by simply unwinding them if a timely diagnosis is made. This often can be performed as a laparoscopic procedure.

Surgery for Ectopic Pregnancy Associated with OHSS

The combination of OHSS and ectopic pregnancy is not encountered frequently. Diagnosis of tubal pregnancy by vaginal ultrasound examination at this stage is not always possible. The presence of large ovaries filling the pelvis makes ultrasound scanning of other structures difficult. Fluid in the cul de sac is of limited diagnostic importance in the presence of ascites.

Other Surgery

Mesenteric resection after massive arterial infarction has been reported. Rarely, vascular surgery is required to treat thromboses that are complicated by recurrent emboli or resistant to medical intervention. Posterolateral thoracotomy and subclavian arteriotomy and thromboarterectomy by the Fogarthy technique have been reported. Inferior vena cava interruption to prevent massive thromboembolism also has been used [10].

Pregnancy Termination

Pregnancy termination may be performed in extreme cases and has been reported to improve the clinical picture of neurological, hematological, and vascular complications [10].

CONCLUSION

OHSS is preventable in the majority of cases. However, there is a narrow margin in which severe cases may still occur, and this remains a challenge for the future [40].

EXPERT COMMENTARY

Ovarian hyperstimulation syndrome remains the most serious complication of ovulation induction. Without understanding the pathophysiology, the treatment will remain empirical in nature. Human chorionic gonadotropin is a central piece that triggers a cascade of events that result in an overproduction of VEGF. Decreasing the dose of gonadotropins and HCG and utilization of other agents such as dopamine agonists and metformin will help in the prevention. Successful prediction and active prevention is more helpful than active treatment.

FIVE YEAR VIEW

OHSS could be successfully prevented over the next five years if a high index of suspicion is exercised and methodical steps are taken for the prevention of the syndrome. Identification of these patients and judicious use of gonadotropins is the beginning of the right path. The use of insulin sensitizers and dopamine agonists will be more refined. Newer technologies as in vitro maturation might completely abolish the syndrome.

KEY POINTS

- Ovarian hyperstimulation syndrome (OHSS) is characterized by bilateral cystic ovarian xpe
- Severe OHSS may result in thromboembolism, adult respiratory distress syndrome, or kidney failure.
- Human chorionic gonadotropin increases VEGF production by granulose cells and endothelial cells, resulting in increased vascular permeability.
- The cornerstone of successful prevention of OHSS is accurate risk prediction.
- Previous history of OHSS or polycystic ovary syndrome is highly predictive of the development of OHSS during ovarian stimulation.
- Ultrasound is essential for the prediction of OHSS before, during, and after the treatment cycle.
- Necklace sign, baseline antral follicle count, and ovarian volume are strongly associated with OHSS.
• The presence of a large number of follicles (>20 per ovary) and an increase in the number of small and intermediate follicles are associated with an increase risk for the development of severe OHSS.

• Increased intraovarian blood flow and low intravascular ovarian resistance are correlated with the severity of OHSS in patients who develop the syndrome.

• The presence of multiple pregnancy increases the risk of the severity and duration of OHSS.

• Primary prevention of OHSS can be achieved by the use of low-dose gonadotropins and, in some cases, ovarian drilling prior to IVF.

• Secondary prevention of OHSS involves delaying hCG (coating), or, in some cases, cancellation of hCG.

• Medical treatment of OHSS involves correction of circulatory volume and electrolyte imbalance.

• Ultrasonographic guidance of transvaginal or transabdominal aspiration of ascites improves the symptoms of OHSS.

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Non-Surgical Treatment Options for Symptomatic Uterine Leiomyomas

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Abstract: Uterine leiomyomas (or uterine fibroids), benign tumors of the uterine smooth muscle cells, can cause significant morbidity and impair quality of life in many women. For those who are asymptomatic, watchful waiting constitutes appropriate management. For women with symptomatic fibroids, hysterectomy and myomectomy are traditionally the standard treatments. In the last two decades, many alternatives to surgery for the management of fibroids have been developed. Most of the current medical treatments involve hormone manipulation. Newer treatments such as mifepristone, asoprisnil and aromatase inhibitors show promising results in fibroid symptom and size reduction; however, larger studies are needed. A safe and effective therapy as an alternative to hysterectomy is uterine artery embolization (UAE), which offers shorter recovery times and fewer major complications, though fertility and pregnancy outcomes after a UAE may be affected. Limited data are available for the efficacy and safety of temporary uterine artery occlusion. Magnetic resonance-guided focused ultrasound surgery (MRgFUS) is the newest treatment option for uterine fibroids. It also shows promising results for symptom relief. The degree of symptom relief depends significantly on the amount of fibroid volume that is non-perfused after treatment and the experience of the physician performing the procedure.

Keywords: Fibroids, hormones, NSAIDs, progestins, oral contraceptives, mifepristone, asoprisnil, gonadotropin-releasing hormone agonists, uterine artery embolization, selective estrogen receptor modulators, transvaginal temporary uterine artery occlusion, magnetic resonance-guided focused ultrasound surgery.

INTRODUCTION

Uterine leiomyomas (uterine fibroids) are one of the most common benign tumors in the female reproductive tract. African-American women generally have a higher incidence of fibroids than white women [1]; current estimates indicate that more than 80% of African-American women and nearly 70% of white women will develop fibroids by the age of 50 [2]. Other risk factors commonly associated with fibroids include nulliparity, advanced age and obesity [3]. The growth of fibroids is hormone driven, as demonstrated by evidence that fibroids develop after puberty, fibroid growth increases with age, and fibroid size increases during the reproductive years and regresses after menopause [3]. Leiomyoma tissues are monoclonal in origin [3, 4]. Many have non-random chromosomal abnormalities such as deletions, translocations and duplication. The most common mutations involve regions in chromosomes 7, 12 and 14, which contain genes essential in cell proliferation, regulation of transcription, or cell cycle control [3, 4].

Fibroids are often discovered incidentally during a physical or radiological examination. For women who are asymptomatic, watchful waiting is an appropriate treatment option as fibroids are rarely malignant and pathology results are not required for the diagnosis. For many other women, however, uterine fibroids can cause significant morbidity and have a detrimental effect on their quality of life. The symptoms related to fibroids include menorrhagia, which leads to iron-deficiency anemia; pressure-related symptoms such as urinary frequency, pelvic pressure, dyspareunia and difficulty with urination or defecation; pain; and infertility.

The treatment for patients with fibroids depends on several factors such as the size, number and location of the fibroids, the severity of the symptoms, and the patient’s child-bearing concerns (Fig. 1). Medical treatment is often used as a first line therapy, although many women might later need surgical intervention. Hysterectomy and myomectomy performed by laparotomy have been the traditional therapies for women with symptomatic fibroids [5]. Hysterectomy is the most common procedure because it is the only definitive option and it eliminates the risk of recurrence. In fact, uterine fibroid is the most common indication for hysterectomy in the United States, and each year, more than 250,000 hysterectomies are performed due to symptoms related to fibroids [6]. Myomectomy is a traditional option for women who anticipate future child bearing; hysteroscopic myomectomy is an appropriate treatment for women with submucosal fibroids; and endometrial ablation is often the treatment of choice for women with menorrhagia who do not wish to undergo major surgery and do not desire to bear children.

In the last two decades, many new therapeutic approaches for symptomatic fibroids have become available (Table 1). Uterine artery embolization is a non-surgical approach that has been extensively studied and for which short- and long-term outcome data are available. New techniques such as magnetic resonance imaging (MRI)-guided focused ultrasound and temporary uterine artery occlusion are being extensively studied and show promising results. Further-
more, several new medications are being studied as a potential long-term treatment for fibroids. The clinical outcomes of the non-surgical approaches to fibroid treatment will be reviewed here.

Fig. (1). Fibroid Uterus.

Table 1. Currently Available Treatment Options for Leiomyomas

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<td>Magnetic resonance-guided focused ultrasound surgery (MRgFUS)</td>
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MEDICAL MANAGEMENT

A number of medical therapies are available for fibroid treatment. Oral contraceptives or non-steroidal anti-inflammatory drugs are often the first line of therapy to decrease pain and bleeding caused by fibroids, but these medications do not decrease the size of the fibroids. The available medical options that could decrease the size of the fibroids, such as GnRH agonists, have multiple side effects, so these options cannot be used long term. Thus, many patients still require surgical therapy. However, many new and promising options for medical treatment are currently being studied for potential long-term use.

COMBINED ORAL CONTRACEPTIVES AND PROGESTINS

Combined oral contraceptives have been used effectively to reduce blood loss in women with idiopathic menorrhagia. Since it is a non-invasive approach, many physicians prescribe oral contraceptives for women with fibroids and menorrhagia before turning to other more invasive options. There are conflicting reports regarding the effect of combined oral contraceptives on fibroids. A large prospective study including over 3,000 patients with fibroids found a positive correlation between fibroid diagnosis and the use of oral contraceptives at a very young age (prior to 17 years old) [7]. Other controlled studies, however, did not find any such relationship [8, 9].

One study found that using combined oral contraceptives containing a newer generation of progestins did not increase the size of the fibroids [10]. Progestins are an effective treatment for idiopathic menorrhagia, but data on the effectiveness of progestins for fibroid treatment is limited. In a study using GnRH agonists for fibroid treatment, an increase in uterine size was found in patients who used progestin for add-back therapy [11]. Another study, however, observed that progestin decreased fibroid size [12]. Due to the conflicting information regarding the effects of estrogen and progestins on fibroid growth, the uterine and fibroid size should be closely monitored during the course of treatment with combined oral contraceptives or progestins [13].

LEVONORGESTREL-RELEASING INTRAUTERINE SYSTEM

The levonorgestrel-releasing intrauterine system (LNG-IUS) releases a daily dose of levonorgestrel locally and continuously into the uterine cavity for at least 7 years. It has been used effectively to reduce blood loss in women with menorrhagia, and it is one of the most effective medical treatments [14]. The studies looking at LNG-IUS for fibroid treatment are limited to case reports and small case series. All of the studies have recruited women with fibroids who had a small uterus (<12 weeks size) and normal uterine cavity [15-20]. LNG-IUS was shown to significantly decrease the amount of bleeding and improve hematocrit in women with menorrhagia due to fibroids. The largest study, which included 46 women with fibroid-related menorrhagia, showed a significant decrease in menstrual bleeding at 3 months after the IUS insertion in all of the subjects. At 6 months, fifty-six percent of the women in the study had become amenorrheic and 22% were experiencing only spotting or oligomenorrhea [15]. The rest of the subjects in the study maintained a normal cycle [15]. No changes, however, in the fibroids or uterine volume were noted during the treatment [15, 16].

NON-STEROID ANTI-INFLAMMATORY DRUGS (NSAIDs)

Non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to be an effective treatment for idiopathic menorrhagia [21]. However, NSAIDs are ineffective in reducing blood loss in women with fibroid-related menorrhagia [22]. NSAIDs are often given to patients with fibroids to
reduce pain symptoms, although their effectiveness for pain relief in this population has not been documented [7].

**DANAZOL AND GESTRINONE**

The androgenic steroids danazol and gestrinone may be effective treatment options for symptomatic fibroids. Danazol is a derivative of 17α-ethinyltestosterone. It acts primarily by inhibiting the luteinizing hormone (LH) surge, thereby inhibiting ovulation and inducing amenorrhea. It also inhibits many of the steroidogenic enzymes and increases free testosterone levels. It has been used in the treatment of endometriosis and menorrhagia. Because danazol induces amenorrhea, it might have a role in reducing menorrhagia and anemia due to fibroids, although data on the effects of danazol on fibroids is very limited. A Cochrane review did not find any reliable evidence from randomized controlled trials showing either that using danazol is beneficial or harmful in the treatment of fibroids [23]. The side effects of danazol are related to its androgenic properties and include weight gain, muscle cramps, acne, hirsutism, hot flashes, fluid retention, fatigue, and decreased breast size.

Gestrinone is a 19-nortestosterone derivative. It has been used in Europe for the treatment of endometriosis but is not available in the United States. Gestrinone, starting at a dose of 2.5 mg three times weekly for 6-24 months, has been shown to decrease the size of fibroids and uterine volume [24, 25]. Most of the patients who used gestrinone for 6 months maintained their decreased uterine size (compared with the pretreatment size) 18 months after discontinuing the medication [24].

**GONADOTROPIN-RELEASING HORMONE AGONISTS (GnRH AGONISTS)**

GnRH agonists have proven to be effective in the treatment of fibroids, although mainly as short-term therapy for up to 3-6 months prior to surgery. Long-term use of GnRH agonists for fibroid treatment is limited because of the hypoestrogenic side effects. The continuous administration of a GnRH agonist causes initial hypersecretion of follicle stimulating hormone (FSH) and LH (flare effect), which lasts approximately 7-10 days, followed by a significant decrease in FSH and LH secretion due to pituitary GnRH receptor down regulation. This in turn causes a reduction in ovarian steroidogenesis and results in a hypoestrogenic state, which is likely the main mechanism causing the reduction in fibroid size. GnRH-agonist treatment also causes a reduction in the expression of several growth factor proteins such as transforming growth factor β, epidermal growth factor, insulin-like growth factor (IGF-I), and IGF II [26]. Microarray analysis has shown alteration in the expression of several genes within the myometrium and fibroid tissues after GnRH-agonist treatment [27, 28].

GnRH agonists have been shown to reduce both fibroid size and uterine size during the course of treatment, with a reduction in the fibroid volume of 35% to 65% noted within the first 3 months of treatment [26, 29]. Fibroid and uterine volume reductions usually occur during the first 3 months of treatment [30, 31]. In one randomized controlled trial, monthly injection of the GnRH agonist depot leuprolide acetate every 4 weeks decreased uterine volume by 36% at 12 weeks and 45% at 24 weeks [30]. Most patients become amenorrheic during the therapy; thus GnRH agonist treatment improves the hematocrit level in patients with anemia.

The effects of GnRH agonists are not sustained after the therapy is discontinued. Although symptomatic improvement still persists in many women [30, 31], the fibroids return to the pretreatment size within 4-6 months after therapy [31]. Long-term use of GnRH agonists, however, is limited by significant side effects, in particular osteoporosis, which may begin to develop after six months of the therapy. Other side effects of GnRH agonists include hot flashes, vaginal dryness, headache, arthralgia, myalgia, depression, insomnia, emotional instability and decreased libido. Nevertheless, most women who develop side effects during treatment tolerate them relatively well and continue with the duration of treatment.

Add-back therapy with estrogen and progestins has been used to reduce the side effects of GnRH agonists during long-term treatment – in particular, to inhibit bone loss. In one study, patients with symptomatic fibroids were randomized to receive add-back therapy with an estrogen-progestin combination or high-dose progestin after 3 months of injection treatment with leuprolide acetate, a GnRH agonist. Patients were continued on leuprolide acetate for a total of 2 years [11]. In both groups, uterine size decreased by 40% and bone mineral density decreased by 2.6% during the first 3 months of the treatment, but there was no significant change in bone mineral density at the end of the study. Interestingly, the uterine size in the progestin-only group increased up to 95% of pretreatment size, while there was no change in the uterine size in the estrogen-progestin group [11]. These results are likely due to the role of progesterone as a fibroid growth promoter.

Other medications have also been studied for use as add-back therapy during GnRH agonist treatment. Tibolone, a synthetic compound structurally related to 19-nortestosterone progestins, has been used to relieve postmenopausal symptoms and osteoporosis. A small randomized trial showed that daily tibolone add-back treatment combined with 6 months of leuprolide acetate treatment reduced vasmotor symptoms and prevented bone loss without compromising leuprolide’s ability to reduce fibroid size [32]. Another randomized controlled trial using tibolone as an add-back therapy during 6 months of goserelin treatment found that patients in the tibolone group had significantly less bone loss than those in the control group, but there was no significant difference in the reduction of fibroid volume in either group [33]. Long-term use of tibolone and leuprolide acetate for 2 years has also been found to reduce hot flashes and prevent bone loss without changing the lipid profile [34]. In another study, raloxifene, a selective estrogen receptor modulator, was administered daily during 18 months of leuprolide acetate treatment for fibroids [35]. After 18 months of treatment, a significant reduction in fibroid size was noted while the bone mineral density and bone metabolic markers remained unchanged from pretreatment levels.

Pretreatment with a GnRH agonist for 3-4 months prior to surgery has many benefits. A Cochrane Database Systemic Review evaluated the role of GnRH pretreatment prior
to hysterectomy and myomectomy [36]. Twenty-one trials were included in the analysis. The review showed significantly higher hemoglobin and hematocrit levels prior to and after the surgery in women who were pretreated with a GnRH agonist. Patients who used a GnRH agonist prior to hysterectomy also experienced significantly less blood loss and a shorter hospital stay than the patients who received no pretreatment, although there was no difference in the transfusion rate. Using a GnRH agonist prior to hysterectomy also allowed many women to undergo vaginal rather than abdominal hysterectomy due to the decrease in their uterine size and lead to shorter operating times. In addition, the decrease in uterine size due to GnRH agonist treatment allows surgeons to use a transverse incision instead of a vertical incision in many patients undergoing hysterectomy or myomectomy. The disadvantage of using GnRH agonist pretreatment prior to myomectomy is the resulting increase in the rate of fibroids recurring after surgery, which is likely because smaller fibroids were not seen or were ignored during the myomectomy. Results from the Cochrane review, however, did not have enough data to support this concept.

GnRH ANTAGONISTS

GnRH antagonists competitively bind to the GnRH receptor, causing an immediate reduction of gonadotropin secretion and a reduction of ovarian steroidogenesis without causing the initial flare effects seen with the use of GnRH agonists. GnRH antagonists have also been shown to directly induce apoptosis in uterine leiomyoma cells [37, 38]. A clinical study using daily subcutaneous injections of gercetrel, a GnRH antagonist, in 20 women with fibroids showed a rapid decrease in fibroid and uterine size (median duration of 19 days, range 1-65 days) [39]. During the first 3 weeks of treatment, the fibroid size was decreased by 42.7% (14% to 77%) and uterine size was decreased by 46.6% (6% to 78%). Another study using the GnRH antagonist cetrorelix also showed a rapid reduction in fibroid size by 16 days of treatment, with a mean fibroid size reduction of 16% [40]. Side effects of the GnRH antagonists are mainly related to hypogonadism. GnRH antagonists are usually administered in daily subcutaneous doses and currently, there are no longer-acting GnRH antagonists available, which make long-term usage impractical.

MIFEPRISTONE

Mifepristone (RU486), a selective progesterone receptor modulator, has been studied for many clinical implications including fibroids. In the presence of progesterone, mifepristone acts as a competitive inhibitor of the progesterone receptor. It also has an antiglucocorticoid property. The first report describing the use of mifepristone for the treatment of fibroids was authored by Murphy et al. [41]. In the study, 10 patients received 50 mg of mifepristone for 3 months. At the end of the study, a 49% reduction in fibroid volume was noted and all of the patients had become amenorrheic. Subsequent to this study, several smaller studies, with a duration of 3 months to 1 year, used different dosages of mifepristone, ranging from 5-50 mg, for fibroid treatment [42-49]. All of the studies revealed a decrease in uterine and fibroid volume ranging from 26% to 74%. A significant decrease in blood loss and a high incidence of amenorrhea were reported in all the studies. A recently study by Eisenger et al. used a much lower dose of mifepristone – 2.5 mg/day for 6 months – than in other studies [50]. An 11% reduction in uterine volume with significant reduction in pain and improvement in quality of life were noted at the end of the study.

The main side effect of mifepristone is vasomotor symptoms. Hot flashes are dose related; no hot flashes have been reported in patients using a 5 mg dose [46]. Mifepristone has no effect on bone density, which makes this medication more attractive than the GnRH agonists. The major concern for long-term use of mifepristone is the potential risk of endometrial hyperplasia from unopposed estrogen. The rates of endometrial hyperplasia range from 2% to 28% [43, 44, 46, and 50]. Two reports on studies using low doses (2.5 and 5 mg) of mifepristone show no evidence of endometrial hyperplasia [50]. In contrast, a report from Bavaria et al. using 10 mg revealed a much higher rate of endometrial hyperplasia (63%) compared with other studies [42]. One patient in the Bavaria et al. study also had complex hyperplasia. The available data available suggests that the incidence of endometrial hyperplasia is likely dose dependent. None of the endometrial biopsies conducted in any of the studies showed evidence of atypical hyperplasia or cancer.

Few randomized controlled trials using mifepristone for fibroid treatment are available. A study by Engman et al. randomized 30 women with symptomatic fibroids to receive 50 mg of mifepristone or a placebo every other day for 3 months [43]. Significant fibroid volume reduction averaging 28% was noted in the mifepristone group but not in the control group. Most of the patients who took mifepristone were amenorrheic after 4 weeks of treatment. There was no evidence of endometrial hyperplasia reported in this study. A double blind, randomized controlled trial comparing the use of 10 mg of mifepristone or a placebo daily for 3 months in 40 women also showed a 26%-32% reduction of uterine volume with mifepristone [42]. In the mifepristone group, 83% of the patients showed improvement in dysmenorrhea but still had other fibroid-related symptoms. As mentioned earlier, this study reported a much higher incidence of endometrial hyperplasia than other studies. A randomized study conducted by Carbonell Esteve et al. compared the efficacy of using daily doses of 5 mg and 10 mg mifepristone for 3 months for the reduction of fibroid volume in 100 women [44]. Both dosages showed comparable reductions in fibroid volume (57% and 45% in the 5 and 10 mg groups, respectively) and uterine volume (36% and 40% in the 5 and 10 mg groups, respectively). Only one patient (2%) in this study, from the 10 mg group, developed endometrial hyperplasia.

Further trials have studied the efficacy of long-term usage of mifepristone. A controlled study using 5 mg mifepristone daily for 6 months and a placebo in the control group showed significant reduction in fibroid volume without evidence of endometrial hyperplasia in the treatment group [45]. Another study comparing the use of 5 mg and 10 mg of mifepristone for 1 year showed a comparable volume reduction and amenorrhea rate in both groups [51]. The efficacy of mifepristone was sustained after treatment for 9 patients in this study, who maintained reduction of fibroids at 6 months.
after treatment [51]. The dosage of mifepristone currently available in the market is much higher (600 mg) than the dosage used in the aforementioned studies, thus limiting the use of this medication for fibroid treatment.

ASOPRISNIL

Asoprisnil is another selective progesterone receptor modulator that has shown some promising results as a medical treatment for fibroids. Unlike mifepristone, asoprisnil is not effective in inducing labor in animals [52]. Asoprisnil has an antiproliferative effect in the endometrium of both animals and humans [52]. *In vitro*, asoprisnil inhibits leiomyoma cell growth and induces apoptosis but has no effect on normal myometrial cell growth [53]. It up-regulates the extracellular matrix metalloproteinase inducer in cultured leiomyoma cells resulting in down-regulation of collagen synthesis [54]. It also down-regulates the expression of several growth factors, such as EGF, IGF-I and TGF-β cultured leiomyoma cells [55].

A very limited number of published studies have reported on the use of asoprisnil for fibroid therapy in humans. A phase one randomized trial comparing the use of different dosages of asoprisnil (5 – 100 mg per day) and a placebo for 28 days showed that asoprisnil delays the onset of the menstrual cycle and increases the cycle length [56]. This effect was observed in women using a dose of 10 mg or higher, and was dose dependent. The largest published study is a phase two, multi-centered, double-blind, randomized controlled trial including 129 patients who were randomized to use 3 different dosages of asoprisnil (5, 10, or 25 mg per day) or a placebo for fibroid treatment for 12 weeks [57]. A 36% reduction in fibroid volume was noted by week 12 in the 25 mg group. A significant decrease in uterine bleeding was observed in all three asoprisnil treatment groups, with the greatest effect seen in the 25 mg group. Amenorrhea was noted in 16%, 36%, and 70% of patients in the 5, 10, and 25 mg groups, respectively. At the same time, significant symptoms were noted in the 10 and 25 mg groups. While no evidence of endometrial hyperplasia was noted in any of the studies, two unique endometrial pathologies called ‘non-physiologic secretory effects’ and ‘secretory patterns, mixed type’ were seen in many patients who took asoprisnil [56-58]. These are characterized by the presence of weak secretory glands with minimal or absent proliferation and variable effects in the stroma [56].

The side effects of asoprisnil are mild, including bloating, flatulence, breast pain and vasomotor symptoms. All of the symptoms were reported as mild and none of the patients stopped the treatment due to side effects [57]. A decrease in uterine artery blood flow was seen in another study when subjects took 25 mg of asoprisnil for 12 weeks [59]. This might serve as one of the mechanisms by which asoprisnil reduces uterine and fibroid volume.

SELECTIVE ESTROGEN RECEPTOR MODULATORS (SERMS)

Data on the effects of SERMs in the treatment of fibroids is limited. Raloxifene has been used for fibroid treatment in several small studies with conflicting results. A study of 70 postmenopausal women with fibroids taking 60 mg/day of raloxifene for 12 months showed a significant decrease in mean uterine and fibroid size starting from 6 months of treatment [60]. Studies in premenopausal women, however, show conflicting results. A study by Palomba *et al.* randomized 90 premenopausal women with asymptomatic fibroids into three treatment groups: raloxifene at 60 mg/day, raloxifene at 180 mg/day, or a placebo. During the 6 months of therapy, no significant changes in uterine or fibroid size or the amount of uterine bleeding were noted among the three groups [61]. Another study by Jirecek *et al.* randomized 25 premenopausal women with fibroids to receive 180 mg/day of raloxifene (the same dose used in Palomba’s study) or no treatment for 3 months [62]. Interestingly, after 3 months of treatment, women in the raloxifene group showed a significant decrease in fibroid size when compared with those in the control group. Jirecek *et al.* speculated that the difference in the results of the two studies might be due to the difference in the average age of the subjects (36 years old and 40 years old in the Palomba and Jirecek studies, respectively) [61, 62]. A Cochrane Database Systemic Review of this topic found no evidence that SERMs reduce the size of fibroids or improve outcomes [63].

In conclusion, there is currently not enough information to conclude that SERMs have any benefit in fibroid treatment or improve outcomes in premenopausal women.

AROMATASE INHIBITORS

Aromatase, an enzyme that converts androgens to estrogen, is very important in the production of estrogen. Aromatase activity and its transcripts have been found in leiomyoma tissues, showing that these tissues can synthesize estrogen locally and promote their own growth [64]. The aromatase mRNA level is much higher in leiomyoma tissues than in normal myometrial tissue, especially in African-American women [65]. This might account for the higher prevalence of fibroids seen in African-American women. Since estrogen plays an important role in the pathophysiology of fibroids, and aromatase, which is essential to the production of estrogen, is found in fibroid tissues, aromatase inhibitors are a potential candidate for fibroid treatment.

Published data on aromatase inhibitors and fibroids is limited to case reports and small trials [66-68]. Two studies using anastrazole at 1 mg/day for 3 months in women with fibroids showed an average fibroid volume reduction of between 9.3% and 55.7% [67, 68], and most patients reported improvement in their fibroid-related symptoms. One of the studies noted a significant change in fibroid volume in women older than 40 years but found no change in women younger than 40 years. A study comparing fibroid treatment with letrozole (2.5 mg/day) with a GnRH agonist for 3 months revealed comparable effects in the reduction of fibroid volume in both groups [66]. No serious side effects were reported in any of these studies.

In conclusion, aromatase inhibitors are a promising medication for the treatment of fibroids. However, randomized controlled trials are needed to confirm the efficacy of this medication for this indication. The long-term effects of this medication in premenopausal women also need to be studied.
UTERINE ARTERY EMBOLIZATION

Uterine Artery Embolization (UAE) is a minimally invasive procedure performed by interventional radiologists. The procedure has been used since the 1970’s for the treatment of obstetric hemorrhage, but it was first utilized as a treatment for fibroids by Ravina et al. in 1995 [69]. Since then, it is estimated that more than 200,000 UAEs for fibroid treatment have been performed worldwide [70].

Of all the minimally invasive treatments for fibroids, UAE is the most thoroughly studied to date. The procedure involves passing an angiographic catheter into each uterine artery through the femoral artery and then injecting the embolization particulate agents to occlude the branches of the uterine arteries that supply the fibroids [71]. The embolization particulate agents normally used include polyvinyl alcohol, tris-acryl gelatin microspheres and gelatin sponge particles [72]. The purpose of the procedure is to significantly decrease the blood supply to the uterus and fibroids, causing permanent ischemia and necrosis of the fibroid tissues while having no permanent effects on normal myometrial tissues [73, 74]. The treatment causes the global reduction of fibroid volume and results in the improvement of the symptoms associated with fibroids. During the procedure, patients normally receive conscious sedation, although some centers use epidural or spinal anesthesia instead [72]. After the procedure, most patients experience moderate to severe pelvic pain for several hours due to ischemia and postembolization syndrome. Thus, most centers admit the patients overnight for pain management. Postembolization syndrome is a side effect generally occurring after the embolization of solid organs, likely from the immune response related to ischemia or degeneration [73, 75]. It occurs in up to 40% of patients who undergo UAE [74]. The symptoms of postembolization syndrome include diffuse abdominal pain, low grade fever, malaise, loss of appetite, nausea, vomiting and leukocytosis [74]. The syndrome is self limiting and lasts from a few hours to a few days [74, 75]. The treatment for the syndrome includes supportive management with anti-inflammatory and antipyretic drugs. Patients usually return to normal activity within 7 to 14 days [71, 76].

Complications after the procedure are few and mostly minor and transient. Data from the Fibroid Registry for Outcome data (FIBROID) registry, the largest prospective registry, which has collected data from more than 3,000 patients who underwent UAE at 72 sites, reports perioperative complications of 2.7% with a rate of major events of 0.66%. Those complications include pain, contrast/drug reaction, urinary retention, groin hematoma, nausea, vessel injury, device related and non-target embolization [76]. Spies et al. reported perioperative complications of 5% in 400 consecutively treated patients following UAE [77]. The FIBROID registry reported adverse events within 30 days after treatment at 26%, with reporting of major events at 4.8 % [76]. The most common major adverse events were inadequate pain control requiring an emergency room visit or readmission (2.1%) and vaginal expulsion of the fibroids (0.7%). Minor adverse events include hot flashes, vaginal discharge, infection, bleeding, headache and passing of fibroid tissues [76-78]. Transvaginal expulsion of the fibroid tissues is a common complication following UAE and sometimes requires surgery [70]. The most serious complication is intrauterine/pelvic infection requiring surgery, which occurs in 2.6% of patients [79]. There was no death reported in any of the large clinical trials. There were, however, 2 case reports of death after UAE due to septicemia [80, 81]. One letter to the editor reported a death after UAE due to systemic non-target embolization from an arteriovenous malformation within the fibroids and patent foramen ovale [82].

Short-term and mid-term outcomes of UAE have been reported by several large case series. Significant decreases in fibroid and uterine volume after treatment range from 40% to 70%. Most patients show improvement in menorrhagia and pain reduction associated with fibroids after the treatment [75, 78, 83-86], as well as a decrease in emotional and somatic concerns [87]. Patients who undergo UAE experience a very high rate of satisfaction (ranging from 85% to 95%) at different time points after the procedure.

Long-term outcomes of UAE are very encouraging. At 3 years after treatment, most patients in the FIBROID registry continued to have significant improvement in their quality of life and 85.68% of patients stated that they would recommend UAE to family members or friends [88]. Fourteen percent of the patients in the studies required additional treatments or repeat embolization to further alleviate their symptoms. Another study including 200 patients showed that 73% of the patients continued to have symptom improvement after 5 years of treatment [89]. If patients do not improve by 1 year or if they have large fibroids, symptoms may still remain at 5 years [89].

The Uterine Artery Embolization (UAE) versus Hysterectomy for Uterine Fibroids trial (EMMY) was a multicenter, randomized trial in which uterine fibroid embolization was compared with hysterectomy in 177 patients. At 24 months after treatment, 24% of the patients in the UAE group underwent hysterectomy [90]. The remaining patients in the UAE group continued to show improvement in health-related quality-of-life outcomes. Uterine and fibroid volume reduction in the UAE patients was 48.2% and 60.5%, respectively [90]. Walker et al. reported long-term clinical outcomes at 5-7 years after UAE in 172 women [91]. More than 80% of the patients continued to experience improvement in fibroid-related symptoms. Five percent of the patients monitored in this report became amenorrheic after UAE and 75% continued to have reduced menstrual flow after surgery. Overall, most of the patients (86%) remained “very satisfied” or “satisfied” with the procedure. Sixteen percent of the patients required additional procedures for fibroid treatment. Eighty-five percent of the patients reported improved quality of life and 88% of the patients would recommend the procedure to others.

COMPARATIVE STUDIES

A number of studies have compared the outcomes of UAE with those of surgical procedures. All showed comparable outcomes between UAE and the surgeries, although for some patients in the UAE group, the therapy failed and they later required additional treatment for their symptoms.

Most of the large studies have compared the outcomes between UAE and hysterectomy. A randomized trial by
Pinto et al. assigned 57 women to a UAE or hysterectomy group for symptomatic fibroid treatment in a ratio of two to one [92]. The controversial randomized method used by Zelen, in which women in the hysterectomy group were not informed about the study or alternative treatments, was used in this study. There was also some overlapping in the randomization in that 3 of the patients who underwent hysterectomy had undergone UAE first. The primary outcome of the study concerned the length of hospital stay. The results showed that the length of hospital stay for the UAE group was shorter than for the hysterectomy group by 14.1 days (1.59 and 5.85 days for the UAE and hysterectomy groups, respectively). Recovery time was also shorter for patients who underwent UAE compared with the hysterectomy patients (9.5 vs. 36.2 days, P<0.001). On the other hand, more patients in the UAE group developed complications after the procedure compared with those in the hysterectomy group. The complications in the UAE group, however, were minor (PES, pelvic pain, urinary tract infection), whereas the hysterectomy group experienced mostly major complications (surgical incision abscess, intra-abdominal abscess plus anemia, and urinary retention).

The EMMY trial (The Uterine Artery Embolization [UAE] versus Hysterectomy for Uterine Fibroids trial) was a multicenter, randomized trial that compared UAE and hysterectomy for treatment of symptomatic fibroids among 177 patients in the Netherlands [78, 93-97]. The primary endpoint of this study was avoidance of a subsequent hysterectomy in at least 75% of patients undergoing UAE. The secondary endpoints were symptom improvement in symptoms and reduction of uterine and fibroid volume. The study met the primary endpoint. At two years after UAE, 23.5% of the patients in the UAE group were required to undergo a hysterectomy; thus, 76.5% of these patients avoided hysterectomy [78]. Patients in the UAE group had a shorter hospital stay than the patients in the hysterectomy group (2.7 vs. 5.1 days in the hospital), and the overall recovery time was much shorter in the UAE patients [96]. Similar improvements in health-related quality of life were noted in both groups. At 2 years after treatment, most patients in both groups reported they were at least “moderately satisfied” (92% and 90% in UAE and hysterectomy groups, respectively) with their treatments [90].

HOPEFUL (Hysterectomy Or Percutaneous Embolisation For Uterine Leiomyomata), a multicenter retrospective study, compared the outcomes for over 1100 women who underwent UAE or hysterectomy in the UK [75, 79]. All of the patients were followed up 2 to 5 years postoperatively in the UAE group and up to 9 years in the hysterectomy group. At baseline, women in the UAE group were younger and were more likely to have had prior pelvic surgery than the hysterectomy cohort (P < 0.001). Obesity, co-morbidity, and history of pelvic surgery increased the risk of complications of the UAE. Patients in the UAE group developed fewer complications than patients who underwent hysterectomy: the odd ratio was 0.60 (95% CI 0.32–1.15) [75]. Interesting, although more women in the hysterectomy cohort reported symptom relief (95% vs. 85%, P < 0.0001), fewer women in that cohort (85%) said they would recommend the treatment to a friend, compared with 91% in the UAE group (P= 0.007) [75].

The Randomized Embolization versus Surgical Treatment for Fibroids (REST) trial randomized 157 patients in the UK in a 2:1 ratio to undergo either UAE or surgery (hysterectomy and myomectomy) for symptomatic fibroids [98]. The primary purpose was to compare the quality of life at 12 months after treatment. The study found no significant difference in health-related quality of life between the two groups at 12 months, although women in the surgery group had significantly greater symptom improvement scores than the UAE group. A high percentage of patients in both groups said they would recommend their treatment to a friend (93% and 88% in the surgery and UAE groups, respectively). The median hospital stay and the recovery time after procedure were shorter for the UAE group than for the surgery group. Twenty of the patients in the UAE group required additional therapy due to continued or recurrent symptoms, whereas only one patient in the surgery group required additional treatment.

A few studies have compared the outcomes of UAE and myomectomy. A study by Goodwin et al. compared the treatment outcomes of 149 patients who underwent UAE and 60 patients who underwent myomectomy [99]. The primary endpoint in the study was an improvement of at least 5 points in the uterine fibroid quality-of-life questionnaire score (UFQoS) from baseline to 6 months after the procedure. Significant and comparable improvement in UFQoS, menstrual bleeding, uterine volume, and overall quality of life were noted in both groups. Patients in the UAE group, however, had a shorter hospital stay (23.8 hr vs. 61.6 hr; P<0.0001) and a shorter recovery time (14.6 days vs. 44.4) than patients in the myomectomy group (P<0.05). At one year, only 1.7% of the patients in the UAE group required additional treatment.

A study by Mara et al. randomized 121 women with fibroids who wished to remain fertile into 2 treatment groups, one to undergo UAE and the other myomectomy [100]. The aim of the study was to compare the safety, efficacy and reproductive outcomes between the two treatments. The patients were followed for 2 years. There were no differences in the rate of treatment failure (10% in both groups) and early complications between the two groups. Patients in the myomectomy group required longer hospital stays than patients in the UAE group. Safety and efficacy in reducing symptoms were found to be comparable in the two groups. However, significantly more patients in the UAE group required additional intervention (32% vs. 3%), the most common reason being the persistence of large fibroids > 5 cm. The reproductive outcomes between the 2 groups also differed. This result is discussed in detail in the section below describing reproductive outcomes after UAE.

**REPRODUCTIVE OUTCOMES AFTER UTERINE ARTERY EMBOLIZATION**

Significant concerns after uterine artery embolization include amenorrhea and premature ovarian failure. The incidence of amenorrhea after UAE has been reported to range from 3% to 7% [86, 89, 101]. Most of the patients who developed permanent amenorrhea were women who were older than 45 years at the time of the procedure [86, 101-103]. The largest available data set comes from the FIBROID registry.
which shows evidence of amenorrhea in 7.3% of UAE patients [89]. Eighty-six percent of these women were 45 years of age or older [102]. The potential mechanism by which UAE causes premature ovarian failure is non-target embolization of utero-ovarian collateral circulation [71], which can cause a reduction in the ovarian blood supply, which in turn results in a decrease in the ovarian reserve or ovarian failure. To evaluate the effects of UAE on the ovarian reserve, a small number of studies have compared basal levels of FSH (an ovarian reserve marker) before and after the procedure [104,105]. All of the studies showed no evidence of a decrease in the ovarian reserve after the procedure, except in patients who were older than 45 years. One study comparing the FSH level and the antral follicle count before and after UAE in 20 women aged 33-39 years showed no difference in the before and after measurements [104]. Another study that compared the difference in FSH levels before and after UAE in 63 patients found, on the whole, no difference before and after surgery [105]. However, when the data were stratified by age, 15% of the patients who were older than 44 years (4 patients) had FSH in the menopausal range after surgery. A study by Healey et al. looked at the ovarian reserve prior to and after UAE and myomectomy and found no difference in the ovarian reserve prior to or after either procedure [106].

Data on pregnancy outcomes after UAE are limited to case reports and case series. Although uncomplicated pregnancies have been reported, several studies show an increased risk of pregnancy complications after UAE, such as miscarriage, malpresentation, postpartum hemorrhage, abnormal placentation and cesarean section [75,100,107-115]. In the HOPEFUL study, 27 women achieved 37 pregnancies after UAE [75]. Among these, 15 miscarriages, 2 ectopic pregnancies, 1 termination and 19 live births were reported. In the Ontario multicenter trial, 24 pregnancies occurred, of which 4 resulted in miscarriages, 14 in full-term deliveries, and 4 in preterm deliveries [110]. Of the 18 deliveries, 9 were vaginal deliveries and 9 were cesarean sections. Three patients with abnormal placentation were also noted, and one of them needed a cesarean hysterectomy because of severe postpartum hemorrhage.

In a report by Walker et al., 108 out of approximately 1200 women who underwent UAE tried to conceive [109]. Thirty three women became pregnant which resulting in 56 pregnancies. Of these, there were 17 (30.4%) miscarriages, 3 stillbirths and 33 deliveries. The cesarean section rate in this study was 72.7% and there were 6 cases of postpartum hemorrhage. It is difficult to conclude that the complications suffered by these women after UAE were related to the procedure alone, since fibroids themselves constitute a risk factor for pregnancy complications. Furthermore, many of these women were in the advance-reproductive age group and their age could have contributed to an increased risk of miscarriage.

A recent systemic review by Homer et al., using the data available in the existing literature, compared pregnancy outcomes in women who underwent UAE with pregnancy outcomes in an age-matched control group of women with untreated fibroids [116]. Two hundred and twenty-seven pregnancies in the UAE group were compared with over 4000 pregnancies in the control group. The study showed no differences in preterm labor, malpresentation, or intrauterine growth restriction between the two groups. However, the rates of cesarean section (66% vs. 48.5%) and postpartum hemorrhage (13.9% vs., 2.5%) were significantly higher in the UAE group. The miscarriage rate in the UAE group was also double that of the control group. (35.2% vs. 16.5%, OR 2.8, 95% CR 2.0-3.8) Since this was a retrospective review, it was difficult to account for confounding factors such as weight or parity. The patients in the UAE group were more likely to be symptomatic than patients who had not received any treatment and might have larger fibroids. Regardless of these limitations, the authors concluded that early pregnancies are significantly vulnerable after UAE, and their data supports the recommendation of ACOG that UAE is relatively contraindicated in women who desire future childbearing [117].

Mara et al. compared the reproductive outcomes of women who underwent myomectomy and UAE [100]. The patients were followed for up to 2 years after treatment. Of 121 patients in the study, 26 from the UAE group and 40 from the myomectomy group attempted to conceive after treatment. Thirteen women (50%) from the UAE group and 31 women (78%) from the myomectomy group became pregnant within 2 years after the procedures. Women who underwent UAE had a significantly lower pregnancy rate, lower delivery rate (19% vs. 48%, 6 and 19 patients), and higher miscarriage rate (64% vs. 23%, 9 and 6 patients) than women who underwent myomectomy in this study. Although the number of patients who became pregnant was very small, the authors concluded that myomectomy is a better choice of treatment for women who desire future fertility. A retrospective study by Goldberg et al. using all the data available from the literature compared pregnancy outcomes between women who underwent UAE and laparoscopic myomectomy [118]. They found 53 pregnancies after UAE and 139 pregnancies after laparoscopic myomectomy. The study found a higher incidence of preterm labor (OR 6.2%; 95% CR 1.4, 27.7) and malpresentation (OR 4.3%; 95% CR 1.0, 20.5) in the UAE group. While there was no significant difference in the rates of postpartum hemorrhage and spontaneous abortion between the two groups, the rates in both groups were higher than those found in the general population.

TRANSVAGINAL TEMPORARY UTERINE ARTERY OCCLUSION

Transvaginal temporary uterine artery occlusion, using a Doppler-Guided Uterine Artery Occlusion Device, is a new procedure originally developed to decrease blood loss during gynecologic surgeries. Its use for fibroid treatment has been described in a very small number of case reports and case series [119-121]. The procedure is based on the theory that fibroid tissues undergo necrosis after uterine ischemia—the same theory that underlies UAE. The device, known as the Flostat™ system (Vascular Control Systems, San Juan Capistrano, CA, USA – now owned by Johnson & Johnson), consists of a guiding cervical tenaculum, a transvaginal vascular clamp with integrated Doppler ultrasound crystals, and a small battery-powered transceiver that generates the Doppler sound. The clamp slides along the guiding tenaculum to...
the lateral vaginal fornices at the 3 and 9 o’clock cervical positions. Using the Doppler ultrasound signal, the right and left uterine arteries are identified. The clamp then advances further into the vaginal fornices and displaces the uterine arteries superior to the point of insertion into the uterus. When the clamp is closed, it occludes both of the uterine arteries against the cervix. For the treatment of uterine fibroids, the instrument is left in place for 6 hours and then removed. During the procedure, patients receive a paracervical block or epidural anesthesia [121]. Minimal pain has been noted during the procedure, with no pain medication required after the patient’s discharge from the hospital [122].

The published data on the use of this system for fibroid treatment is still preliminary. A case report by Vilos et al. showed a significant decrease in uterine fibroids and uterine volume of more than 44% in a patient treated with the instrument [122]. At three months after treatment, the patient reported significant improvements in her symptoms. A feasibility study by Hald et al. recruited eight patients for this procedure [121], of which only 4 patients had successful occlusion. The authors reported two cases of hydronephrosis, and 2 of the 4 patients required additional therapy for fibroid treatment.

This procedure has some advantages over UAE in that there is no radiation exposure, less pain after the procedure and no risk of non-target embolization. However, there is very limited data on both short-term and long-term outcomes on which to base a recommendation. Because the uterine arteries are occluded only temporarily using this instrument, the degree of fibroid ischemia is potentially less than in UAE. Thus, this treatment might not be favorable to UAE in the long term for this reason. Furthermore, the potential for urethral injury noted in the feasibility study needs to be addressed in larger studies.

MAGNETIC RESONANCE-GUIDED FOCUSED ULTRASOUND SURGERY

Magnetic resonance-guided focused ultrasound surgery (MRgFUS) has been used in the treatment of uterine fibroids since 2000. This technique combines the use of high-energy focused ultrasound technology with real-time MRI guidance. High-energy focused ultrasound is a thermal ablation procedure in which high-intensity ultrasound waves are transmitted from transducers outside the body to a small area within the targeted treatment region deep inside the body. Within seconds, the temperature of the targeted tissues rapidly increases, causing tissue necrosis, while the surrounding areas remain unaffected. MRI guidance provides accurate imaging of the tissues and surrounding organs, allowing extreme precision with planning, monitoring, and treatment. During treatment, MRI thermal imaging provides real-time temperature monitoring and thus assures the proper energy exposure in the target tissues and prevents injury to the surrounding organs. This technology is currently being used not only for uterine fibroids, but also for different types of tumors [123].

MRgFUS for the treatment of fibroids is normally done on an outpatient basis. During the procedure, the patient lies prone on the MRI table with her abdomen positioned over the ultrasound transducer. Conscious sedation is usually provided with intravenous medications given to the patient throughout the duration of the procedure. The treatment itself consists of a series of high-energy ultrasound pulses (sonications), each lasting approximately 20 to 40 seconds. The interval between each sonication is approximately 90 seconds to allow the tissue temperature to normalize. Multiple sonications are required over one to four hours to treat each fibroid, depending on its size. The patient normally returns home within 1 to 2 hours after the procedure. Post-procedure pain is usually manageable with over the counter analgesics. Patients typically return to normal activity within three days. The ExAblate 2000 (InSightec, Inc. Haifa, Israel) was the first MRgFUS system approved by the U.S. Food and Drug Administration in 2004 for the treatment of symptomatic fibroids in women who had completed childbearing.

Because MRgFUS has only recently come into use for fibroid treatment, only short-term outcome data are available in the literature. The treatment has been shown to improve fibroid symptoms, with the degree of improvement varying significantly according to the treatment volume. In an early study, Hindley et al. report a case series including 109 patients who underwent MRgFUS for fibroid treatment [124]. The protocol used in the study resulted in a treatment volume of approximately 25% and a mean fibroid volume reduction of approximately 13.5% at 6 months. Interestingly, despite the small degree of volume reduction, 79.3% of the patients experienced significant improvement of their symptoms as measured by the Uterine Fibroid Symptoms and Quality of Life Questionnaire score. The follow-up report from the same study showed that 51.2% of the patients who still remained in the study at 12 months (42 of 82 patients) still had significant improvement in their symptoms [125]. Nine serious adverse events were reported in the study, although only one patient had nausea after the procedure that was thought to be related to the surgery [124]. The rest of the adverse events were related to patients’ underlying conditions (fibroids) or treatment failure. Five percent of the patients reported minor skin burns after treatment, which were due to incomplete removal of the abdominal wall hair. Previous studies have shown an increased risk of minor skin burn from abdominal wall hair, likely from air becoming entrapped in the particular areas [126]. One patient reported leg and buttock pain from sciatic nerve palsy (which was shown in the MRI images to be in the far field of the sonication path). The patient was noted to have recovered completely from the symptoms, using only symptomatic management, at a 12-month follow-up visit. Since the incident, the treatment protocol has changed to ensure at least a 4 cm distance between the treatment area and any major nerve bundles that are situated in close proximity to the bone surface of the sacrum.

Later studies modified the treatment protocol to include a larger treatment volume. A case series by Fennessey et al. compared the outcomes of 96 patients who were treated with the original protocol with that of 64 patients who were treated with the modified protocol [127]. The modified protocol leads to a larger treatment area and longer treatment time. Repeat treatment, if needed, was also allowed within 2 weeks in the modified protocol. In both protocol groups, the greatest symptom improvement was noted at 3 months and
the effects were sustained up to the 12 month time point of the study. At 3 months, patients in the modified protocol group reported greater improvement in symptoms than the original protocol group, and 91% and 72% of patients in the modified and original protocol groups continued to report symptom improvement at 12 months. Thus, the degree of symptom improvement seems to be associated with treatment volume. At the end of the procedure, 28% and 37% of the patients in the original and modified protocol groups required alternative therapy.

The longest outcome report was authored by Stewart et al. [128]. In that study, 359 patients who were treated by MRgFUS were followed for 24 months. Because 4 different protocols were used to treat patients, the relationship between the outcomes and treatment protocols was standardized by the degree of treatment volume. As expected, patients who had higher treatment volume (>20%) had significantly more improvement in their fibroid symptoms than the patients with lower treatment volume (<20%) both at 3 months and beyond 3 months. This study also found that treatment volume has a significant effect on the number of patients who need additional therapy and the increase in hematocrit in women with anemia.

The advantages of MRgFUS are that it is a non-invasive, outpatient procedure, requiring no surgical incision, and results in a short recovery time after surgery compared with hysterectomy or myomectomy. Unlike UAE, after which most patients develop severe pain from post-embolization syndrome immediately after the procedure, post-MRGFUS pain can be managed with oral analgesics. MRgFUS has many limitations as well, including limits on the size, type, and location of fibroids that can be treated with this method [129]. The cost of treatment and the very limited availability, due to few treatment centers in the U.S., are also major limitations. The procedure cannot be performed if the patient has abdominal wall scar tissue or if the fibroids are too close to the neurovascular bundle or other vital organs (such as the bowel or bladder) or if there are structures (for example, bowel loop) that could interfere with the ultrasound beam. The procedure is also not suitable for patients with multiple fibroids due to the duration of the treatment, which takes 2 to 4 hours per fibroid. Thus, only 1 or 2 fibroids can be treated at a time. The procedure is currently limited to the treatment of six or fewer fibroids that are less than 10 cm in diameter. Smart et al. reported a protocol that involved pretreating patients who have fibroids larger than 10 cm with a GnRH agonist for 3 months prior to MRgFUS, with promising results [130]. In this study, patients who were pretreated with a GnRH agonist had a larger treatment area than the patients who did not receive a GnRH agonist. The authors hypothesized that GnRH agonists decrease the vascularity in the fibroids, which in turn contributes to the rate of temperature increase in the fibroids during MRgFUS treatment and increases the extent of the tissue necrosis [130].

The effect of MRgFUS in patients who desire future fertility is still not well studied. Thus far, there are only 4 case reports and one case series that have reported pregnancy outcomes after MRgFUS [131-135]. All of the case reports reveal patients who had vaginal delivery at term after MRgFUS [131-134]. In the case series, there were 54 pregnancies in 51 women who were treated with MRgFUS [135]. Forty-one percent (22 patients) of the pregnancies resulted in delivery and 20% were ongoing. Twenty-six percent of the pregnancies resulted in miscarriage (13% of those were elective terminations). Two patients had placenta previa whereas 12 of 22 patients delivered without complications, with all but one delivery at full term. One patient developed uterine atony and severe postpartum hemorrhage after a cesarean section due to breech presentation and intramural fibroids. Although the sample size was small, the pregnancy outcomes and complications are favorable in comparison to the pregnancy outcomes after UAE. Currently, the trial using MRgFUS for patients with fibroids who have infertility is ongoing and likely will provide us with more information in the future.

In conclusion, MRgFUS is a promising new technology for the treatment of fibroids that appears to be safe and effective. However, the procedure also has limitations, and long-term outcomes are not yet available.

EXPERT COMMENTARY

Currently, there are several treatment options available for fibroid treatment. Hysterectomy is the only definitive treatment for relief of fibroid symptoms and the only procedure that prevents the recurrence of fibroids. For symptomatic women who wish to preserve their fertility or simply do not want to undergo invasive surgical procedures, many minimally invasive or radiological procedures are available. Hormone and NSAID therapy are appropriate for first-line symptoms, particularly those related to menorrhagia and anemia, but they have no effect on the size of fibroids. GnRH agonists are effective in improving hemoglobin and hematocrit as well as in reducing uterine size. Due to the side effect of bone loss with long term use, GnRH-agonist usage is limited to short term use (between 3 to 6 months) for decreasing uterine and fibroid size prior to surgery. The levonorgestrel-releasing intrauterine system has been shown to increase hematocrit in women with menorrhagia due to fibroids who have a normal uterine cavity, although the presence of fibroids theoretically could increase the chances of expulsion of the device. Uterine artery embolization is a safe and effective alternative to hysterectomy. It provides a shorter recovery time and fewer major complications than hysterectomy. Patients, however, should be aware that approximately 20% to 25% of patients will later require further treatment due to treatment failure. MRgFUS has promising short-term outcomes, but data on long-term outcomes are currently not available. It is important that patients talk with their physicians about their treatment options and the relative risks and benefits of each procedure prior to undergoing any procedures or treatments.

FIVE-YEAR VIEW

Current therapy options for women with symptomatic uterine fibroids are primarily limited to mechanical methods of excision, ablation, or devascularization. With increased experience using conservative, non-surgical procedures to treat uterine fibroids, non-invasive technologies will continue to improve to expand eligibility, and allow safe and effective long-term resolution of fibroid-related symptoms.
The approach to fibroids will also benefit from a comprehensive approach, including the incorporation of evolving medical management options in the fibroid treatment armamentarium. Ultimately, the future of fibroid management may involve multimodal therapy that will allow early intervention to prevent growth of fibroids that are asymptomatic or reduce rate of fibroid recurrence after treatment.

As we continue to increase our understanding of the pathophysiology of fibroids, we are learning about several compounds involved in fibroid growth, which may provide therapeutic targets in the future. The influence on growth of leiomyoma cells is multifactorial. Although modulation of hormonal effects, for both estrogen and progesterone, on leiomyoma cells is an area of particular research interest, many studies show that there are other important cytokines and growth factors that play a critical role in the development of fibroids. Many of these growth factors are still being studied in cell culture systems or they are in early phases of clinical trials. However, these limited studies show therapeutic potential for inhibition of leiomyoma cell growth pathways or interference with collagen production as promising adjuncts to the non-hormonal management for fibroids [136].

Interferon-alpha (IFN-α) has been shown to inhibit leiomyoma cell growth in vitro [137]. One case report found persistent shrinkage of a single fibroid in a patient who received IFN-α for hepatitis treatment [138]. Pirfenidone, an antifibrotic agent being studied for treatment of pulmonary fibrosis, has been shown to inhibit DNA synthesis and inhibit mRNA production for collagen type I in leiomyoma cells and normal myometrial cell cultures in a dose-dependent manner [139]. A heparin-like compound, RG13577, has been shown to inhibit DNA synthesis of normal myometrial and leiomyoma cells, with the effect more pronounced in leiomyoma cells [139]. Halofuginone significantly inhibits cell proliferation, collagen production, and TGF β mRNA levels in both normal and leiomyoma cells [140]. This medication is not currently used in humans and its toxicity is unknown [136]. Pioglitazone, a peroxisome proliferation-activated receptor-gamma (PPARgamma) ligand, has also been shown to inhibit cell proliferation of both normal and leiomyoma cells in a dose-dependent manner [141]. In addition, various methods of gene therapy are currently being studied in animal models as a potential non-surgical treatment intervention for fibroids [142-144].

KEY ISSUES

• Several non-surgical treatment options for fibroids treatment are available.
• NSAIDs, progestins, and combined oral contraception are often prescribed to alleviate symptoms related to fibroids, although they do not decrease the size of fibroids.
• GnRH agonists are effective in decreasing fibroid and uterine size as well as increasing hemoglobin/ hematocrit in patients with anemia. Due to their side effects with long-term administration, they are used only short-term prior to surgery.
• GnRH agonist pretreatment prior to surgery allows many women to have a vaginal hysterectomy, which might not have been possible prior to treatment due to uterine size. The pretreatment might also allow surgeons to use transverse incision instead of vertical incision.
• Mifepristone and asoprisnil have been shown in small studies to be effective in reducing fibroid size and improving fibroid-related symptoms. Large randomized controlled trials are needed to confirm their efficacy.
• The levonorgestrel-releasing intrauterine system is effective in decreasing the amount of bleeding in women with fibroids who have a normal uterine cavity.
• Uterine artery embolization has been shown to be an excellent alternative to hysterectomy for the treatment of fibroids; resulting in shorter hospital stay, shorter recovery time, and fewer major complications.
• Approximately 20%-25% of patients who undergo UAE will require further treatment.
• The short-term and long-term outcomes of UAE are promising, with more than 80% of the patients still satisfied with their treatment 3 to 5 years after treatment.
• Pregnancy outcomes after UAE are limited. Although successful pregnancies have been reported, several reports showed increased risks of pregnancy complications in UAE patients when compared with the general population.
• MRgFUS is the newest minimally invasive treatment for fibroids. The short-term outcomes of this procedure are promising.

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Articles of special notes have been marked as:
• of interest
•• of considerable interest.

** This is an excellent review article about etiology and pathogenesis of fibroids.
* This large prospective study found a positive correlation between the diagnosis of fibroid and oral contraceptive used.
Non-Surgical Treatment Options for Symptomatic Uterine Leiomyomas

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Non-Surgical Treatment Options for Symptomatic Uterine Leiomyomas

Current Women’s Health Reviews,


[76] This is one of the large cohort study comparing hysterectomy and UAE.


[78] *This paper reviewed the short-term outcomes data in patients who underwent UAE from the FIBROID registry.


[81] *EMMY trial is another large randomized trial compared the outcome of UAE and hysterectomy.


[83] *HOPEFUL study is another large cohort study compared outcomes of UAE and hysterectomy.


Non-Surgical Treatment Options for Symptomatic Uterine Leiomyomas

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Surgical Management Options for Patients with Infertility and Endometriosis

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Abstract: Aim: Endometriosis is an important cause of infertility. The disease is both diagnosed and treated surgically. Its pathogenesis is not entirely known; however, retrograde menstrual flow and a pro-inflammatory state in the peritoneum are thought to support its development. Many studies have been done to help better assess the effects of the disease on fertility rates and how surgical removal of disease can improve these fertility rates. The aim of this study was to review the current literature on the effects of endometriosis on infertility and the benefit of surgical treatment for these patients.

Methods: Review of recent publications through Pubmed and the Cochrane data base.

Results: The effects of minimal and mild disease on infertility are debatable, and studies have shown inconsistent results. Surgery through laparoscopic removal has been shown to be beneficial for women with moderate and severe endometriosis; however, those with severe disease may not benefit as much. Removal of ovarian endometriomas by an excisional process appears to be superior to fenestration and coagulation for spontaneous pregnancy outcomes.

Conclusion: Laparoscopic removal of endometriosis is an important treatment option for patients with endometriosis-related infertility. Questions remain, however, and further research should be done on the effects of Stage I/II disease and bowel endometriosis on infertility.

Keywords: Infertility, endometriosis, laparoscopy, ovarian endometrioma.

INTRODUCTION

Endometriosis is the presence of ectopic endometrial glands and stroma outside the uterine cavity and it is frequently associated with infertility. The disease and its pathogenesis are not entirely understood, but continued research is helping to better understand this disease. Women can present with a spectrum of disease severity with the most severe disease leading to deep adhesions and alteration of normal anatomy. The main treatment options for infertile patients with endometriosis include surgical removal of disease and in vitro fertilization (IVF). The focus of this article is to briefly review the pathogenesis of endometriosis and review current literature on disease treatment for infertility with a focus on laparoscopy.

PATHOGENESIS

The most widely accepted theory to explain the development of endometriosis was described by Sampson in 1927. His theory of retrograde menstruation describes menstrual debris escaping through the fallopian tubes into the pelvis [1]. It has been found that patent fallopian tubes during the time of menstruation significantly increase the likelihood of finding blood in the peritoneal cavity [2, 3]. This increase in peritoneal blood is thought to be from refluxed menstrual blood; however, actual endometrial cells in the peritoneal cavity have yet to be identified [3].

The anatomic distribution of endometriosis seen in the abdomen and pelvis give additional support to the theory by Sampson [4]. Gravity appears to have an influence on location of endometriosis lesions with a greater proportion of endometriosis adhesions/deep implants being located in the pelvis, particularly the posterior (most dependent) pelvic compartment [5, 6]. Additional support is that locations of peritoneal fluid stasis show increased endometriosis implants when compared with other compartments in the peritoneal cavity. Specifically, the Pouch of Douglas, the region of the appendix, the region of the sigmoid colon, and the right paracolic gutter are areas in which peritoneal fluid can get trapped, and these regions correspond to areas where endometriosis lesions are commonly seen [4, 6, 7]. If retrograde menstruation is indeed an important step in the disease process, then it would seem understandable that regions exposed to increased menstrual blood would be more likely to develop the disease. Gravity would cause the majority of the blood to be deep in the pelvis, leading to more disease in the more dependent regions of the pelvis, and upper abdominal lesions could be formed with menstrual blood trapped in regions of peritoneal fluid stasis.
The mere presence of blood, however, is not the only causal factor leading to the development of endometriosis. Considering that women with and without the disease have been found to have increased blood in the peritoneal cavity during menstruation, other factors need to be present for endometriosis lesions to develop. Endometrial cells would need to attach to the peritoneal cavity. This attachment is thought to be due to abnormalities of both the endometrium as well as the patient’s immune system. Endometrial lesions need a vascular supply for support after attachment to the peritoneal layer. Increased vascularization has been documented in the red endometriotic lesions [8]. Angiogenic factors are thought to play a critical role in the increased vascularization and endometriosis disease process. In particular, vascular endothelial growth factor (VEGF) has been found in increased amounts in the peritoneal fluid of patients with endometriosis when compared with controls [9, 10].

Other endometrial abnormalities thought to lead to development of endometriosis include disorders of apoptosis. Apoptosis is thought to remove endometrial cells during the late secretory and menstrual phases of the menstrual cycle. In patients with endometriosis, apoptosis is decreased, leading to increased survival of endometriotic cells [11]. Matrix metalloproteinase (MMP) also has been implicated in the pathogenesis of endometriosis. This family of enzymes participates in normal tissue remodeling, and altered expression of MMPs has been noted in women with endometriosis [12]. Further research is needed to help clarify the roles of these endometrial abnormalities as they pertain to the development of endometriosis.

Immune response abnormalities also seem to play a role in the development of endometriosis. The immune system should provide a defense to the formation of endometriosis, and its alteration has been found in patients with the disease. Cytokines are associated with a pro-inflammatory state that is hypothesized to promote endometriosis implantation. Several studies have demonstrated increased levels of various cytokines in the peritoneal fluid of women with endometriosis [13–15]. However, other studies have failed to confirm these findings and further investigations are warranted [16].

Other areas of the immune system that have been studied in regards to their association with endometriosis include natural killer cells and macrophages. Macrophages specifically are thought to exacerbate inflammation, and studies have found increased number of macrophages in endometrial tissue in patients with endometriosis [17]. Studies continue to support the idea that women with an altered immune response leading to a pro-inflammatory state are more susceptible to developing endometriosis (Table 1).

### Endometriosis and Infertility

Endometriosis is a known cause of subfertility and infertility. Stage III/IV disease leads to infertility with adhesions and distortion of normal anatomy. There is more debate, however, on the extent of infertility caused by Stage I/II disease. Several studies have looked at the peritoneal fluid in these patients in the hopes of discovering a potential cause of decreased fertility. The increase in cytokines and inflammatory cells found in the peritoneum of these patients is thought to contribute to decreased fertility. The elevated cytokines seen are thought to disrupt the normal cell cycle, leading to increased infertility [18]. Other observations that have been noted include reduced sperm motility on exposure to interleukin-6 [19]. Increases in inflammation seen in the peritoneum of these patients can lead to an increase in oxidative stress and reactive oxygen species thought to further hinder fertility.

### Treatment of Stage I/II Endometriosis

As stated previously, some controversy exists regarding the association between minimal or mild endometriosis and infertility. Several studies give conflicting results when looking at fecundability of patients with minimal and mild disease. Some studies have shown no significant difference in fertility rates when comparing these patients to those with unexplained infertility [22, 23]. A more recent retrospective study looked at natural conception rates between women with Stage I/II disease and women with unexplained infertility. Patients were diagnosed by visualization of disease and no treatment was given. The study reports that these patients were followed for three years and found that patients without endometriosis had a significantly greater chance of pregnancy [24]. To add to the discrepancies, the removal of minimal and mild endometriosis has been shown to have mixed results in regards to fertility outcomes. Several studies have suggested a benefit for treating minimal and mild disease for both natural cycles and cycles using controlled ovarian hyperstimulation and intrauterine insemination [25, 26]. Others, however, have failed to replicate these results [27, 28]. Several of these studies are flawed, however, due to their retrospective nature and small study numbers.

Two groups have studied the effect and treatment of minimal and mild endometriosis in larger prospective studies...
in an attempt to settle this debate. A Canadian group first published its result on infertility associated with minimal and mild disease. The group’s first objective was to look at the effects of mild and minimal endometriosis on fertility. Women enrolled in the study underwent diagnostic laparoscopy for infertility. The first part of the study looked at women with Stage I/II endometriosis that had only diagnostic laparoscopy without any treatment or disease removal. These women were compared with women with unexplained infertility (no endometriosis found on laparoscopy). The women were followed for 36 weeks after laparoscopy to compare pregnancy rates between the two groups. In total 168 women with minimal and mild endometriosis were compared with 263 women with unexplained infertility. The probability of becoming pregnant and carrying the pregnancy to 20 weeks gestation did not differ statistically between the two groups [29].

The same researchers then looked at the effects of treating minimal and mild endometriosis on fertility outcomes. This portion of the study compared women randomized to laparoscopic treatment of disease with laparoscopic diagnosis only. This study compared 172 women with excision or ablation of disease to 169 women in the diagnosis only group and again followed them for 36 weeks. Removal of disease was associated with a clear advantage to patients. Treatment increased the cumulative probability of pregnancy by 73% with 30.7% of the treatment group achieving pregnancy and 17.7% of the diagnosis group achieving pregnancy in the 36 week follow up period [30].

The second prospective randomized trial to examine the effects of treatment on Stage I/II endometriosis came from a group in Italy. Women were randomized at time of laparoscopy to treatment or diagnosis alone and followed for one year postoperatively. Unlike the Canadian study, the study in Italy failed to show that surgery was beneficial to women with minimal and mild disease. In the treatment group, 12/51 (24%) women achieved pregnancy compared with 13/45 (29%) of the no-treatment group. This was not statistically significant. After spontaneous abortions, only 10/51 (20%) of the treatment group and 10/45 (22%) delivered. This again was not significant [31]. Due to the discrepancies found in these two studies, additional, larger studies are needed to help clarify the impact minimal and mild endometriosis have on fertility.

**Treatment of Stage III/IV Endometriosis**

There is less debate surrounding the effect of moderate to severe endometriosis on infertility. Dense adhesions/fibrosis can lead to a loss of normal anatomy and obvious hurdles in achieving pregnancy. The best treatment for these patients to give them the highest chance for success, however, is still being investigated.

Laparoscopic removal of disease can be utilized to treat infertile patients. Early studies reported a benefit to laparoscopic removal of severe disease in regards to fertility outcomes [32, 33]. Although technically challenging, laparoscopic removal is minimally invasive and cost-effective and is the preferred treatment method for moderate and severe disease [34]. However, pregnancy rates still may remain low after laparoscopic removal, especially for patients with the most severe disease. One prospective study found the pregnancy rate for infertile women after laparoscopic removal of Stage III disease to be 44%. Women in the same laparoscopic study with Stage IV had a pregnancy rate of only 16.7% after disease removal [35].

Some women with severe endometriosis will have bowel involvement. Bowel resections have been done for these patients to relieve pain symptoms associated with the deeply infiltrating disease, but the utility of bowel resection in regards to improving fertility outcomes in these patients is unclear. Few studies have started to look at fertility outcomes in this subgroup of patients with severe disease. One retrospective study looked at pregnancy rate and live birth rates after bowel resection. In this study, 22 women desired pregnancy after bowel resection, and 10 of these women achieved pregnancy after surgery with a median time to conceive after surgery of 8 months. Although this small study showed promising results for fertility outcomes in patients after bowel resection, it also discussed potential complications. Two patients in this study had complications. One developed a rectovaginal fistula and the other developed a pelvic abscess. Both of these women required further surgery, reminding us that bowel resection is not a benign surgery [36].

Another prospective non-randomized study compared pregnancy rates in infertile patients after bowel resection by either laparotomy or laparoscopy. This study found improved outcomes in the group that underwent laparoscopic surgery with 57.6% of the women in the laparoscopic group becoming pregnant compared with only 23.1% of the women in the laparotomy group [37]. Another interesting study looking at the influence of bowel endometriosis on fertility compared three groups of patients: Group A had surgery for endometriosis with colorectal resection, Group B had surgery for endometriosis and evidence of endometriosis in bowel but did not have a colorectal resection, and Group C had surgery for moderate or severe endometriosis with no evidence of disease in the bowel. These women were then followed for four years with pregnancy rate being a primary outcome. Pregnancy rate was found to be significantly lower in the group that had bowel endometriosis present (Group B). The monthly fecundity rate in Group A was 2.3%, Group B 0.84% and Group C 3.95% [38]. These studies appear to indicate that removal of bowel endometriosis may not only benefit a patient’s pain symptoms, but may also improve their chances for successful pregnancy.

Although surgery for infertility related to moderate-severe endometriosis has been shown to improve fertility outcomes, many patients will not achieve success after surgical resection. Pregnancy rates are the highest in the months immediately following surgery [39]. Many physicians will use one year as the time point for additional intervention if pregnancy has not occurred. Six months also can be used, especially in older patients. Options for the patient at that point include re-operation or IVF. Two studies have compared these choices. One looked at women who had laparotomies for endometriosis and compared those who underwent repeat laparotomy verses those who underwent...
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IVF. Pregnancy rates in this study were low and only one cycle of IVF was compared, but this study found a 22% chance of achieving pregnancy within one year of re-operation and a 12% chance of pregnancy after one cycle of IVF [40]. Another study looking at laparoscopic reoperation versus IVF found cumulative pregnancy rates to be 5.9%, 18.1%, and 24.4% at 3, 7, and 9 months out from reoperation. This was significantly lower than the pregnancy rate after two cycles of IVF (69.9%) [41]. In the absence of other symptoms, moving to IVF may be more beneficial for patients than repeat surgery.

Treatment of Ovarian Endometriomas

One area that has been studied extensively is the effect of ovarian endometriomas on fertility and infertility treatments. Ovarian endometriomas can usually be easily detected with transvaginal ultrasound. Several studies have looked at the risks and benefits of removing these masses for both spontaneous pregnancies and prior to IVF. A potential risk of removing endometriomas would be incidental removal of normal ovarian tissue. Ovarian endometriomas can be more challenging to remove than other ovarian cysts. That being said, the removal of normal ovarian tissue along with the cyst has been shown to be more likely when removing endometriomas compared with other ovarian cysts [42]. In addition to loss of normal ovarian tissue, removal of ovarian endometriomas also potentially can lead to decreased blood flow to the ovary if damage to its vascular supply occurs during the removal. Loss of normal ovarian tissue or decreased vascular supply to tissue can lead to a decreased ovarian response during controlled ovarian hyperstimulation. Alternatively, one could leave the endometrioma and proceed to IVF. Somigliana et al. looked at women with one ovary affected by at least one endometrioma during IVF stimulation and found that larger endometriomas or multiple endometriomas resulted in a decreased response in the ovary with disease [43].

Several other retrospective studies have looked at the effects of ovarian endometriomas on IVF cycles with mixed results. Some studies suggested that removal of endometriomas did not affect the number of follicles obtained during IVF cycle [44, 45]. This is in contrast to other studies that have found a significant reduction in the number of follicles generated after laparoscopic cystectomy for endometriomas [46]. A prospective randomized trial compared women with endometriomas of 3 - 6 cm during IVF cycles. They were randomized into two groups: Group I underwent cystectomy prior to IVF; Group II had no surgical intervention prior to IVF. They found that Group I had more stimulation and had less oocytes retrieved then the group that did not have surgery. However, there was no difference in fertilization, implantation, or clinical pregnancy rates between these two groups [47]. Larger prospective studies should be done to help clarify the mixed results seen regarding the effects of endometriomas and their removal prior to IVF.

After deciding to operate on an ovarian endometrioma for fertility treatment, a choice should be made on the treatment method to be used. A Cochrane review was published comparing excisional versus ablative treatment of endometriomas that looked at both spontaneous pregnancy outcomes and controlled ovarian hyperstimulation [48]. The review included three randomized, controlled trials that evaluated the two laparoscopic surgical techniques on patients with ovarian endometriomas of at least 3 cm [49-51].

Two prospective studies in the Cochrane review considered the effect of these two surgical techniques on spontaneous pregnancy outcomes [49, 50]. In one study of 62 infertile patients with endometriomas, 32 were treated with cystectomy, and 30 were treated with fenestration and coagulation. Patients were followed for one year during which no infertility medications or procedures were administered or performed. The group that had cystectomies had a significantly higher pregnancy rate (59.4%) compared with the group that underwent fenestration and coagulation (23.3%) [49]. The other study included in the Cochrane review also compared spontaneous pregnancy outcomes after laparoscopic cystectomy versus drainage and coagulation of endometriomas. This was a smaller study that compared 9 infertile patients in the cystectomy group to 17 patients in the coagulation group. Pregnancy rates were monitored for 24 months, and the cystectomy group exhibited a statistically higher pregnancy rate (66.7%) than the coagulation group (23.5%) [50]. The Cochrane review used these two studies to conclude that overall spontaneous pregnancy rates favor excision with an odds ratio of 5.21 [48].

The same Cochrane review also compared follicular response to ovaries during controlled hyperstimulation after cystectomy versus fenestration and coagulation. A single prospective study compared fenestration and coagulation versus cystectomies prior to controlled ovarian hyperstimulation. In one arm of the study, patients had bilateral endometriomas with one removed by cystectomy and one by fenestration and coagulation. Patients in the other two arms had one endometrioma and were randomized to be treatment by cystectomy or by fenestration and coagulation. This study failed to find a significant difference in the number of follicles obtained regardless of treatment method. Neither was there a difference in the number of follicles obtained between treated ovaries and normal ovaries that did not require surgery [51]. The Cochrane review stated that evidence is insufficient at this time to state which surgical technique should be employed prior to controlled ovarian hyperstimulation [48].

CONCLUSION

Endometriosis is believed to arise from a combination of menstrual blood refluxed through the fallopian tubes and a peritoneal environment that is supportive of its implantation. An alteration of immune response leading to a pro-inflammatory state is thought to promote its development. Endometriosis is classified based on the extent of disease found during surgery. Stage I and II are also termed minimal and mild disease, and their effects on infertility remain controversial. Further prospective studies should be done to help clarify the role of minimal and mild disease in sub- and infertility and what effects removal of disease has on fertility outcomes.
Unlike Stage I/II disease, clear evidence supports the negative impact that Stage III/IV disease has on fertility. Moderate and severe endometriosis can lead to extensive adhesions and deep infiltrating disease. Surgical removal of Stage III/IV disease will improve fertility rates; however, those with the most severe disease may not benefit as much with surgery alone. Recent studies have suggested the removal of bowel endometriosis may also improve fertility outcomes in this subset of patients, although studies remain limited on this subject. Finally, large or multiple endometriomas can lead to a decreased ovarian response in controlled ovarian hyperstimulation. Further studies need to be done to determine what surgical technique for endometrioma removal will give the best results during controlled ovarian hyperstimulation. On the other hand, it has been shown that removal of endometriomas by excisional procedures appears to be superior to ablation of endometriomas in improving spontaneous pregnancy outcomes. It is clear from the studies reviewed here that laparoscopy has a significant role for the treatment of endometriosis-related infertility. Many questions remain, however, and continued research is needed to help specify these roles so that the best recommendations can be offered to this patient population.

EXPERT COMMENTARY

This article’s intention was to briefly highlight the pathogenesis of endometriosis and then review current clinical evidence for treatment of this disease in regards to improving fertility outcomes. We still have much to be learned about the disease mechanism, and continued research will not only help understand this disease but also will help formulate improved treatment options. Additional investigations are needed on the molecular level to help clarify the roles of various cytokines and inflammatory cells. This will give us a better understanding of why endometriosis occurs in some women and may lead to prevention and treatment options for these patients.

In addition to improving our understanding of disease on the molecular level, further clinical studies will help us provide optimal management for our patients with endometriosis. Additional studies are needed to clarify the role of minimal and mild endometriosis on infertility. Another area of interest for future research involves assessing the effects of bowel endometriosis on infertility. Larger studies should be done to validate whether removal of bowel endometriosis can improve fertility rates. As additional knowledge is gained through continued clinical and in vitro studies, improved outcomes for these patients should be achieved.

FIVE YEAR VIEW

As stated previously, continued research is being done on the molecular level to help better understand this disease. Potential benefits of this work could include possible serum markers to help identify patients with endometriosis. This would be significantly less invasive than the surgical diagnosis that is needed to diagnose the disease today. Improved prediction models can not only help identify patients with endometriosis but also could potentially be used to follow patients after treatment. Elevated markers potentially could alert the physician to disease recurrence. Current trends are moving towards less invasive procedures, and serum markers have the potential to play an important role in the diagnosis and management in endometriosis patients.

As treatment evolves in this direction, the role of diagnostic laparoscopy for infertile patients is becoming uncertain. Specifically, the value of diagnostic laparoscopy for patients that do not suffer from pain and have normal imaging studies is in question. Limited data currently support the removal of minimal and mild endometriosis for infertility. Due to the controversial role that Stage I/II endometriosis have on infertility, recommendations are moving away from performing diagnostic laparoscopies on infertile patients. Ultimately, this will lead to less surgery for these patients and increased medical management for patients with infertility-related endometriosis.

KEY ISSUES

- Mixed data on the effects of treating Stage I/II endometriosis.
- Pregnancy rates are highest in the months immediately following surgery.
- Removal of bowel endometriosis may improve fertility rates.
- If surgery fails to restore fertility, IVF may yield a higher success rate.
- Cystectomy for endometriomas gives superior natural pregnancy results.
- Mixed data on the effects of endometriomas or their removal prior to IVF.

REFERENCES


Surgical Strategies for Fertility Preservation in Women with Cancer

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Abstract: Survival has significantly improved for women diagnosed with cancer during the reproductive years. The majority of these women will desire children after completion of their cancer therapy. Future fertility often is eliminated or impaired by surgical removal of reproductive organs for the treatment of gynecologic cancers or by radiation or chemotherapy for the treatment of other cancers. As a result, several strategies have been developed in an attempt to preserve fertility in these women, including alternate surgical approaches, protecting reproductive organs during treatment, and removing and storing oocytes prior to cancer treatment for future use. Surgical approaches used in women with early gynecologic cancers include unilateral oophorectomy for ovarian cancer, radical trachelectomy for cervical cancer, and progestin therapy to avoid surgery for endometrial cancer. Ovarian function and fertility can be preserved in women requiring pelvic irradiation by ovarian transposition away from the treatment field. In women requiring systemic chemotherapy, surgical removal of ovarian tissue followed by cryopreservation and subsequent transplantation after the patient is determined to be in remission has been shown to restore ovarian function and result in pregnancy. To provide the best chance of future fertility, these surgical approaches to fertility preservation must be implemented as part of the initial cancer therapy, prior to definitive radiation or chemotherapy.

Keywords: Fertility preservation, gynecological cancers, ovarian transplantation.

INTRODUCTION

Survival has significantly improved for women diagnosed with cancer during the reproductive years primarily as a result of significant advances in the field of cancer treatment [1]. The overall cancer death rates for women decreased by 11.4% from 1991 to 2005, particularly because of advances made in the diagnosis and treatment of breast and colorectal cancers. Between 1991 and 2005, more than 160,000 cancer deaths were averted in women in the United States [1].

Approximately 70% of reproductive-aged women diagnosed with cancer during the reproductive years primarily as a result of significant advances in the field of cancer treatment [1]. The overall cancer death rates for women decreased by 11.4% from 1991 to 2005, particularly because of advances made in the diagnosis and treatment of breast and colorectal cancers. Between 1991 and 2005, more than 160,000 cancer deaths were averted in women in the United States [1].

Approximately 70% of reproductive-aged women diagnosed with cancer during the reproductive years primarily as a result of significant advances in the field of cancer treatment [1]. The overall cancer death rates for women decreased by 11.4% from 1991 to 2005, particularly because of advances made in the diagnosis and treatment of breast and colorectal cancers. Between 1991 and 2005, more than 160,000 cancer deaths were averted in women in the United States [1].

The increasing need to consider fertility preservation in these women is well appreciated. The American Society of Clinical Oncology recommends that cancer patients in the reproductive years be counseled about fertility preservation [4]. The President’s Cancer Panel released a strong recommendation addressing the need for more research on fertility preservation [5].

Several strategies for fertility preservation have been developed for reproductive-age women diagnosed with cancer. These strategies include 1) altering surgical treatment of gynecologic cancer to preserve fertility, 2) protecting reproductive organs during radiation or chemotherapy, and 3) removal and cryopreservation of ovarian tissue prior to cancer treatment for future use. Surgical approaches used in women with early gynecologic cancers include unilateral oophorectomy for ovarian cancer, radical trachelectomy for cervical cancer, and progestin therapy to avoid surgery for endometrial cancer. Ovarian function and fertility can be preserved in women requiring pelvic irradiation by ovarian transposition away from the treatment field. In women requiring systemic chemotherapy, surgical removal of ovarian tissue followed by cryopreservation and subsequent transplantation after the patient is determined to be in remission has been shown to restore ovarian function and result in pregnancy. To provide the best chance of future fertility, these surgical approaches to fertility preservation must be implemented as part of the initial cancer therapy, prior to definitive radiation or chemotherapy.

GYNECOLOGIC CANCERS: SURGICAL TREATMENTS THAT PRESERVE FERTILITY

Treatment for most gynecologic cancers includes removal of the uterus, ovaries, and tubes, often rendering the patient sterile. However, for reproductive-age women with early disease, fertility-sparing surgery sometimes is possible. This section will address fertility-preserving surgical approaches to cancer of the cervix, ovary and endometrium (Table 1).

Cervical Cancer

Cervical cancer is the most common gynecologic cancer diagnosed in the reproductive years. Although cervical cancer accounts for only 16% of all cancers in women, 40% are diagnosed before the age of 45 [6]. In the United States, the age-adjusted incidence rate is approximately 8 per 100,000...
Early invasive cervical cancer is radical trachelectomy, another fertility-sparing approach for patients with very low birth weight infants [12, 13].

This approach increases the risk of both pre-term delivery and other complications, including a recent large meta-analysis, have suggested that cone biopsy on obstetric outcomes. While some studies reported no increase in adverse outcome [10, 11], other studies, including a recent large meta-analysis, have suggested that this approach increases the risk of both pre-term delivery and low birth weight infants [12, 13].

### Standard Therapy

Therapy for cervical cancer depends on the extent of the disease (i.e., stage) at diagnosis. Approximately 50% of women with cervical cancer will be Stage II-IV, where the cancer is found to have spread beyond the cervix, either to adjacent structures, to regional lymph nodes, or to more distant sites [6]. Standard therapy for most of these women is a combination of chemotherapy and radiotherapy.

The remaining women will be found to have Stage I cervical cancer, where the disease is confined to the cervix [6]. Surgical therapy for women with this condition who no longer desire fertility is radical hysterectomy with pelvic lymphadenectomy [3]. Cases have been reported in which women who have undergone this procedure have had children utilizing in vitro fertilization (IVF) and the assistance of a surrogate mother to carry the pregnancy [7, 8]. Depending on recurrence risk factors, adjuvant pelvic radiotherapy may be administered after radical hysterectomy. However, the ovary has poor tolerance to radiation, and standard pelvic radiotherapy doses of 45 to 50 Gy will reliably induce premature ovarian failure. Successful surrogate pregnancies have been reported when either oocytes were removed from the ovaries or the ovaries were surgically transposed out of the pelvic radiation field at the time of radical hysterectomy [7, 8].

### Fertility-Sparing Surgery

#### Cone Biopsy

Observational data suggest that lesions meeting criteria for microinvasive cervical carcinoma, designated as “Stage IA1,” have a negligible risk for lymphatic metastasis and can be excised using less radical procedures, such as cold knife conization [9]. Controversy exists regarding the impact of cone biopsy on obstetric outcomes. While some studies report no increase in adverse outcome [10, 11], other studies, including a recent large meta-analysis, have suggested that this approach increases the risk of both pre-term delivery and low birth weight infants [12, 13].

#### Radical Trachelectomy

Another fertility-sparing approach for patients with very early invasive cervical cancer is radical trachelectomy, where the cervix is removed along with the surrounding vaginal and parametrial tissue while preserving the uterine fundus [14-17]. This approach might be used for smaller, cervix-confined lesions, such as lesions otherwise meeting criteria for microinvasion but are accompanied by lymphovascular invasion, Stage IA2 lesions; and Stage IB1, visible lesions < 4 cm confined to the cervix with superficial invasion and no vascular space invasion [9].

Radical trachelectomy can be performed using an abdominal approach combined with pelvic lymphadenectomy to exclude lymphatic spread [18]. Alternatively, this procedure can be performed vaginally with the addition of laparoscopic lymphadenectomy [19]. After excision of the cervix and parametria, a permanent cerclage is typically placed and the vaginal mucosa is re-approximated to the uterine stump.

The recurrence and survival rates for this approach appear to be similar to traditional radical hysterectomy in appropriate early-stage patients. Three studies with a total of 151 patients followed for 23-60 months reported acceptable recurrence rates of 2.8% to 5% [15, 20, 21]. Data focusing on oncologic outcomes in women with larger cervical lesions is more limited.

Radical trachelectomy is often effective in preserving fertility, although obstetric complications are increased. While first trimester miscarriage rates are similar to that seen in the general population, rates of both second trimester losses and pre-term delivery are significantly increased in patients who have undergone trachelectomy. In a review of 520 patients treated with radical vaginal trachelectomy, 43% attempted to conceive after surgery and 70% of these women achieved pregnancy [22]. Of these 227 pregnancies, 21% ended in first trimester miscarriage and 8% in second trimester miscarriage, 21% were born prematurely, and 50% were born at < 36 weeks. As many will have a permanent cerclage placed at the time of their trachelectomy, delivery by elective cesarean section is typically warranted.

### Ovarian Cancer

Ovarian cancer was ranked as the fifth leading cause of cancer deaths in the United States in 2009 [1]. Although the peak age of occurrence of ovarian cancers is after menopause, up to 17% of all ovarian cancers occur in women <40 years of age [23]. Many women in this age group wish to preserve their reproductive potential and ovarian endocrine function.

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**Table 1. Fertility-Sparing Surgery for Reproductive-Age Women with Gynecologic Cancers**

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Standard Therapy</th>
<th>Fertility-Sparing Approach</th>
<th>Requirements</th>
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| Cervical      | Total abdominal hysterectomy, bilateral salpingoophorectomy | 1. Cone biopsy  
2. Radical trachelectomy                                      | 1. Stage IA1  
2. Stage IA2-IB1                                                 |
| Ovarian       | Total abdominal hysterectomy, bilateral salpingoophorectomy | Unilateral oophorectomy                                        | 1. Borderline tumors  
2. Stage IA-C Grade I or II                                      |
| Endometrial   | Total abdominal hysterectomy, bilateral salpingoophorectomy | Surgical staging without hysterectomy or oophorectomy; Progestin therapy | Stage I, Grade I endometriod cancer                               |
Survival rates can be predicted by the stage and histological grade of ovarian cancer at the time of diagnosis. In general, women with ovarian cancer confined to the ovary (Stage I) have >90% five-year survival rate, whereas those with Stage III-IV tumors have <40% five-year survival rate. Because >75% of ovarian cancers are Stage III-IV at diagnosis, the overall five-year survival rate for women diagnosed with ovarian cancer is <45% [6, 24].

Ovarian Cancer Subtypes

Ovarian cancer can be divided into three major categories: epithelial ovarian tumors, germ cell tumors, and stromal tumors. Each histologic subtype is associated with clinical features that are relevant to determining the advisability of a fertility-sparing procedure, such as age at presentation, typical stage at presentation, and incidence of bilateral ovarian involvement. Fortunately, many of the histologic subtypes seen more commonly during the reproductive years are amenable to less radical surgical approaches.

Epithelial ovarian malignancies usually arise from the ovarian surface epithelium or within epithelial inclusion cysts. They account for at least 80% of ovarian malignancies [25]. Approximately 75% of invasive epithelial cancers present in stages III or IV; however, most are diagnosed in postreproductive age women [24]. An important group to distinguish are tumors of low malignant potential, or borderline epithelial tumor. These relatively indolent lesions are found most frequently in the reproductive age group. Moreover, they tend to remain ovary-confined for long periods of time and can often reach an impressive size prior to diagnosis. Approximately 90% present with Stage I disease (confined to one or both ovaries) but will be bilateral in 25% of cases [26, 27].

Germ cell tumors account for approximately 10-15% of ovarian tumors, but malignant germ cell tumors are rare. While germ cell malignancies account for fewer than 5% of all ovarian cancers, they account for 2/3 of the ovarian malignancies seen in the first two decades of life [28]. Ovarian dysgerminoma is the most common malignant germ cell tumor, accounting for 40% of germ cell cancers. Approximately 75% are Stage I at diagnosis; however, 15% present with bilateral ovarian involvement. Other germ cell malignancies, such as immature teratoma, endodermal sinus tumor, and choriocarcinoma are rarely bilateral.

Ovarian stromal tumors, such as granulosa-theca and Sertoli-Leydig cell tumors are quite rare and usually low-grade; approximately 70% are Stage I at presentation. They are rarely bilateral [24, 29].

Standard Therapy

Surgical therapy for women with ovarian cancer who no longer desire fertility is hysterectomy and bilateral salpingo-oophorectomy. For staging purposes, many patients also undergo lymphadenectomy and partial omentectomy. For those with sufficient risk for recurrence, adjuvant combination chemotherapy may be advised postoperatively [25].

Fertility-Sparing Surgery

Fertility-sparing surgery for ovarian malignancies refers to unilateral oophorectomy, usually with removal of the adjacent fallopian tube. Often staging lymphadenectomy and relevant biopsies are performed as well. However, the contralateral ovary and uterus are not removed. There are many situations in which removal of the contralateral ovary and uterus does not impact overall oncologic outcomes or prognosis. In these cases, fertility-sparing surgery is quite reasonable practice.

Most unilateral germ cell and sex cord stromal malignancies will be amenable to fertility-sparing surgery. Fortunately, aggressive histologic subtypes and/or advanced surgical stage are uncommon in the reproductive age groups. In these cases, adjuvant multi-agent chemotherapy often is recommended to reduce the risk of recurrence [30]. One series reported a 90% 10-year overall survival following fertility-sparing surgery for germ cell malignancies; moreover, successful subsequent pregnancies are reported, even among women who received chemotherapy [31].

Unilateral borderline tumors usually are amenable to fertility-sparing surgery, and there is little evidence that oncologic outcomes are compromised, even in the setting of metastatic disease [32]. In one series, the recurrence rate was approximately 5%, regardless of whether patients were treated with radical or fertility-sparing surgery. That being said, bilateral ovarian involvement is common in borderline malignancies, and the remaining ovarian tissue was the most common site of tumor recurrence in those opting for fertility-sparing procedures, thus indicating a need for close surveillance of the remaining ovary [32]. In this series, 18% of 184 women subsequently had successful pregnancies after fertility-sparing surgery.

Invasive epithelial ovarian cancers can be treated with conservative fertility-sparing surgery in selected unilateral Stage I cases [33]. When fertility-sparing surgery is planned for these women, complete staging is still recommended. Pelvis washings, lymph node sampling, and omentectomy will reveal occult metastases in 15% of women presenting with apparent ovary-confined disease [25, 34]. Some experts suggest performing a wedge biopsy on the contralateral normal-appearing ovary with intraoperative frozen section to exclude occult malignancy [35]. Except in cases of low-grade histology, most women diagnosed with invasive epithelial ovarian carcinoma of any stage will be treated with adjuvant chemotherapy after surgery.

Even after unilateral oophorectomy and chemotherapy, many women with malignant epithelial ovarian tumors will be able to conceive. The majority will experience resumption of menstrual function, and in one series more than 70% subsequently had a successful pregnancy [25, 36].

Endometrial Cancer

Endometrial cancer is the most common gynecologic cancer, accounting for approximately 6% of all cancers in women [37, 38]. However, the majority of women diagnosed with endometrial cancer are post menopausal. Less than 5% of endometrial cancers are diagnosed in women <40 years of age, and in this cohort these neoplasms tend to be well differentiated with an indolent biology and confined to the uterus at diagnosis [6]. Excess estrogen, such as that seen in obesity and anovulation, is the main risk factor for the de-
velopment of low-grade endometrial neoplasms. Indeed, approximately 25% of young women who develop endometrial cancer will have polycystic ovary syndrome (PCOS) [38]. The overall five-year survival rate for this so-called “low risk” endometrial cancer is in excess of 90%.

**Standard Therapy**

The standard therapy for endometrial cancer is hysterectomy and bilateral salpingo-oophorectomy [39]. Adjuvant postoperative radiation therapy or chemotherapy may be recommended for high-risk histology or advanced disease. Since the majority of women with endometrial cancer are beyond the reproductive years, fertility-sparing surgery is rarely considered.

**Fertility-Sparing Therapy**

In select younger women with low-grade endometrial carcinoma who are motivated to preserve their fertility, fertility-sparing approaches may be considered. These approaches typically involve medical treatment with progestin therapy. This approach is appropriate only in young women with non-invasive (Stage Ia), hormone-sensitive lesions (typically Grade I histology) who desire fertility preservation and have an otherwise reasonable chance for conception after therapy [40-42].

**Pre-Treatment Studies**

Thorough endometrial sampling though D&C and accurate pathology review are essential prior to embarking on a strategy of medical management for endometrial carcinoma [40]. Immunohistochemistry studies evaluating progesterone receptor content are to be helpful in predicting response to progestin therapy. However, in a study of metastatic endometrial carcinoma, 37% of women with tumors demonstrating progesterone content responded to progestin therapy, whereas only 8% of those responded whose tumors did not [43]. Most FIGO Grade I endometrial neoplasms will express progesterone receptors to varying degrees. The value of further quantifying progesterone content in women considering for medical management has not yet been evaluated.

As non-invasive or minimally invasive lesions are considered the only candidates potentially amenable to medical management for endometrial carcinoma, an important part of pre-treatment evaluation is determining depth of myometrial invasion. The most accurate imaging technique available for this purpose is the contrast-enhanced MRI [44,45]. Pelvic MRI is also highly sensitive for detection of neoplastic lymph node involvement, albeit lymphatic metastasis is otherwise a very uncommon occurrence for non-invasive and early-invasive FIGO Grade I endometrial lesions [46].

**Progestin Therapy**

To avoid the need for hysterectomy, progestin therapy is used in an attempt to eradicate early-stage, well-differentiated endometrial cancer [6, 47, 48]. Although there is no standard treatment regimen, two commonly use progestin regimens are megestrol acetate, 80 mg/day or medroxyprogesterone, 200 mg/day. Medroxyprogesterone doses as high as 1000 mg/day have been advocated by some; however, this higher dose has not been found to be more effective than 200 mg/ day for women with advanced disease, and thromboembolic complications were markedly increased [43].

**Follow-up**

Definitive assessment of treatment response requires endometrial sampling, either biopsy or D&C, which has been recommended every 1 to 3 months [41]. Once D&C verifies that the endometrium has reverted to normal histology, pregnancy can be attempted [40]. Monthly transvaginal ultrasonography and serum CA-125 might be useful as early indicators of disease progression [41, 49].

**Outcomes**

Approximately 50% of well-differentiated tumors show a complete response to hormonal treatment. As a result of ongoing risk factors, approximately 25% of responders will suffer a relapse after initial successful therapy [50]. Thus, patients should be counseled regarding the potential for failure with this approach.

**Subsequent Fertility**

Several authors have reported successful pregnancies after progestin therapy for endometrial carcinoma [40]. Although the reports are somewhat limited, it appears that as many as 1/3 of women will conceive after such therapy. Unfortunately, as stated before, recurrence is common, and so continued close follow-up is required as long as the patient retains her uterus [40]. Unfortunately reported conception rates are low, reflecting both inadequate regression of endometrial pathology as well as inherent fertility issues in this typically subfertile patient population. As PCOS and anovulation are common in this population, ovulation induction is often necessary.

**PROTECTING OVARIES AND OOCYTES DURING CANCER TREATMENT**

Cancer therapy often has devastating effects on ovarian function and future fertility. Pelvic irradiation can be curative for a number of cancers common in young women, including gynecologic cancers and lymphomas. However, if the ovaries are within the treatment field, premature ovarian failure is common. Likewise, systemic chemotherapy is an effective treatment for these types of cancers in addition to cancers outside the pelvis such as breast cancer and leukemia. Multi-agent chemotherapy results in ovarian failure in approximately 40% of women < 40 years and more than 75% in women > 40 years and is likely to impair fertility in an even greater number [51].

The key to fertility preservation in women requiring pelvic irradiation or systemic chemotherapy is protection of oocytes from damage. Both non-surgical and surgical methods for fertility preservation have been developed for women undergoing these cancer therapies.

**Non-Surgical Approaches**

Although the focus of this manuscript is surgical approaches to fertility preservation, existing non-surgical approaches should be mentioned. These approaches fall into
two categories: oocyte retrieval for *in vitro* manipulation and cryopreservation, and pituitary down-regulation during chemotherapy.

**Oocyte Retrieval for In Vitro Manipulation**

Oocyte removal for *in vitro* manipulation is the most effective non-surgical approach to fertility preservation [52]. The highest pregnancy rates have been achieved with a combination of hormonal stimulation followed by timed transvaginal retrieval of mature oocytes, *in vitro* fertilization and embryo cryopreservation (IVF-ET). The cumulative pregnancy rate with this approach after thawing and intrauterine transfer is currently > 60% per embryo [53]. Oocyte cryopreservation can be used if the woman does not desire fertilization of her oocytes; the current pregnancy rate after thawing, fertilization, and intrauterine transfer is 57% per embryo [54]. The disadvantages of these techniques is that they delay planned cancer therapy for two or more weeks from the beginning of the next menstrual period and expose the women to higher than normal estrogen levels during ovarian stimulation.

To decrease the time until retrieval and avoid high estrogen levels in women with estrogen dependent cancers (e.g. breast, endometrial), methods have been developed to retrieve immature oocytes [55, 56]. The oocytes can be hormonally treated using a process called *in vitro* maturation (IVM). The resulting mature oocytes can be fertilized and the embryos frozen, or the oocytes can be frozen without being fertilized. Although there have been reports of pregnancy rates comparable to those attained with standard IVF-ET, these approaches have yet to become widely applied [57].

**Pituitary Down-Regulation During Chemotherapy**

A pharmacologic approach to decreasing ovarian damage from chemotherapy is down-regulation of the pituitary with gonadotropin-releasing hormone agonist (GnRHa) [58]. Several hypotheses exist to explain why this approach might limit the gonadotoxic effects of chemotherapy. First, inhibition of FSH secretion might hinder recruitment of preantral follicles. Second, decreased FSH and LH stimulation might diminish ovarian perfusion. Finally, decreased FSH stimulation of the ovary up-regulates sphingosine-1-phosphate which might serve as an ovarian cytoprotectant [52, 58, 59].

The ability of pituitary down-regulation to inhibit recruitment of preantral follicles has been called into question. It has been observed that primordial follicles continued to grow in a patient with Hodgkin disease treated with GnRH-a [60]. *In vitro* studies of human ovarian tissue have suggested that preantral follicles exhibit FSH independent growth, and this might explain why pituitary down-regulation does not completely inhibit antral follicle development [52]. Other researchers have observed that primordial follicles continue to initiate growth during GnRH-a therapy and during the hypogonadal state before puberty [61].

A recent review of clinical studies of GnRH-a use during chemotherapy concluded that, although there is some evidence that GnRH-a therapy reduces chemotherapy-related gonadotoxicity, it has yet to be proven statistically [62]. In the nine studies they analyzed the incidence of ovarian failure after chemotherapy was 11% in patients who received GnRH-a compared with 56% in those who had not. However, because most of these studies were small, retrospective, and non-randomized, the benefit of GnRH-a remains uncertain.

**SURGICAL APPROACHES FOR PRESERVING OVARIAN FUNCTION**

Radiation therapy and chemotherapy are commonly used treatments for gynecologic cancers and other cancers in the reproductive years, most notably breast cancer and lymphomas. Since both pelvic irradiation and systemic chemotherapy can damage the ovaries, surgical methods have been developed that attempt to preserve ovarian function. Two surgical approaches have been reported for this purpose: 1) ovarian transposition and 2) ovarian tissue cryopreservation and reimplantation (Table 2).

**Ovarian Transposition**

Ovarian transposition (or oophoropexy) is the surgical mobilization of the ovary with its vascular pedicle, followed by relocation of the ovary out of the planned field of radiation therapy. The goal is to minimize ovarian radiation exposure to preserve ovarian function and possibly fertility [63]. Techniques and results for ovarian transposition have been reported for women undergoing local pelvic or abdominal radiotherapy for a number of cancers, including cervical, vaginal, dysgerminoma and Hodgkin disease [64-67].

Standard external beam radiation and brachytherapy are designed to deliver a radiation dose to the pelvis of >4000 cGy [68]. Doses to the ovary of > 1200 cGy will result in premature ovarian failure in >50% of women. After transposition from the pelvis, ovaries receive approximately 5–10% of the total pelvic dose as a result of scatter [69]. As a result of this, ovaries can be preserved by relocating them out of the radiation field of the planned therapy [70].

**Table 2. Surgical Methods for Preserving Ovarian Function for Reproductive-Age Women with Cancer Undergoing Pelvic Irradiation or Systemic Chemotherapy**

<table>
<thead>
<tr>
<th>Method</th>
<th>Indication</th>
<th>Approaches</th>
<th>Effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian transposition</td>
<td>Cancer requiring pelvic radiation therapy</td>
<td>• Lateral transposition</td>
<td>• Often preserves ovarian function</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Medial transposition</td>
<td>• Might require IVF to restore fertility</td>
</tr>
</tbody>
</table>
| Ovarian transplantation| Cancer requiring pelvic irradiation or systemic chemotherapy | 1. Laparoscopic removal of ovarian tissue  
2. Cryopreservation  
3. Transplantation of ovarian tissue | • Remains experimental  
• Preservation of ovarian function and fertility is possible |
of both radiation scatter and changes in circulation resulting from surgery, ovarian function is not uniformly spared by surgical transposition.

**Technique**

Ovarian transposition was originally performed via laparotomy [70, 71]. More recent reports utilize a laparoscopic approach [67]. A standard technique begins with occlusion and division of the utero-ovarian ligament followed by separation of the fallopian tube from the ovary [71]. The ovaries are mobilized by incising the peritoneum along the infundibulo-pelvic ligament. Finally, the ovaries are laterally displaced and fixed to the peritoneum high in paracolic gutters.

Alternatively, the ovaries can be repositioned behind the uterus when lateral pelvic radiation therapy is required, as is often the case with lymphomas [67]. The theoretical disadvantage of the midline oophoropexy is higher radiation exposure of the ovaries as a result of internal scatter.

**Ovarian Function Preservation**

Ovarian transposition prior to pelvic irradiation has been reported to preserve ovarian function in 50 to 79% of women [66, 70]. These relatively short-term studies have not determined how many patients will subsequently experience premature ovarian failure.

**Fertility Preservation**

Ovarian transposition is less effective for preserving fertility than ovarian hormonal function. Separation of the ovaries from the fallopian tubes and lateral displacement is likely to significantly decrease fertility. In one series of 37 patients, 16 pregnancies occurred spontaneously, although in 12 of these women the ovaries were not separated from the fallopian tubes [66].

In some cases where the ovaries have been separated from the fallopian tubes and spontaneous pregnancy does not occur, oocyte retrieval followed by IVF-ET has been successful. However, transvaginal oocyte retrieval is likely to be difficult following lateral transposition and might require a transabdominal or laparoscopic approach for retrieval.

**OVARIAN TISSUE TRANSPLANTATION**

For many patients with cancer, especially adolescent girls and children, ovarian tissue transplantation is an experimental approach with significant potential. Ovarian tissue is surgically removed prior to radiation or chemotherapy and cryopreserved. After the patient has completed cancer therapy and is in remission, the ovarian tissue is transplanted back into the patient (e.g. autologous transplantation). This experimental approach has been used to preserve both ovarian function and fertility.

Two approaches have been used for surgical removal of ovarian tissue for later transplantation: 1) removal of the entire ovary or large sections of the ovary with a vascular pedicle, and 2) removal of ovarian cortex tissue. Ovarian cortex tissue can be transplanted back on to the ovary (termed “orthotropic”) or to subcutaneous sites distant from the pelvis (termed “heterotopic”) Fig. (1) shows different sites and approaches for ovarian transplantation.
Ovarian Tissue Transplant with Vascular Pedicles

Surgical removal of ovarian tissue with a vascular pedicle with subsequent orthotopic transplantation back into the adnexal region has been used to re-establish ovarian function in women with premature ovarian failure by transplanting the ovary of her identical twin into her adnexa [72]. Currently, microvascular anastomoses are performed via laparotomy, although laparoscopic methods are being investigated [73].

This approach remains experimental. It has been shown to be feasible in the animal model where the ovaries were harvested with their vascular pedicles and then autotransplanted. The ovarian vessels were anastomosed to the deep inferior epigastric vessels using either end-to-end, end-to-side, or fish mouth modification. The end-to-end anastomosis yielded the highest patency rate of the vascularized grafts [73].

Ovarian Cortex Transplant

Surgical removal of ovarian cortex followed by cryopreserved subsequent orthotopic transplantation back to the donor’s adnexal region has had some success in humans [74]. Heterotropic transplantation to a subcutaneous site distant from the adnexal has also been reported in humans [75-77].

Orthotopic Transplantation

Reimplantation of human ovarian cortex back to the ovarian surface has been reported by several investigators [78-81]. Reestablishment of ovarian functions after transplantation usually takes more than 4 months [82]. The length of time that grafts remain viable after transplantation is variable and has been reported to range from a few months to more than 5 years [82]. Although this approach can restore menstrual function in women with previous ovarian failure, basal FSH levels remain elevated; indicating decreased ovarian reserve [83].

Fertility Preservation

Live births have been reported after autologous transplantation of frozen-thawed cortical tissue to orthotopic sites [78, 79, 81]. The majority of these pregnancies occur spontaneously after establishment of menstrual function and normalization of the FSH and most often occurred during the first year after transplantation. When IVF is attempted in the transplanted ovaries, oocytes harvested from transplanted ovarian cortex might tend to be immature and of poor quality [84].

Heterotopic Transplantation

Reimplantation of human ovarian cortex to subcutaneous sites distant to the pelvis has also been reported [53, 85]. It avoids the risk of a second surgery required to transplant tissue back onto the ovary. However, the ischemia experienced by the tissue prior spontaneous neovascularization is likely to be detrimental to subsequent functionality of an ovarian tissue transplant. In addition, subsequent fertility will require oocyte retrieval and IVF. To date, no pregnancies have been reported with this technique.

Expert Commentary

The purpose of this article is to review the surgical approaches to preserving fertility in reproductive-age women with cancer. A subset of reproductive-age women and gynecologic cancers can be treated with fertility-sparing surgery. Appropriate patient selection can result in survival rates comparable to traditional radical surgery, while sparing fertility. Select women with ovarian cancer that is border line or Grade I, Stage I can be treated with unilateral oophorectomy. Women with Stage I cervical cancer can be treated with radical trachelectomy. Young women with Stage I endometrial cancer can be treated with progestins so that hysterectomy can be avoided.

Surgical approaches for fertility preservation can also be used for reproductive-age women diagnosed with cancer who require pelvic irradiation or systemic chemotherapy. Ovarian translocation prior to pelvic irradiation has been demonstrated to preserve subsequent ovarian function. When systemic chemotherapy is planned, experimental methods are being developed to surgically remove and cryopreserve ovarian tissue. After the patient is determined to be in remission, transplantation of ovarian tissue back into the patient has been demonstrated to restore both ovarian function and pregnancies.

Methods continue to be developed to preserve ovarian function and fertility in reproductive-age women undergoing cancer treatment. These methods must be implemented as part of the initial approach to cancer therapy and prior to definitive radiation or chemotherapy if the patient is to have the best chance of continued ovarian function and fertility.

Five-Year View

The future of fertility preservation for reproductive-aged women with cancer is likely to involve removal of ovarian tissue, followed by in vitro follicle culture of the tissue and removal of oocytes [86]. Immature eggs thus obtained will be subjected to in vitro maturation. More effective techniques are being developed for cryopreservation of both oocytes and embryos.

KEY ISSUES

- Survival has significantly improved for women diagnosed with cancer during the reproductive years.
- The majority of these women desire children after completion of their cancer therapy.
- For women with early gynecologic cancers, fertility-sparing surgery is often possible.

For women requiring pelvic irradiation or systemic chemotherapy for a variety of cancers, surgical methods for preserving ovarian function include ovarian transposition, and removal of ovarian tissue prior to therapy for cryopreservation and later transplantation.
REFERENCES

Papers of special note have been highlighted as:

- of interest
- of considerable interest


[2] This article demonstrated an overall overview on cancer statistics.


Surgical Strategies for Fertility Preservation in Women with Cancer

Current Women's Health Reviews, 2010, Vol. 6, No. 2 175


[77] This study demonstrated promising results after orthotopic ovarian tissue transplantation in 5 women.


Innovative Roles for Surgical Robotics in Reproductive Surgery

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Abstract: In the area of reproductive surgery and infertility, minimally invasive techniques appear to have outcomes similar to those of laparotomy for tubal reanastomosis and myomectomy. Studies have shown that robotic use in these surgeries is feasible and that it may be used as an alternative for conventional laparoscopic surgery. The robot can overcome many of the limitations seen with conventional laparoscopy through improved dexterity and ergonomics. In this review, we will evaluate the current applications of robotics in reproductive surgery.

Keywords: Robotic surgery, reproductive medicine, tubal reanastomosis, myomectomy, Da Vinci.

HISTORY OF ROBOTIC SURGERY

The first appearance of the robot in surgery was in 1985 when the PUMA 560 robot was used to perform CT-guided brain tumor biopsies [1]. The next step in the surgical use of the robots occurred at the Imperial college, London in 1988 where a device named PROBOT was involved in performing transurethral resection of the prostate guided by a preoperative construction of a three-dimensional image [2]. In 1992, the ROBODOC machine was used in orthopedic surgery to perform total hip replacement in humans [3]. These early robots were designed to function autonomously after preoperative mapping [4].

The biggest step in the development of the surgical robot was the evolution of robotic telepresence technology, which gives the surgeon the ability to operate remotely. This was created in a collaboration between the Stanford Research Institute, United States Department of Defense and the United States National Aeronautics and Space Administration (NASA) [5]. The main goal for developing this system was to provide immediate surgical care to soldiers in a battlefield area. Robotic arms were designed to be mounted on an armored vehicle that would travel to the wounded soldiers guided by a surgeon from a remote location [6]. This technology was subsequently commercialized, and the surgical robot was no longer autonomous but functioned only with help from surgeon’s movements [4].

In this review, we will evaluate the current applications of robotics in reproductive surgery.

SURGICAL ROBOTS USED IN GYNECOLOGY

Three surgical robot systems have been described in the area of gynecologic surgery. The first was the AEOSP (Automated Endoscopic System for Optimal Positioning Computer Motion Inc., Goleta, CA) device, which was the first commercially available surgical robotic technology. It was used to hold a laparoscopic camera guided by a voice command system [7].

The second system was the ZEUS robot (Computer Motion Inc., Goleta, CA, USA). It was developed in the early 1990’s. This system was composed of three robotic arms attached to an operating table and a robotic console where the surgeon was sitting. One of the robotic arms was used to hold a laparoscope camera, and it was voice activated. The surgeon controlled the other two arms using handles on the console. The tip of ZEUS instrument had an articulation that allowed some wrist movement but was never intuitive [8].

The third robotic system used in gynecologic surgeries is the da Vinci. In 2000, the FDA approved the da Vinci robot system for use in abdominal surgeries. This system (Intuitive surgical, Mountain View, California) consists of a surgical console where the surgeon sits and a surgical tower at the bedside. The insight vision system provides binocular vision with a three-dimensional view. The console has two handles that control the robot arms [9].

The patient tower has three to four robotic arms one holds the camera and the others hold surgical instruments such as graspers or scissors. The use of the da Vinci robot provides a three-dimensional view of the operative field as well as increased dexterity and precision in both dissection and suturing procedures. The use of the endowrist instruments that imitate the human wrist gives seven degrees of freedom in movement, facilitating a wide range of motion. There are some limitations with the use of the surgical robot, however, that include the initial system cost, maintenance costs and the expense of disposable instruments. The system is very bulky, limiting patient access. Lack of tactile feedback during the procedure requires use of visual cues to properly perform surgical tasks. There are no randomized clinical trials comparing robotic to conventional laparoscopy or laparotomy [10]. Also, it is unclear how many surgeries are needed before a surgeon obtains technical proficiency.
The first application of the surgical robot in reproductive surgery was tubal reanastomosis. After an initial success, it was implemented in myomectomies, complex hysterectomies and more intensively in gynecologic oncology—particularly for endometrial cancer staging.

ROBOTICALLY ASSISTED TUBAL REANASTOMOSIS

Tubal reanastomosis surgery was traditionally performed through a small Pfannenstiel incision; microsurgical technique was used for the reconstruction. The disadvantages of this procedure were postoperative pain and prolonged convalescence. A minilaparotomy approach can be performed as an outpatient procedure with good results [11]. However, this approach has not been generally adapted. With the development of minimally invasive surgery, tubal reanastomosis was performed laparoscopically with shorter hospital stays and recovery times, smaller incisions and decreased postoperative pain levels [12].

The use of laparoscopy in such a procedure requires precise dissection, delicate handling, and suturing of the fallopian tubes. In addition, the learning curve of conventional laparoscopy is high and requires a multitude of laparoscopic surgical skills [13]. The skill set needed to perform such procedures has made this approach less popular. Consequently, use of the minimally invasive approach, which could obviate all these limitations, is desirable. Robotic assistance in tubal reanastomosis appears to be a good alternative to conventional approaches [14].

Use of the surgical robot managed to overcome many of the limitations seen with conventional laparoscopy. The first reported case of robotic tubal reanastomosis was with the Zeus robot in 1999 [15]. The most commonly used system for robotic surgery is the da Vinci system, which provides a three-dimensional view of the tubal architecture. The endowrist instruments provide seven degrees of freedom in dissection and knot tying. In addition, it eliminates the tremors resulting from user fatigue and the use of fine sutures [16-18].

We reviewed the literature for previous evaluations on the use of the robot in tubal reanastomosis surgery. Eight publications were identified and are summarized in Table 1.

The first attempt at implementing robotics in reproductive surgery was in 1998 at Cleveland Clinic when an animal study using a pig model was performed. Three approaches to tubal reversal-type surgery were compared: open and laparoscopic with or without robotic assistance. This animal experiment concluded that the use of Zeus robot is helpful in performing tubal microsurgical reanastomosis and results in shorter operative times compared with those of conventional laparoscopy [19].

A case report of full robotic assistance in tubal sterilization reversal was published in 1999 by the same group. Surgery with the Zeus Robot was accomplished successfully without conversion to laparotomy. Tubal patency was documented at the end of the operation [15]. In further work by Falcone et al., a human pilot study with the Zeus robot was conducted with 10 patients undergoing tubal reanastomosis. The procedure was completed successfully except in one patient with dense intraperitoneal adhesions, which allowed only unilateral access of the tube. There was 100% patency.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Robot Used</th>
<th>Number of Robotic Cases</th>
<th>Tubal Patency Rate</th>
<th>Pregnancy Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Margossian, 1998</td>
<td>1998</td>
<td>Zeus System</td>
<td>1 Pig animal</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>2 Falcone, 1999</td>
<td>1999</td>
<td>Zeus System</td>
<td>A case Report</td>
<td>100 %</td>
<td>Not followed up</td>
</tr>
<tr>
<td>3 Degueldre, 2000</td>
<td>2000</td>
<td>Da Vinci</td>
<td>8 patients</td>
<td>100%</td>
<td>2 of 8 (25%)</td>
</tr>
<tr>
<td>4 Falcone, 2000</td>
<td>2000</td>
<td>Zeus System</td>
<td>10 patients</td>
<td>97.5%</td>
<td>5 of 10 (50%)</td>
</tr>
<tr>
<td>5 Goldberg, 2003</td>
<td>2003</td>
<td>Zeus System</td>
<td>10 patients</td>
<td>89.5%</td>
<td>5 of 10 (50%)</td>
</tr>
<tr>
<td>6 Rodgers, 2007</td>
<td>2007</td>
<td>Da Vinci</td>
<td>26 patients</td>
<td>NA</td>
<td>19 pregnancies of 14 patients (61%) (11%) ectopic 16% spontaneous abortion</td>
</tr>
<tr>
<td>7 Vlahos, 2007</td>
<td>2007</td>
<td>Da Vinci</td>
<td>5 patients</td>
<td>100%</td>
<td>4 of 5 (80%)</td>
</tr>
<tr>
<td>8 Dharia Patel, 2008</td>
<td>2008</td>
<td>Da Vinci</td>
<td>18 patients</td>
<td>NA</td>
<td>(62.5%) (28%) intrauterine (22%) ectopic (11%) sp pregnancy loss</td>
</tr>
</tbody>
</table>
in all reanastomosed tubes with a 70 ml mean blood loss. By 12 months, 5 patients (50%) had achieved pregnancy [18].

In 2003, Goldberg and Falcone compared 10 patients who underwent robotically assisted tubal reanastomosis surgery with a control group of 15 patients who underwent the same procedure by conventional laparoscopy. Five pregnancies (50%) were reported in the robotic group and all carried their pregnancies to term. The recorded operative time was significantly longer in the robotic group. A statistically but not clinically significant higher blood loss was detected in the robotic group [20]. The Zeus robot is no longer available.

In 2000, a feasibility study described 8 women who desired fertility restoration after tubal ligation surgery. Using the da Vinci surgical system, bilateral tubal reanastomosis was performed in all the patients. There was no recorded perioperative or postoperative complications. Short and long-term follow up data showed patent tubes on HSG (hysterosalpingogram) in four patients and pregnancy in 2 patients within 4 months after surgery [14].

Rodgers et al., in 2007, compared minilaparotomy as an outpatient procedure with the use of robotics in performing tubal reanastomosis surgery. In 26 patients, the da Vinci robot was used whereas the minilaparotomy was the preferred approach in 40 other patients. The operative time was longer and costs were higher with the use of robotics. In addition, there was no significant difference between the 2 groups in hospitalization times, but there was a significantly decreased convalescence time seen in the robotic patients. There was no significant difference in pregnancy rates: 61% of the patients in the robotic group and 79% of patients in the minilaparotomy group became pregnant. The ectopic pregnancy rate was 11% in the robotic group versus 13% in the minilaparotomy group. The rate of spontaneous abortion was 16% in the robotic group and 38% in the minilaparotomy group [21].

In 2007, Vlahos et al. published a study that reviewed 5 patients who underwent robotic tubal reanastomosis surgery. All procedures were completed successfully without major complications. They also noticed a trend in the time needed to prepare for surgery by robot positioning and set up and perform the robotic surgery both declined as the surgeons became more familiar with using the technology. Four of the five patients conceived resulting in two viable pregnancies and x (11%) spontaneous pregnancy losses versus x (30%), x (10%) and x (10%), respectively, in the laparotomy group [16].

**ROBOTICALLY ASSISTED MYOMECTOMY**

The evolution of robotics in gynecologic surgery offered the chance for a minimally invasive approach to be used for complicated surgical procedures that were challenging by conventional laparoscopy. Hysterectomy and colposacropexy are widely performed with robotic assistance [17, 23] as are procedures in gynecologic oncology [24].

Myomectomy remains the best choice of treatment for symptomatic fibroids in patients desiring to preserve their fertility, even with newer modalities such as uterine artery embolization [25, 26]. Open myomectomy was the preferred approach until the emergence of the minimally invasive surgical technique. Laparoscopy was used to perform myomectomy with better cosmesis and shorter postoperative pain and hospital stay but the procedure was highly challenging for the surgeon. With conventional laparoscopy, precise dissection of the fibroid without unnecessary breaching of the endometrial cavity is key. Moreover, it is necessary to suture the fibroid bed in layers with precise approximation of edges to prevent rupture of the uterus during labor [27]. Laparoscopic suturing is a difficult skill to master. These challenges limited the acceptance of this technique, and open myomectomy remained the approach of choice.

We reviewed the literature for previous experiences in robotic myomectomy 7 studies were identified (Table 2).

The first work was reported by Advincula P, et al. in 2004. This was a preliminary study that included 35 patients who underwent robotically assisted myomectomy between 2001 and 2004. The number; weight and diameter of the removed myomas were recorded. All cases were performed with the da Vinci Robot the conversion rate to laparotomy was 8.6%. The average estimated blood loss was 169 ±198.7 ml and the median length of stay was 1 day [27].

A case report was published by Mao SP, et al. in 2007 that featured a 38-year-old gravida 2 para 2 female patient with an anterior wall subserosal myoma measuring 7x 8 9 cm. The myoma was successfully removed with the use of the da Vinci Robot in 3 hours with an estimated blood loss of 150 ml. There were no recorded adverse events [28].

Bocca S, et al. in 2007 documented for the first time an uncomplicated full-term pregnancy in a patient who had undergone robotically assisted myomectomy. The surgery was successfully completed with the use of da Vinci robot, which removed a 3-cm single fundal intramural uterine myoma. Approximately four months later, the patient became pregnant after clomiphren citrate and HCG (human chorionic gonadotropin) treatment. Delivery of a full-term healthy baby was done by Caesarian section at 38 weeks [29].

In 2007, Advincula et al. compared robotically assisted myomectomy with open myomectomy. This study included 58 patients; 29 in the robotic group and 29 in the open group.
No statistically significant differences were seen between the two groups in regards to age, BMI and weight of the removed myomas. The patients in the robotic group had significantly less blood loss and a shorter length of stay but the Hospital costs were higher in this group. The operative time was longer in the robotic group, but the rate of complications was higher in the laparotomy group [30].

More recently, in 2009, Nezhat C, et al. compared 15 cases of robotically assisted myomectomy with 35 cases of standard laparoscopic myomectomy. No differences were seen between the 2 groups in regards to patient age, BMI, parity, and previous abdominopelvic surgery. No significant difference was seen between both groups in the size, number and location of the removed myomas. However, the mean surgical time was longer in the robotic group. There were no differences between the 2 groups in regards to estimated blood loss, length of hospital stay or rate of postoperative complications [30].

A retrospective data analysis was conducted by George A, et al. that included 77 cases of robotically assisted myomectomy with 35 cases of standard laparoscopic myomectomy. No differences were seen between both groups in the size, number and location of the removed myomas. However, the mean surgical time was longer in the robotic group. There were no differences between the 2 groups in regards to estimated blood loss, length of hospital stay or rate of postoperative complications [30].

Bedient CE, et al., compared robotic and laparoscopic myomectomy in a retrospective chart review of 81 patients--40 patients were in the robotic group and 41 patients were in the laparoscopic group. No differences were recorded between the 2 groups regarding the operative time, mean blood loss or length of stay. There was a significant difference, however, noted in the laparoscopic group they had a larger preoperative uterine size, a larger mean size and a larger number of removed fibroids [33]. When adjusted for uterine size, there was no difference in regards to intra-operative or postoperative complications.

CONCLUSION

Robotic surgery has been successfully implemented in reproductive surgery. The surgical robot offers 3-D vision, improved dexterity, and easier precise suturing, which appears to be critical in fertility preserving surgeries. The surgical robot can provide the surgeon with the dexterity of an open approach while keeping the minimally invasive advantages. The disadvantages of using this technology remain the cost and the bulky size of the robot. Further studies are warranted to evaluate the potential role of the robot in randomized clinical trials. The cost effectiveness and long-term post surgical reproductive outcomes of these surgeries need to be critically evaluated.

EXPERT COMMENTARY

The main objective of this article is to review the current experience of robotic surgery in the field of reproductive medicine. The robotic approach has been used successfully
to perform tubal reanastomosis and myomectomy. The use of the robot has overcome many of the challenges seen previously with conventional laparoscopy such as precise laparoscopic suturing. The surgical robot provides the three-dimensional vision required for depth perception and improved dexterity, which helps the surgeon in precise dissection and suturing. There seems to be improved immediate postsurgical outcomes by reducing the blood loss and length of the hospital stay with subsequent rapid return of the patient to normal activities. The loss of haptic feedback and the high cost are limitations in its widespread use and application. Randomized controlled trials will be required to study the value of the surgical robot in these procedures with respect to short and long term outcomes. Cost effectiveness studies will be important to determine the role of this technology.

FIVE YEAR VIEW

The use of surgical robotics in reproductive medicine is expected to continue to expand. In the United States, it is expected that more surgical procedures will be performed using the robotic approach. Expansion of robotics outside the States will be slower mostly because of the high costs. In the States, public health policy may require clear evidence that the surgical robot will be important to determine the role of this technology.

KEY ISSUES

• The surgical robot was developed to perform complex surgical tasks with minimally invasive techniques.
• For certain procedures, the robot can help surgeons overcome the reported limitations of conventional laparoscopy.
• The surgical robot can provide the surgeon improved ergonomics.
• In gynecology, robotic surgery has been used for many surgical procedures with reported success in benign gynecology, gynecologic oncology and reconstructive pelvic surgery.
• Reduced hospital stay and blood loss with the use of the robot were recorded in many retrospective studies.
• The bulky size, high cost and loss of the tactile feedback are still considered disadvantages with the use of the surgical robot.

REFERENCES


Surgical Management of Müllerian Duct Anomalies

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Abstract: Developmental anomalies of the müllerian duct system represent an interesting field of disorders in obstetrics and gynecology as they can affect any of the reproductive organs from the Fallopian tubes to the hymen. The purpose of this article is to review the available treatment options for müllerian duct anomalies with special emphasis on simple and advanced surgical approaches. Surgical options are presented based on a novel treatment plan classification system adapted from the American Fertility Society classification of müllerian duct anomalies. Care was taken to include all previously termed unclassified anomalies as well as the important category of longitudinal fusion defects. Important diagnostic approaches are discussed with special emphasis on detection of associated anomalies of the urinary system and other relevant systems. Early establishment of an accurate diagnosis is important for planning management options and preventing complications in the genital organs and surrounding systems. Classifying müllerian anomalies based on the available treatment options seems logical and the inclusion of previously unclassified entities is important for a comprehensive understanding and management of this group of disorders. The surgical approach for the correction of müllerian duct anomalies is individualized to the type of malformation. The value of a given surgical procedure should be assessed on terms of its capability to improve a patient's postoperative ability to have healthy sexual relations and achieve successful reproductive outcomes.

Keywords: Müllerian duct anomalies, vaginoplasty, vaginal agenesis, bicornuate uterus, septate uterus, transverse vaginal septum.

INTRODUCTION

Developmental anomalies of the müllerian duct system represent an interesting spectrum of disorders in obstetrics and gynecology [1]. The exact prevalence is unknown, and the present classifications have inherent limitations. The main objective of current treatment modalities is to conserve or restore all or some of a patient’s reproductive goals. These goals include restoring menstrual functions through treatment of crypto menorrhea, which eliminates pain, prevents endometriosis and other consequences, and gives the patient a true chance for natural conception. In other cases where functioning uterine or vaginal tissue are not present, treatment goals are directed towards achieving a normal sexual life via the creation of a neovagina and appropriate psychosexual support. The later field has undergone continuous improvement, especially in the past five years—for example, patients who have undergone partial or total construction or reconstruction of the vulvovaginal complex can initiate sexual activity as early as one week after surgery. In the future restoration, of full reproductive potentials for those women without functioning endometrial tissues through stem cell therapy and/or uterine transplant may become possible.

Embryological Backgrounds

The genital systems of male and female embryos are morphologically identical at 6 weeks. Both embryos have two sets of paired genital ducts: the müllerian ducts and the wolffian ducts. In females, the wolffian ducts regress, and the vestiges provide a template for the developing müllerian ducts. This explains the frequent associations observed later between müllerian defects and renal-urinary system malformations [2–4]. The traditional hypothesis maintains that the müllerian ducts are fused in a caudal-cranial direction. However, the müllerian anomalies characterized by a septate uterus, cervical duplication, and longitudinal vaginal septum supports the alternative hypothesis in which fusion of the müllerian ducts is segmental and bidirectional [4].

Recent data, both experimental and observational, [2, 5, 6] support early 20th century studies that identified the sino-vaginal bulbs as being derived from the caudal aspects of the wolffian ducts and the müllerian ducts. These bulbs were designated as wolffian bulbs. The hymen is a vestige of the endodermal membrane that separates the vaginal lumen from the UGS cavity; it usually ruptures perinatally and remains as a thin mucous membrane [7, 8].

Etiological Backgrounds

The diversity of structural anomalies seen in müllerian duct defects results from interruption or inappropriate regulation in müllerian-duct development at various stages of morphogenesis. Well-known factors, such as intrauterine and extraterine elements, genetics, and teratogens (eg, diethylstilbestrol [DES], thalidomide), have been associated with müllerian duct anomalies [7]. The genetics of müllerian duct anomalies are complex. In general, they occur sporadically, and most familial cases are multifactorial. Other modes of inheritance, including autosomal dominant, autosomal reces-
gressive, and X-linked disorders, also exist. Müllerian anomalies may also represent a component of a multiple malformation syndrome [9, 10].

**Classifications**

The form of classification that includes agenesis or hypoplasia, lateral fusion defects, vertical fusion defects, and DES-related abnormalities, is not mutually exclusive because many müllerian duct anomalies often coexist. Classifying müllerian duct anomalies using the method described by Buttram and Gibbons and others [8, 12-18] bears merit because it correlates anatomic anomalies with arrests in morphogenesis. However, this method is awkward and confusing.

The most widely accepted method of categorizing müllerian duct anomalies is the American Fertility Society (AFS) classification [11]. There are some limitations of the AFS classification, however. We suggest the treatment plan classification, which will be discussed in this article (Table I).

**SURGICAL MANAGEMENT OF MÜLLERIAN DUCT ANOMALIES**

According to the treatment plan classification, the surgical management of müllerian duct anomalies is organized

**Table 1. AFS and Treatment Plan Classification of Müllerian Duct Anomalies**

<table>
<thead>
<tr>
<th>Classification</th>
<th>AFS Classification</th>
<th>Treatment Plan Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>Segmental or complete agenesis or hypoplasia.</td>
<td>Class Ia – Affecting Fallopian tubes.</td>
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<tr>
<td></td>
<td></td>
<td>Class Ib - Affecting the uterine fundus.</td>
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<tr>
<td></td>
<td></td>
<td>Class Ic- müllerian aplasia (MRKH) syndrome.</td>
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<tr>
<td></td>
<td></td>
<td>Class Id- cervical aplasia (isolated or associated with vaginal aplasia).</td>
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<td></td>
<td></td>
<td>Class Ie- isolated vaginal aplasia.</td>
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<tr>
<td>Class II</td>
<td>Unicorne uterus with or without a rudimentary horn.</td>
<td>Class IIa-Unicornuate uterus without a rudimentary horn.</td>
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<tr>
<td></td>
<td></td>
<td>Class IIb- Communicating horn with or without functioning endometrium.</td>
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<tr>
<td></td>
<td></td>
<td>Class IIc-Non-communicating without functioning endometrium.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Class IId-Non-communicating with functioning endometrium</td>
</tr>
<tr>
<td>Class III</td>
<td>Didelphys uterus.</td>
<td>Class IIIa – Partial bicornuate uterus</td>
</tr>
<tr>
<td>Class IV</td>
<td>Complete or partial bicornuate uterus.</td>
<td>Class IIIb - Complete bicornuate 2 separate horns and single cervix.</td>
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<td></td>
<td></td>
<td>Class IIIc- Partial duplication with two cervices.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Class IIId- Complete duplication of the uterus, cervix, and vagina.</td>
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<tr>
<td></td>
<td></td>
<td>Class IIIe- any of the above with unilateral or bilateral obstruction of menstrual outflow</td>
</tr>
<tr>
<td>Class IV</td>
<td>Complete or partial septate uterus.</td>
<td>Class IVa - A complete or partial midline septum is present within a single uterus without fundal depression,</td>
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<td></td>
<td></td>
<td>Class IVb- A complete or partial midline septum is present within a single uterus with fundal depression,</td>
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<td></td>
<td></td>
<td>Class IVc – Any combination of the above in addition to septate cervix and vagina.</td>
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<td></td>
<td></td>
<td>Class IVd – Any combination of the above with unilateral or bilateral obstruction.</td>
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<tr>
<td>Class V</td>
<td>Longitudinal fusion defects</td>
<td>Class Va- partial transverse vaginal septum</td>
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<tr>
<td></td>
<td></td>
<td>Class Vb-complete transverse vaginal septum.</td>
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<tr>
<td></td>
<td></td>
<td>Class Vc- lower segmental vaginal atresia with upper heamtocolpos.</td>
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<tr>
<td></td>
<td></td>
<td>Class Vd- imperforate hymen.</td>
</tr>
<tr>
<td>Class VI</td>
<td>Arcuate uterus</td>
<td>Class VI (insignificant &amp; historical anomalies).</td>
</tr>
<tr>
<td>Class VII</td>
<td>DES-related abnormalities</td>
<td>Class VIa – Arcuate uterus.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Class Vb - DES-related abnormalities</td>
</tr>
</tbody>
</table>


based on the anatomical hierarchy of each class. Also included in the organization is the clinical significance of the available interventions as well as the presence or absence of current surgical options.

For example, the first category includes segmental or complete agenesis or hypoplasia of the tubes, uterus, or vagina. According to the anatomical hierarchy presentation, tubal anomalies will be addressed first followed by uterine anomalies then cervical and vaginal anomalies.

### Treatment of Class I (Segmental or complete agenesis or hypoplasia) (Table 2- Fig. 1)

#### Class Ia – Segmental or Complete Agenesis or Hypoplasia Affecting the Fallopian Tubes

Congenital fallopian tube disorders are either asymptomatic or presenting with infertility, ectopic pregnancy or acute abdominal pain due to torsion of an accessory tube [12-14]. Types of tubal anomalies range from aplasia, hy-

<table>
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<tr>
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<tbody>
<tr>
<td>Class I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Segmental or complete agenesis or hypoplasia</td>
<td>Agenesis and hypoplasia may involve the vagina, cervix, fundus, tubes, or any combination of these structures. Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome is the most common example in this category.</td>
<td>Class Ia – Segmental or complete agenesis or hypoplasia affecting the Fallopian tubes. Class Ib - Segmental or complete agenesis or hypoplasia affecting the uterine fundus. Class Ic - müllerian aplasia (Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome Class Id - cervical aplasia (isolated or associated with vaginal aplasia Class Ie - isolated vaginal aplasia</td>
</tr>
</tbody>
</table>

**Table 2. Description of AFS and Treatment Plan Classification of Class I Müllerian Duct Anomalies**

**Fig. (1). Shows segmental or complete agenesis or hypoplasia affecting the fallopian tubes, uterine fundus, (Class Ib) müllerian aplasia (Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome, (Class Ic) cervical aplasia (isolated or associated with vaginal aplasia Class Id) and isolated vaginal aplasia (Class Ie).**
poplasia, non-canalization, segmental atresia, accessory ostia, congenital diverticula, and elongation to accessory tubes and duplication. Few anomalies are amenable to surgical correction through open microsurgery, laparoscopic microsurgery or robotic surgery. Others may require corrective surgery and/or excisions in the event of torsion or ectopic pregnancy. Assisted reproductive technology (ART) represents an important effective backup for uncorrectable isolated tubal anomalies such as aplasia, hypoplasia and non-canalization.

Class Ib - Segmental or Complete Agenesis or Hypoplasia Affecting the Uterine Fundus

In cases of isolated uterine aplasia and/or hypoplasia with a functioning vagina, there are no treatment options if the present vaginal pouch permits adequate marital relations [1].

Class Ic - Müllerian Aplasia (Mayer-Rokitansky-Kuster-Hauser Syndrome)

Vaginal agenesis occurs in 1 out of 4,000-10,000 females. The most common cause of vaginal agenesis is müllerian aplasia or agenesis (Mayer-Rokitansky-Kuster-Hauser [MRKH] syndrome). The hormonal profile helps distinguish the MRKH syndrome from androgen insensitivity syndromes [15-17].

Trans-abdominal and trans-rectal ultrasonographic and MRI findings can add support to the clinical findings and detect the presence of normal ovaries [18-20]. Fusion of the cervical vertebrae, a component of Klippel-Feil syndrome, can result in cervical rigidity that may substantially interfere with the intubation procedure [21]. Discovery of a pelvic kidney is important in planning corrective surgery because its presence may limit the potential space available for graft placement [22, 23]. Laparoscopy is confirmatory for diagnosis and is one of the indispensable methods for treatment [24, 25]. There are three main well known treatment strategies for MRKH syndrome—a fourth one was recently introduced [26]. The first strategy, and the least invasive one, is the non-surgical use of successive dilators, which requires high compliance and patience, and good results are achievable over months of dilatation only if the patient is sufficiently motivated. The second strategy is the surgical correction via the creation of a surgical space at the site of the absent vagina. It is covered with a graft, and then a form is used to maintain the graft. The third strategy is creation of neovagina from native tissue through traction that is applied from above using an acrylic olive placed on the vaginal dimple.

The fourth and newest treatment option is balloon vaginoplasty where traction is applied from above on a silicon coated balloon catheter, creating a natural neovagina over a shorter period of time [24]. Interestingly, with balloon vaginoplasty, there is a possibility of manipulating both the depth as well as the width of the neovagina using different schedules of catheter traction and balloon distention [25].

Any surgical treatment should be well thought-out only when the patient can participate in the decision making wishes to become sexually active [27]. In certain countries, the operation is only performed after planning for marriage or after actual marriage [24-26].

Whereas a number of vaginoplasty methods have been developed, refined, and modified, no state-of-the-art surgical approach has been established. This is due to a number of factors including regional differences, surgeon experience and preference for a method, and patient choice [26, 28, 29].

The goal of vaginoplasty is to develop a space between the bladder and the rectum. This is followed by grafting—either by a full or split-thickness skin graft. The later has had high rates of success and patient satisfaction. Scar formation at the graft site has been a concern [30]. Human amnion has also been used as a graft for vaginoplasties.

Transposition flaps have been used successfully in some cases. One method is to use a de-epithelialized vulvar flap as the graft. Another method is a pudendal thigh fasciocutaneous flap, which has been described as having good cosmetic and functional outcomes [31]. Most recently, a triple flap relying on the use of labia minora and suburethral tissue has been reported with high success rates.

Autologous buccal mucosa has also been used as a graft source. These approaches used harvested bilateral full-thickness buccal mucosa grafts that are expanded with several stab incisions and sutured over a condom-covered soft stent. They are then placed in the newly created space.

Artificial dermis and absorbable adhesion barriers have been used as exogenous graft sources in vaginal reconstruction with promising results. An acrylic resin form was covered with artificial dermis, inserted, and fixed to the newly created vaginal space. The form was usually removed after 7 days; good results were reported with continued use of the form at nighttime [32].

Absorbable adhesion barrier (Interceed; Ethicon, Somerville, NJ) was used in a similar technique where neovaginal epithelialization was reported in 1-4 months [33-34].

A laparotomy procedure for peritoneal mobilization was reported in 1969 by Davydov. The peritoneum from the uterocecal space (pouch of Douglas) is advanced in such a manner that a vaginal canal is created. Recently, a laparoscopic modification was developed where the peritoneum is pulled through the newly created vesicorectal space using high tension and by approximating it at the introitus. A stent is then used for vaginal dilation. There are several benefits of this procedure, including minimal scarring and functional vaginas associated with comfortable intercourse [35].

Bowel vaginoplasty involving the distal sigmoid colon to line the neovaginal space has gained popularity with some pediatric surgeons. Classically, this approach requires concomitant laparotomy and bowel anastomosis. However, recent reports describe a laparoscopic approach [34, 36, 37]. Bowel vaginoplasty does not require persistent dilation, and the neovagina is self-lubricating [38].

The goal of split-thickness grafting is to generate a sufficient space between the urethra and/or bladder. The graft is placed over a sterilized stent with the epidermis facing the surface of the stent and the dermis facing out. The labia mi-
Serious complications include postoperative fistula (4% risk) 6-10 weeks after surgery [22, 39, 40]. To use the form continuously for 6 weeks, removing it only for urination and defecation. Six weeks later, a silicone form is used nightly for one year. In most cases, the vagina is functional 6-10 weeks after surgery [22, 39, 40].

Serious complications include postoperative fistula (4% risk) and enterocoele [22, 41]. Other complications including hemorrhage, infection, graft failure, graft contracture and excess granulation tissue have been reported [22]. Rarely, primary malignancy of the neovagina has been reported and for this reason, yearly Pap smears are recommended as part of long-term follow-up care [42, 43].

The Williams vulvovaginoplasty procedure is useful for patients with a previously failed vaginoplasty or for patients who have undergone radical pelvic surgery in areas where other better alternatives are not affordable. The vagina created by this approach is not anatomically similar to a normal vagina as its axis is directly posterior and horizontal to the perineum. However, the vagina is functional and well received by patients, and dilation is required for only 3-4 weeks [44].

Native epithelium covered neovaginas are developed from by expanding the natural epithelium at the dimple. Tissue expansion is classically achieved either by pushing from below with graduated dilators or by tracton from above as in the Vecchietti operation and its laparoscopic version. Recently, a bidirectional (axial and circumferential) tissue expansion technique was developed through introduction of balloon vaginoplasty where axial expansion which increases vaginal length is effected through traction on the catheter stem and circumferential expansion that increases the neovaginal width is achieved through increasing balloon distension. A native epithelium covered neovagina is more favorable to one made from foreign tissue.

The original Vecchietti operation was developed in 1965. Through laparotomy, sutures are attached to the traction device, which are connected to an oval plastic olive placed at the vaginal dimple. By gradually increasing suture tension, upward traction and continuous pressure lengthens the neovaginal space over a period of one week.

The laparoscopic Vecchietti vaginoplasty (LVV) was developed in 1992 with outcomes similar to those of the original technique. The long-term clinical and sexual function outcomes were evaluated after LVV in 106 patients with müllerian aplasia. Sexual function was successfully achieved in 97% of cases and was comparable with that reported in the control group [45, 46].

Another procedure is termed transretropubic traction (TRT) vaginoplasty. In this procedure, a plastic olive is placed on the vaginal dimple and is lifted by a mesh tape inserted through the space of Retzius and anchored to the anterior abdominal wall. Traction is placed on the mesh tape and maintained by applying a plastic clamp over a special supporting plate [47, 48].

Balloon vaginoplasty was recently introduced by El Saman et al. using a laparoscopic approach. A silicon coated balloon catheter is manipulated by a specially designed inserter, which is passed transperitoneally and through the pelvic floor where the balloon is positioned at the vaginal dimple. An upward, gradual (1-2cm/day) traction is applied on the catheter stem from the abdominal side for one week. A concomitant increase in balloon capacity (5ml every other day) to increase the width of the neovagina is also done [26]. Sexual relations were reported as early as one week after surgery [24].

The concept of manipulating the neovaginal depth and width is a novel characteristic of balloon vaginoplasty. The manipulation is done using different distensions and traction schedules. This only served to worsen existing dyspareunia. This resulted in more initial dyspareunia compared to cases predominant distension. On the other hand, patients who underwent a predominant increase in the balloon distention developed a neovagina that was wide but a bite shallower, and this resulted in minimal or no initial dyspareunia. With time, both groups showed improvement in dyspareunia.

The idea of retropubic balloon vaginoplasty was born when a patient with vaginal aplasia was referred to the As-siut University Women Health Center for laparoscopic balloon vaginoplasty. However, because of her past medical and surgical history, laparoscopy was considered risky due to extensive pelvic adhesion. Thus, the retropubic space was thought to be a safe pass for catheter insertion. Through a small supra pubic puncture, the catheter inserter was passed into the retropubic space just behind the pubic bone and guided to the center of the vaginal dimple. Then, a cystoscopic examination was performed to ensure bladder and urethral integrity. This was followed by gradual controlled distention of the balloon and traction on the catheter stem as described in laparoscopic balloon vaginoplasty [49]. One inherent limitation of balloon vaginoplasty is the need for a customized set of instruments such as the catheter inserter, supporting plate, and vaginometer for assessment of outcomes. The authors of original balloon vaginoplasty modified ed their the technique to allow its performance with conventional laparoscopic instruments and other commercially available accessories. The procedure was successfully preformed with comparable outcomes [24].

**Class Id - Cervical Aplasia (Isolated or Associated with Vaginal Aplasia)**

Cervical aplasia and / or hypoplasia are rare but challenging Müllerian anomalies. Hysterectomy was recommended by some authors when canalization procedures fail or are not viable for successful relief of the related symptoms [50]. Others adopted a conservative policy [50, 51]. Recently, two endoscopic procedures were described. The principal advantage is that dissection in atretic areas is not necessary as the uterovaginal anastomosis can be established endoscopically. The first technique is endoscopically monitored canalization of isolated cervical atresia. In this procedure, a special inserter with a silicon drain attached to its caudal end is passed
performed uteroneovaginal anastomosis. Uterovestibular anastomosis is treated by uterovestibular anastomosis or vaginoplasty and metra with or without upper hematocolpos. This condition patients with combined cervical and vaginal aplasia. The retropubic balloon vaginoplasty is the fastest way to create a neovagina. This was done using upward traction on a silicon coated catheter that was placed across the retropubic space to the vaginal dimple. Another balloon catheter was manipulated from below to the distended uterine cavity to drain. Hematometra; down traction is exerted on the lower uterine segment. Both up and down tractions are applied for few days to one week, and the balloons are monitored by transrectal US when they are suspected of being too close (kissing balloons). The final step is utero-neovaginal anastomosis [53].

Class Ie - Isolated Vaginal Aplasia: Segmental or Complete Agenesis or Hypoplasia Affecting the Vagina

Patients with this class of anomalies present with hema-tometra with or without upper hematocolpos. This condition is treated by uterovestibular anastomosis or vaginoplasty and uteroneovaginal anastomosis. Uterovestibular anastomosis is performed via combined abdominal and perineal approaches that mobilize the uterus from above and create the anastomo-sis from below. However, the logical management plan should involve creation of a neovagina by any of the aforementioned techniques followed by utero-neovaginal anasto-mosis. Because balloon vaginoplasty is the fastest way to create a functional neovagina, it is the first choice of treat-ment in cases of isolated vaginal aplasia [53].

Management of Class II (Unicornuate Uterus) Table 3 Fig. (2)

A unicornuate uterus may be not be diagnosed until the end of the reproductive years, especially if there is no func-
tioning rudimentary horn. MRI reliably helps make this diagnosis and represents the gold standard diagnostic tool for all subclasses of unicornuate uterus [18-20, 54-56]. High-resolution ultrasonography, intravenous urography and/or renal ultrasonography assist in the evaluation of ipsilateral renal agenesis, horseshoe kidney, and ipsilateral pelvic kidney [57].

Women with unicornuate uterus subclasses IIa, IIb and IIc (Fig. 2) are not normally considered for reconstruction metroplasty [7, 58]. The main indication for surgery is the presence of functioning endometrium in the accessory horn. Laparoscopic hemihysterectomy of the rudimentary horn is the treatment of choice [59-61]. The pedicle of the rudimen-tary horn is coagulated using bipolar coagulation, and it is excised (scissor excision) along with the ipsilateral Fallopian tube; the functional ovary is not removed. Morcellation may be required when the rudimentary horn is bulky. Successful pregnancy in the major horn has been reported after laparo-scopic removal of the accessory horn.

Hysteroscopic endometrial ablation of the accessory horn endometrium as well as hysteroscopic drainage of a hema-tometra in a noncommunicating accessory horn using electrocautery to create a communication between the horns has been reported [62, 63]. No specific major complications apart from those associated with laparoscopy and postsurgical obstet-ric outcomes have been reported [59, 60].

Class III - Uterus Didelphys and Bicornuate Uterus Table 4

Both a bicornuate and a didelphys uterus arise when mid-line fusion of the müllerian ducts is arrested [64]. An extra-ordinary capacity of the didelphys uterus is that, in many cases, intercourse is often possible in both vaginas. Moreover, simultaneous pregnancies in each uterus can occur, although this is rare. Each pregnancy may be considered a separate entity. In fact, a twin may be delivered after a long

<table>
<thead>
<tr>
<th>Classification</th>
<th>AFS Classification</th>
<th>Treatment Plan Classification</th>
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<tbody>
<tr>
<td>Class II</td>
<td></td>
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<tr>
<td>Unicornuate uterus with or without a rudimentary horn</td>
<td>When an associated horn is present, this class is subdivided into communicating and noncommunicating. The noncommunicating type is further subdivided on the basis of whether an endometrial cavity is present in the rudimentary horn. The clinical significance of this classification is that they are invariably accompanied by ipsilateral renal and ureter agenesis.</td>
<td>Class IIa- Unicornuate uterus without a rudimentary horn. When a rudimentary horn is present, (asymmetric lateral fusion defects) it is classified as follow: Class IIb – Communicating horn with or without functioning endometrium. Class IIc – Non-communicating without functioning endometrium. Class IId – Non-communicating with functioning endometrium.</td>
</tr>
<tr>
<td>Class Ila</td>
<td>No surgical treatment</td>
<td></td>
</tr>
<tr>
<td>Class IIb</td>
<td>No surgical treatment vs. excision</td>
<td></td>
</tr>
<tr>
<td>Class IIc</td>
<td>Usually no surgical treatment is required</td>
<td></td>
</tr>
<tr>
<td>Class IId</td>
<td>Laparoscopic hemihysterectomy is required as early as possible.</td>
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Table 3. Description of AFS and Treatment Plan Classification of Class II Müllerian Duct Anomalies
interval, ranging from 3 hours to 5 days to 8 weeks, after delivery of its sibling [65-67].

Without obstruction, all subclasses are usually asymptomatic until menarche. These patients are not candidates for surgical unification. The condition is associated with favorable obstetrical outcomes and usually remains undiagnosed until cesarean delivery or other procedures reveal its existence [39, 68, 69]. Undeniably, some authorities contend that the results of unification surgery, especially for the uterine didelphys, may be disappointing. Furthermore, cervical unification is technically difficult and can result in cervical stenosis or incompetence [70].

**Management of Class IIIa – Partial Bicornuate Uterus**

The partial bicornuate uterus is characterized by two separate horns with unified lower uterus and cervix originat-
ing from failure of fusion. Its reproductve performance is
good, and Strassmann metroplasty of the partial bicornuate
uterus is very rarely, if ever, required [71].

Management of Class IIIb – Complete Bicornuate Uterus

Complete bicornuate uterus rarely requires surgical re-
construction. The condition is associated with favorable ob-
estetrical outcomes and usually remains undiagnosed until
cesarean delivery or other procedures reveal its existence
[39, 68, 69]. Metroplasty should be reserved for women who
have a history of recurrent spontaneous abortions, midtrimes-
ter loss, and premature birth and in whom no other etio-
logic factor has been identified [58]. The Strassmann proce-
dure removes the septum by wedge resection with subse-
quent unification of the two cavities. Transabdominal metro-
plasty can considerably improve the reproductive perform-
ance of women with a bicornuate uterus who have had recur-
rent spontaneous abortions or premature deliveries before
surgery [72].

Management of Class IIIc- Partial Duplication with Two
Cervices = Didelphys Uterus

The decision to perform metroplasty should be individu-
alized, and only selected patients with a long history of
recurrent spontaneous abortions or preterm deliveries may
benefit from Strassmann metroplasty [70].

Management of Class IIIId- Complete Duplication of the
Uterus, Cervix, and Vagina

The management of a nonobstructing longitudinal sep-
tum is simple and appears to be associated with improve-
ment of fecundability. Excision of the septum allows simul-
taneous insemination of both hemiuteri during a single act of
coitus. Cold knife/ scissor excision and diathermy excision
are reported to have similar outcomes. The management of a
nonobstructing longitudinal septum in pregnancy is not clear.
Some authors advocate excision whereas others recommend
leaving it undisturbed unless it becomes obstructing during
labor [39].

Class IIIe- any of the above with Unilateral or Bilateral
Obstruction of Menstrual Outflow

In hemivaginal obstruction, the clinical presentations are
variable and depend on the degree of obstruction and
whether the obstruction is complete or incomplete [73]. The
obstructed unilateral vagina is a clear indication for resection
of the vaginal septum. Uterine didelphys with obstructed
unilateral vagina requires full excision and marsupialization
of the vaginal septum. After the septum has been excised,
laporoscopic evaluation and treatment of associated endome-
triiosis, adhesions, or both is recommended [74]. Excision of
an obstructing vaginal septum during pregnancy requires
leaving an adequate pedicle to help minimize possible bleed-
ing should the vaginal mucosa retract. In addition, leaving a
generous pedicle behind allows the surgeon to place hemo-
static sutures in basal parts of the septum rather than in the
walls of the vagina [39].

Hemihysterectomy with or without salpingo-oophorecto-
my is rarely indicated and should be avoided to provide the
best opportunity for a successful reproductive outcome.
Favorable obstetrical outcomes were reported in ten intra-
terine pregnancies. Five resulted in term delivery, four re-
sulted in preterm delivery, and one resulted in early sponta-
neous abortion [74].

Class IV: Complete or Partial Septate Uterus with or
without Fundal Depression or Obstruction Table 5

Management of Class IVa - A Complete or Partial Midline
Septum is Present within A Single Uterus without Fundal
Depression

A uterine septum can be diagnosed by HSG, hystero-
scopy, and laparoscopy. HSG reveals a 2-chambered uterus.
Laparoscopy can help the surgeon determine whether the
fundal contour is normal, which is the best approach for dis-
tinguishing between these entities. The partial septum does
not extend to the os. In the case of partial septa that is less
than 1 cm in length, there are usually no adverse effects on

<table>
<thead>
<tr>
<th>Classification</th>
<th>AFS Classification</th>
<th>Treatment Plan Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class V Complete or partial septate uterus</td>
<td>A complete or partial midline septum is present within a single uterus..</td>
<td>Class IVa - A complete or partial midline septum is present within a single uterus without fundal depression, Class IVb - A complete or partial midline septum is present within a single uterus with fundal depression, Class IVc – Any combination of the above in addition to septate cervix and vagina, Class IVd – Any combination of the above with unilateral or bilateral obstruction.</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Subclass of TP Classification</th>
<th>Available Treatment Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class IVa</td>
<td>Hysteroscopic metroplasty is usually required with or without laparoscopy</td>
</tr>
<tr>
<td>Class IVb</td>
<td>Hysteroscopic metroplasty is usually required with concomitant laparoscopy</td>
</tr>
<tr>
<td>Class IVc</td>
<td>Excision of vaginal septum &amp; hysteroscopic metroplasty with concomitant laparoscopy</td>
</tr>
<tr>
<td>Class IVd</td>
<td>Excision of vaginal septum, unification of obstructed hemi uterus is required as early as possible followed by hysteroscopic metroplasty.</td>
</tr>
</tbody>
</table>
reproductive outcomes, and operative intervention is not indicated based on data obtained from residual septa after hysteroscopic metroplasty [75]. Not all women with a partial septate uterus require surgery. However, those with recurrent spontaneous abortions, a single second-trimester loss, or histories of preterm delivery are considered candidates for correction [76-78].

Hysteroscopic metroplasty with concurrent laparoscopy is the state-of-the-art treatment plan [75, 79]. Laparoscopy helps reduce the risk of uterine perforation and diagnose associated pelvic pathology [80]. As an alternative, ultrasonographic guidance has been used as a monitoring tool for hysteroscopic metroplasty [81]. Hysteroscopic metroplasty can be performed by using microscissors, electrosurgery, or a laser. Thick septa with broad base are best excised with a resectoscope using monopolar or bipolar diathermy. In the septate uterus with cervical extension, the cervical portion is incised at the proximal aspect using Metzenbaum scissors, laser, or needle electrode followed by hysteroscopic metroplasty [82]. Division of the septum is considered complete when the hysteroscope can be moved freely from one cornual end to the other without obstruction [80, 82].

**Class IVb - A Complete or Partial Midline Septum is Present within A Single Uterus with Fundal Depression**

The fundal depression in this subclass results from incomplete fusion of the paramesonephric ducts at the fundus. Failure of resorption in the remaining part makes the down prolongation of the dividing septum. Its presentation and diagnosis is the same as that of the septate uterus. On laparoscopy, there is a shallow fundal depression. However, confusion may result by relying on the anatomic appearance of the external uterine fundus without correlating the depth of fundal depression with the depth of the internal division. Thus, it is important to incorporate the results of other investigations (MRI, US & HSG) with the laparoscopic appearance. The depth of the groove and length of the uterine septum depend in the adult uterus on the length of the incompletely fused müllerian ducts in the fetus. Symptomatic cases with this disorder are candidates of hysteroscopic metroplasty but are at a greater risk of perforation. The use of laparoscopy for monitoring is important for avoiding uterine perforation.

Reproductive performance appears to be considerably improved after surgery [82-87]. Definitive resection of an incomplete transverse vaginal septum is not required often. However, this anatomic congenital defect may contribute to primary infertility [91]. Dilation under anesthesia followed by regular dilation is sufficient for patients who experience difficult or painful coitus. In other cases where symptoms are inadequately addressed with dilation alone, definitive resection is required. Asymptomatic cases may manifest themselves for the first time during delivery where a generous episiotomy may be needed for a low-seated vaginal septa. High seated and thick septa, especially those involving a long segment of the upper third of the vagina, are best managed by cesarean section with definitive management accomplished at a later time [92, 93].

**Class IVc – Any Combination of the above in Addition to Septate Cervix and Vagina**

Excision of the vaginal septum is done either by cold knife/scissor division or diathermy resection. The decision to perform metroplasty should be individualized; only selected patients with a long history of recurrent spontaneous abortions or preterm deliveries may benefit from hysteroscopic metroplasty [70]. Hysteroscopic metroplasty is less morbid than abdominal metroplasty which is cost-effective and associated with limited risk of pelvic adhesions. Recovery is rapid with no prolonged postoperative delay in conception, and it can be performed in an outpatient setting. In addition, it allows for subsequent vaginal delivery [89].

**Management of Class IVd – Any Combination of the Septate Uterus, Cervix and Vagina with Unilateral or Bilateral Obstruction of Menstrual Outflow**

An obstructed unilateral vagina is a clear indication for resection of the vaginal septum. A septate uterus with an obstructed unilateral vagina requires full excision and marsupialization of the vaginal septum. After the septum has been excised, laparoscopic evaluation and treatment of associated endometriosis, adhesions, or both is recommended [74]. Excision of an obstructing vaginal septum during pregnancy requires the same precautions as mentioned for obstruction of hemivagina uterine didelphys [39]. However, definitive resection of a cervicouterine extension of the septum.

**Class V Longitudinal Fusion Defects Table 6**

This important class represents a group of defects that are not classified by the AFS system. Appropriate timely diagnosis and management will alleviate many unfavorable consequences on the reproductive organs. Fortunately, this group of defects is amenable to complete surgical correction.

**Class Va- Partial Transverse Vaginal Septum**

Incomplete transverse vaginal septum (TVS) allows menstrual flow to escape periodically, but hematocolpos and hematometra often develop over time. Complaints include foul-smelling vaginal discharge, dyspareunia secondary to a short vagina, and infertility. The existence and severity of the above mentioned symptoms varies according to the caliber of the opening. In addition, TVS can cause soft tissue dystocia in patients who eventually become pregnant [90].

Definitive resection of an incomplete transverse vaginal septum is not required often. However, this anatomic congenital defect may contribute to primary infertility [92]. Dilation under anesthesia followed by regular dilation is sufficient for patients who experience difficult or painful coitus. In other cases where symptoms are inadequately addressed with dilation alone, definitive resection is required. Asymptomatic cases may manifest themselves for the first time during delivery where a generous episiotomy may be needed for a low-seated vaginal septum. High seated and thick septa, especially those involving a long segment of the upper third of the vagina, are best managed by cesarean section with definitive management accomplished at a later time [92, 93].

**Class Vb-Complete Transverse Vaginal Septum**

TVS can occur at nearly all levels in the vagina, but most of these septa are located in the superior vagina [94]. Because management of a transverse vaginal septum is more technically difficult, treatment should occur at a tertiary-care center with a qualified surgical team. Cases presenting with acute pain can be treated with laparoscopic drainage, which provides a novel approach to the acute management of a transverse vaginal septum, providing pain relief without compromising the success of definitive surgery that can be performed at a later date [95]. Cases with concurrent imper-
Vaginal atresia, retropubic balloon vaginoplasty may be reatretic segments [105, 106]. In extreme cases of segmental gery to cover the raw area created from excision of long through or pull through vaginoplasty or reconstructive sur-

Segmental atresias usually require some form of push anomy from cervical agenesis, which is quite rare [104].
The presence of a cervix, which should distinguish this

Anomalies from vaginal agenesis [102, 103]. MRI can aid in detecting the most commonly observed uterine anomaly detected by HSG [122, 123]. It is a clinically benign anomaly--adverse obstetric outcomes are rare--and it may not affect reproductive outcomes [69, 122]. HSG reveals a single uterine cavity

forate hymen and transverse complete vaginal septum have been reported [95, 97].

Excision of the septum should be performed under transabdominal or transrectal ultrasound guidance. The widest possible excision should be made by making two crossing X-shaped incisions followed by excision of the four margins. Careful approximation of the upper (proximal) and lower (distal) edges at the base of the cut septum with fine delayed absorbable sutures is important in preventing recurrence [98].

Cyclical hematuria is a rare presentation of transverse vaginal septum and occurs by menstrual blood flow out of the lower urinary tract because of a vesical-vaginal communication. One treatment that has been reported for this condition is reconstruction using a transvaginal and transabdominal approach to create a direct anastomosis between the proximal vaginal segment and the distal vaginal pouch [99].

Class Vc- Lower Segmental Vaginal Atresia with Hemato-

colpos

Vaginal atresia occurs when the urogenital sinus (UGS) fails to contribute to the inferior portion of the vagina. The müllerian structures are usually normal, but fibrous tissue completely replaces the inferior segment of the vagina. Non-surgical methods may be recommended as the first approach in managing vaginal atresia. When nonsurgical methods fail, surgical approaches are recommended. Children with this anomaly may develop pyometrocolpos and present with obstructive uropathy, septicemia, or renal failure [100-102]. Transperineal ultrasonography reveals the presence of ovaries, a uterus, a cervix, and an obstructed blind-ending super-

Adequate surgical excision & end to end anastomosis +/- vaginoplasty.

Adequate surgical excision.

No surgical treatment, vs. dilatation v. excision.

Hymenectomy, hymenotomy, or circular excision to preserve hymeneal structural integrity in certain societies

Table 6. Description Treatment Plan Classification of Class V Müllerian Duct Anomalies (longitudinal fusion defects) which is not Classified by the AFS

<table>
<thead>
<tr>
<th>Classification</th>
<th>AFS Classification</th>
<th>Treatment Plan Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class V</td>
<td>Not mentioned</td>
<td>Class Va- partial transverse vaginal septum</td>
</tr>
<tr>
<td>Longitudinal fusion defects</td>
<td></td>
<td>Class Vb-complete transverse vaginal septum.</td>
</tr>
<tr>
<td>Class Vc</td>
<td>Adequate surgical excision &amp; end to end anastomosis +/- vaginoplasty.</td>
<td>Class Vc- lower segmental vaginal atresia with upper heamtocolpos.</td>
</tr>
<tr>
<td>Class Vd</td>
<td>Hymenectomy, hymenotomy, or circular excision to preserve hymeneal structural integrity in certain societies</td>
<td>Class Vd- imperforate hymen</td>
</tr>
</tbody>
</table>

Subclass | Available Treatment Options

Class Va | No surgical treatment, vs. dilatation v. excision.
Class Vb | Adequate surgical excision.
Class Vc | Adequate surgical excision & end to end anastomosis +/- vaginoplasty.
Class Vd | Hymenectomy, hymenotomy, or circular excision to preserve hymeneal structural integrity in certain societies
with a saddle-shaped fundal indentation. MRI findings show convex or flat external uterine contour. The indentation is broad and smooth. Aberrant vascularity within the fundal myometrium has been suggested [122, 124]. Arcuate uterus rarely, if ever, requires surgical correction. It may be managed similarly to septate uterus, but only in selected patients who fulfill poor reproductive performance criteria after exclusion of all other factors.

**CONCLUSION**

Early establishment of an accurate diagnosis is indispensable for planning treatment and preventing complications in the genital organs and surrounding systems. Classifying müllerian anomalies based on the available treatment options seems logical, and the inclusion of previously unclassified entities is important for comprehensive understanding and management of this group of disorders. The surgical approach for correction of müllerian duct anomalies is individualized to the type of malformation. The value of a given surgical procedure should be assessed on terms of its ability to improve a patient’s postoperative ability to have healthy sexual relations and achieve successful reproductive outcomes.

**Key Points**

Mullerian duct anomalies (MDA) represent an open ended spectrum of disorders that present either in isolation (affecting one organ e.g. tubes, uterus, cervix or vagina), in combination (affecting more than one organ) or in associations with other body systems anomalies (renal, skeletal, etc).

Appropriate and early diagnosis of MDA is of special importance in the prevention of complications. The diagnostic workup should include a search for associated anomalies and complications.

Treatment should be individualized, and only symptomatic cases with poor reproductive outcomes should be considered for surgical correction. Obstructive cases are given a unique priority for earliest corrective interventions. Simplified approaches for treatment of vaginal aplasia (e.g. balloon vaginoplasty) and cervical atresia (e.g. endoscopic canalization) should be adopted as they are technically simple to perform and safety and have a high efficacy.

Fertility preserving procedures should be adopted, refined and offered to cases with MDAs as a first choice treatment options.

**Expert Commentary:** The past five years have seen the introduction of balloon vaginoplasty where postoperative dilatation was not needed and sexual activity could be initiated as early as on the day of discharge from hospital (one week after surgery). Compared to other procedures, this is considered a great advance in management of vaginal aplasia where vaginal functions were initially achieved only after months of postoperative care. Also, the vagina created by balloon vaginoplasty mimics the natural vagina and represents an excellent choice for cases with cervical aplasia associated with vaginal aplasia where in these cases a uterus-vaginal anastomosis represents an attractive, effective and logical management option. We hope the future carries more benefits for cases with MDAs through blends of traction/distension vaginoplasty, refined corrective surgeries, uterine transplants and stem cell therapy.

**REFERENCES**


