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REVIEW

Oocyte developmental competence and embryo development: impact of lifestyle and environmental risk factors

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Alex C Varghese has been an active embryologist and researcher on IVF since 1997 and received his PhD in 2005 from University of Calcutta, India. In 2008, after his post-doctoral tenure at Centre for Reproductive Medicine, Cleveland Clinic, Ohio, USA, he became scientific director and chief embryologist at Fertility Clinic and IVF Department, AMRI Hospital. Alex founded lifeinvitro.com, a knowledge-sharing platform. His particular interests are molecular causes and prevention of male infertility, genomics and metabolomics in embryology and cryotolerance of reproductive cells. He is Secretary of Institute of Reproductive Health and Toxicology and Vice-President of Academy of Clinical Embryologists, India.

Abstract Oocyte development is the end result of a sophisticated biological process that is hormonally regulated and produced by highly specialized cellular lines that differentiate in early embryo/fetal development. Embryo development is initially regulated by maternal transcripts until replaced by embryonic genomic expression. Then, an assortment of hormones and local environmental factors in various concentrations along the reproductive tract (e.g. fallopian tube, endometrial lining) provide the protection, nutrients and means of communication for the embryo to implant and develop. Both oocytes and embryos are susceptible to environmental, occupational and lifestyle exposures that can exert direct toxic effects and disrupt hormones. While some exposures may produce reversible changes, others, especially those damaging germinal cells *in utero* or during prepuberty, may result in permanent sequelae that continue in future generations. This article reviews the main factors that affect female fertility and their possible influence on human reproduction. Some lifestyles, xeno-oestrogens and heavy metals are already known to compromise female reproductive function. Nonetheless, many questions remain and little is known about the effect of many other factors on female fertility. 

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Introduction

Significant refinements in IVF have emerged in the current decade because of active research with an emphasis on clinical pregnancy outcome. In recent times, substantial improvements have been made with ovulation induction, such as medications and regimens, and with culture technology, with a plethora of culture media available for preimplantation embryos. It is estimated that more than 3 million babies have been born worldwide through the application of some form of assisted reproduction technology (Andersen et al., 2007).

However, despite the fact that IVF is now a well-established treatment for infertility, success rates remain low, with only about 23% of women who undergo treatment becoming pregnant (HFEA, 2000). A large proportion of embryo loss appears to occur during preimplantation stages, with only 50% of embryos cultured *in vitro* reaching the blastocyst stage by day 6 (Hardy et al., 2001) and <15% of transferred embryos developing into a baby (HFEA, 2000). Although major advances have occurred in the field of assisted reproduction during the past 30 years, researchers and clinicians are still grappling to identify additional factors other than female age, quality of embryos that are transferred, quality of gametes (spermatozoa and oocytes) and response to hormonal stimulation that affect the success rates of IVF (Craft and Brinsden, 1989; Gerris, 2009; Macklon et al., 2006). This work reviews the current evidence on the associations between oocyte developmental competence and embryo development with lifestyles and environmental exposure risk factors.

Defining a good-quality oocyte

A good-quality oocyte is an oocyte at metaphase I (MI) or metaphase II (MII) with a clear zona pellucida, clear or moderately granular cytoplasm and a small perivitelline space, whereas a poor-quality oocyte is at the germinal stage, is post-mature or has a fractured zona pellucida (Balaban and Urman, 2006; Robker, 2008; Veeck, 1988). Oocyte quality (or maturation) also refers to the completion of the first meiotic division and complementary processes essential for subsequent fertilization and embryo development. This process consists of two general components, cytoplasmic maturation and nuclear maturation; both must take place to achieve healthy and competent oocytes (Swain and Pool, 2008).

Cytoplasmic maturation initiates the oocyte maturation process by producing specific factors, redistributing organelles and actively undergoing post-transcriptional modification of mRNA in preparation for fertilization and embryonic developmental competence (Smith, 2001; Heikinheimo and Gibbons, 1998).

Nuclear maturation begins shortly after with the continuance of meiosis following oocyte arrest at prophase I to form a haploid complement from the diploid state. During nuclear maturation, several events occur: (i) the nucleus undergoes germinal vesicle breakdown in which the nuclear membrane dissolves; (ii) chromatin condenses into discrete bivalents that align on the metaphase plate; and (iii) bivalents completely separate at MII (Heikinheimo and Gibbons,

1998; Smith, 2001). At this point, the mature oocyte and first polar body arrests and awaits sperm penetration (Heikinheimo and Gibbons, 1998; Smith, 2001). The quality of the developing embryo is directly related to the maturity and the quality of the oocyte, with poorer-quality oocytes being associated with lower fertilization rates and lower subsequent embryo development (Metwally et al., 2007).

In conclusion, a good-quality oocyte is an imperative initial prerequisite for preimplantation embryo development and survival, the establishing and maintaining of pregnancy and the development of the fetus.

Health and lifestyle

There are several factors that may adversely affect the female reproductive system and, in particular, that may reduce the quality of oocytes necessary for optimal fertilization. Among these considerations are physical, environmental, behavioural and socioeconomic factors. Periconception and pregnancy are extremely important stages that can have an adverse influence on future fertility and pregnancy outcomes (Younglai et al., 2005). The production and development of oocytes depends on the follicular environment, which can be altered by the mother's health and lifestyle. When the mother is diseased or under- or over-nourished or exposes herself to toxicants, such as drugs, alcohol and other chemicals, she also increases her risk for problems with fertility (Mtango et al., 2008; Younglai et al., 2005). As a result, reproductive processes may be negatively disrupted as well as production of an optimal-quality oocyte for fertilization and subsequent successful embryo development.

Nutrition and obesity

Nutrition is a critical factor in human survival. DNA replication and protein synthesis requires a source of energy obtained through food consumption. Therefore, it is important for mothers to maintain a healthy and balanced diet in the preimplantation and periconception periods. Otherwise, an insufficient amount of energy and building blocks will be available to maintain the body's natural processes. As a result, the female body will maximize the supply of resources for essential processes such as cell maintenance, circulation and neural activity by decreasing non-essential activities such as reproduction and fat storage (Wade and Jones, 2004). The availability of energy substrates can regulate the developmental processes of oocytes within the antral follicles by suppressing or inducing meiosis (Sutton et al., 2003).

Studies have examined the association of malnutrition and fertility and have concluded that undernourished women have irregular menstrual cycles or no cycle at all (ESHRE Capri Workshop Group, 2006). A nutritionally suppressed menstrual cycle is reversible, as shown in female athletes whose menstrual cycle returns after a decrease in energy expenditure or increase in body fat (Loucks, 2003). Maternal micronutrient intake periconceptionally, mainly of folic acid and vitamin B12 which act as methyl donors, is also important in DNA and protein modification; these micronutrients have long-lasting effects that contribute to

reduced frequency of neural tube defects in later development (Fleming et al., 2004).

There is also anecdotal evidence from IVF oocyte-donation programmes in developing countries, where females from very low economic conditions may opt to donate oocytes (Varghese et al., data not shown). It has been observed that the corona–cumulus mass is very sparse in oocytes from these donors, which reflects the reproductive system's ability to compensate for the reduced nutrients/essential growth factors by cutting down many of the number of somatic cells (Varghese et al., data not shown). The consequences of such rescue may be reflected in the oocyte developmental competence or future health status of the fetus.

In an animal study using heifers, Adamiak et al. (2005) demonstrated the negative effects of the moderately fat condition and a high level of feeding, which impaired oocyte quality. These heifers were hyperinsulinaemic and the very high plasma insulin concentrations had detrimental effects on blastocyst yields (Adamiak et al., 2005).

Likewise, evidence has also shown fertility outcomes are compromised when the body is overnourished. Obesity is closely related to reproductive performance and has been linked to a number of adverse reproductive outcomes including anovulation, infertility and poor response to assisted conception treatments (Metwally et al., 2007). Metwally et al. (2007) examined the oocyte quality and embryo quality among obese women in various studies and concluded that women who are obese have no adverse effect on oocyte quality but a significantly poorer embryo quality and also reduced fertilization outcomes than women with normal body mass index.

Obesity can also compound with other health issues to decrease reproductive success. Morbid obesity in conjunction with polycystic ovarian syndrome (PCOS), the most common cause of infertility due to ovulatory dysfunction (with anovulatory dysfunction accounting for 75% of the cases) has been shown to negatively affect embryo quality and subsequent pregnancy outcome (Hull, 1987; Jungheim et al., 2009; Knochenhauer et al., 1998; Patel and Carr, 2008). Oocytes from PCOS patients show different gene expression profiles in comparison with normal patients (Wood et al., 2007, 2003). Basically, different gene expression profiles have been detected for putative androgen receptors, peroxisomes and genes associated with chromosome alignment and segregation during cell division. This suggests that androgens and other activators of nuclear receptors may play a role in differential gene expression in PCOS oocytes. In addition, defects in meiosis or early embryonic development may contribute to the reduced developmental competence that are also found in these oocytes (Wood et al., 2003, 2007). Microarray data shows that a number of the genes whose transcript abundance is altered in the PCOS oocyte contain putative nuclear receptor binding sites (androgen receptor, proliferator-activated receptor γ (PPAR- γ) and/or PPAR- γ –retinoic X-receptor within the proximal promoter of several genes differentially expressed in PCOS oocytes). A study by Jungheim et al. (2009) demonstrates that embryos of patients with morbid obesity had a higher degree of fragmentation on day 3 and pregnancy rates were decreased compared with non-morbidly obese PCOS patients, an observation that suggests

that aggressive weight loss may be an important step in IVF treatment to improve the quality of the embryo and improve the chances of pregnancy for PCOS patients who are also morbidly obese.

Not only is oocyte quality reduced in obese women, but lower antral follicle counts are observed as well (Robker, 2008). This observation is supported by a systematic review demonstrating that a majority of reports indicate that a higher dose of gonadotrophins is required to elicit a response in obese IVF patients (Maheshwari et al., 2007). This may be due to altered absorption and/or metabolism of gonadotrophins, thus contributing to a reduced number of oocytes (Robker, 2008).

Increased insulin resistance in obesity is another primary factor in oocyte quality and embryo development (Minge et al., 2008). Robker (2008) examined the association between oocyte and embryo quality and obesity. General observations were that obese women may have altered oocyte developmental competence and suboptimal early embryo development, which is likely to result in decreased pregnancy rates in these women. A further study by Robker et al. (2009) demonstrated that obese women exhibit an altered ovarian follicular environment, particularly increased metabolite, C-reactive protein and androgen concentrations, which may be associated with poorer reproductive outcomes observed in these patients (Robker et al., 2009). Obese women may be able to normalize their fertility through modest weight loss, moderate exercise regimens and/or medical treatments that regulate insulin concentrations (Robker, 2008).

Obesity can also influence blood flow and negatively impact developmental outcomes. Poor follicular vascularity can affect follicular temperature and surrounding tissue, thus increasing endothermic activity within ovulatory gradients (Sutton et al., 2003). In addition, poor vascularity and low oxygen concentrations may decrease the viscosity of follicular fluid, which helps to facilitate entry into the Fallopian tube (Sutton et al., 2003).

Cholesterol and other lipids are essential for the development of several hormones, including sex hormones. However, abnormally high concentrations of some lipids, such as cholesterol, pose health risks for obesity, diabetes and heart disease. Although there is little evidence about the role of lipids, Sutton et al. (2003) suggested that abnormally high lipid concentrations are negatively correlated with oocyte maturation and follicular environment quality, as they inhibit germinal vesicle breakdown, which prevents the oocyte from proceeding beyond the arrested prophase I stage (Sutton et al., 2003). The follicular environment programmes the oocyte developmental competence by controlling the germinal-vesicle stage. Initially, higher lipid concentrations act as an energy source during the early stages of oocyte maturation but have been shown to decline over the course of oocyte maturation *in vitro*. High lipid concentrations may consequently inhibit oocyte release from the follicle (Kim et al., 2001; Ferguson and Leese, 1999). Undoubtedly, oocyte growth and development are dependent on the nurturing capacity of the follicle.

In a more recent study, excessive nutrient exposure prior to and during conception was shown to impair early embryo development by way of up-regulating mitochondrial activity (Igosheva et al., 2010). More interestingly, there was an

observed change in the distribution of mitochondria within the oocyte of obese women. In the oocytes of lean women, there was an even distribution of mitochondria throughout the cytoplasm. Yet Igosheva et al. (2010) observed a dense clustering of mitochondria in the outer region of the ooplasm and surrounding the nucleus in obese women. This observation is supported by other research which has shown omega-3 polyunsaturated fatty acid ($n - 3$ PUFA), a popular lipid supplement known for its health benefits, has been linked to adverse effects to the development of mouse oocyte and embryo (Wakefield et al., 2008). In comparison with unexposed mouse oocytes, those exposed to high concentrations of $n - 3$ PUFA during periconception were observed to have twice the concentration of reactive oxygen species (ROS) and a shift in the distribution of mitochondria (and mitochondrial calcium) to the cortical region of the oocyte (Wakefield et al., 2008). ROS are known to have deleterious effects on oocytes (Halliwell and Gutteridge, 1988; Tarin, 1996) but the precise functional significance of perturbations in mitochondrial calcium is not known (Wakefield et al., 2008). The fertilization of mouse oocytes exposed *in vitro* to high concentrations of $n - 3$ PUFA was more likely to be arrested at the 2-cell stage or various cleavage stages than the control group (Wakefield et al., 2008). Similarly, fragmented embryos were found in the oviducts in two out of five obese human females that failed to produce blastocysts (Igosheva et al., 2010). In conclusion, high lipid concentrations, as a consequence of maternal obesity or voluntary supplement intake, have been associated with mitochondrial changes, oxidized redox state and increased oxidative load. Ultimately, these changes result in poor implantation rates and subsequent pregnancy rates.

Maternal stress

Chronic exposure to high stress levels during pregnancy has been demonstrated in some studies to have negative effects on pregnancy outcome (Mulder et al., 2002), in which maternal stress was characterized by a physiological response that stimulates glucocorticoid secretion by the adrenal medulla in response to energetic, health or psychological stressors. Maternal stress may have an effect on the preimplantation embryo in very early pregnancy (Cikos et al., 2007), in which adrenergic receptors for epinephrine and norepinephrine were detected in ovulated mouse oocytes and preimplantation embryos, thus suggesting these catecholamines could directly affect embryo development in the oviduct during periconception and providing evidence of the influence of maternal stress on very early embryonic stages. In a more interesting longitudinal study of the cumulative effects of 'real life' stress, the daily changes in the concentrations of urinary cortisol and reproductive hormones were measured (Nepomnaschy et al., 2004). The results indicated that an increase in cortisol concentrations is associated with increases in gonadotrophins and progesterone concentrations. When the combination of higher cortisol concentrations and lower progesterone concentrations occur during the midluteal phase between cycle days 4–10, implantation may be adversely affected (Nepomnaschy et al., 2004). High midluteal progesterone concentrations have been shown to be associated with an increase

in the success of embryo implantation and subsequent pregnancy (Baird et al., 1999). More randomized controlled studies with human subjects are needed to confirm that both findings are relevant.

Intense exercise

Exercise has been touted to have many health benefits and many women are encouraged to participate in fitness activities. In recent decades, an increasing number of women are engaging in intense exercise training, including marathons and triathlons. Yet chronic intense exercise training in female athletes has been linked to clinical reproductive abnormalities including delayed menarche, primary and secondary amenorrhoea and oligomenorrhoea in 6–79% of women (Warren and Perlroth, 2001). Menarche refers to the first menstrual cycle, usually occurring around 12 years of age in the USA (Anderson et al., 2003). Moreover, the American Society of Reproductive Medicine specifically defines primary amenorrhoea as a delay of menarche to 15 years of age whereas secondary amenorrhoea refers to cessation of menstruation for 6 consecutive months in females who have attained menarche and oligomenorrhoea refers to amenorrhoea that involves less than nine cycles a year (American Society for Reproductive Medicine, 2004). These reproductive abnormalities are a result of an energy deficit when female athletes expend more energy than they consume in their diet. Furthermore, amenorrhoea is associated with the absence of ovarian follicular development, ovulation and luteal function, which are all necessary for a successful pregnancy (Nattiv et al., 2007).

Warren and Perlroth (2001) conferred two hormone profiles: hypo-oestrogenism in female athletes participating in sports that emphasize thinness (i.e. ballet, gymnastics, figure skating) and hyperandrogenism in female athletes involved in sports that emphasize strength (i.e. swimming, rowing). Specifically, hypo-oestrogenism resulted from the suppression of the hypothalamic pulsatile release of gonadotrophin-releasing hormone, which normally occurs every 60–90 min and limits pituitary secretion of LH and, to a lesser extent, FSH, which in turn limits ovarian stimulation and oestradiol production. Alternatively, in hyperandrogenism, the overproduction of androgens associated with hirsutism (abnormal growth and distribution of thick hair) and virilism (appearance of secondary male characteristics) in females may be the underlying cause of oligomenorrhoea (Hagmar et al., 2009). The mechanism is unknown.

No studies have been found to determine the direct effects of chronic intense exercise on oocyte developmental competence or embryo quality in female athletes.

Smoking

Exogenous factors, such as smoking, can greatly affect follicular and oocyte maturation. Approximately one-third of women of reproductive age in the USA and Europe are smokers (Soares and Melo, 2008). Tobacco cigarettes and smoke are composed of more than 4000 chemicals, including toxicants and carcinogens (Cooper and Moley, 2008). Most

research to date has been focused on the effects of nicotine (toxic alkaloid), cadmium (heavy metal in cigarettes), cotinine (major metabolite of nicotine) and benzo[a]pyrene (polycyclic aromatic hydrocarbon) and their links to disease states of the female reproductive system (Cooper and Moley, 2008; Zenzes, 2000; Zenzes et al., 1995). Tobacco smoke not only contributes to cardiac disease and cancer, but it also compromises several areas within the female reproductive tract, including steroidogenesis, folliculogenesis and ovulation (Cooper and Moley, 2008). Oocyte maturation and quality and embryo quality are also negatively affected by exposure to tobacco smoke (Soares and Melo, 2008; Zenzes, 2000). The nicotine in tobacco smoke alters spindle formation and blocks oocytes in MI of meiosis, ultimately leading to nondisjunction and an increased likelihood for aneuploid embryos (Cooper and Moley, 2008).

Smoking can also increase the vascular endothelial growth-factor fms-like tyrosine receptor-1 (sVEGFR-1), which complexes with vascular endothelial growth factor A to inhibit angiogenesis and therefore compromises the vascularity within the ovaries (Cooper and Moley, 2008; Motejlek et al., 2006). It is unclear which of the toxicants in cigarette smoking causes the endothelial cells to up-regulate sVEGFR-1 (Motejlek et al., 2006). Yet the compromised blood flow to the ovaries in this scenario may decrease delivery of necessary resources to maintain oocyte quality.

Chemicals within tobacco smoke inhibit steroid hormone production and decrease oestrogen availability. They reduce the oestradiol production in the follicles, thus negatively impacting the oocyte and its maturity (Younglai et al., 2005). In addition, tobacco smoke accelerates follicular depletion and diminishes ovarian reserve early in reproductive age so that menopause occurs 1–4 years earlier in women who smoke (Cooper and Moley, 2008; Freour et al., 2008; Soares and Melo, 2008). Tobacco smoke also decreases expansion of the cumulus–oocyte–complex (COC) and interferes with the COC retrieval rate (Cooper and Moley, 2008). Thus, heavy smokers are more susceptible to premature ovarian failure than non-smokers with little to no exposure of environmental tobacco smoke.

Furthermore, tobacco smoke also impairs several additional tubal and uterine processes that increase the risk for infertility. It may impair uterine receptiveness and increase zona pellucida thickness and follicular growth inhibition. Toxic compounds in the reproductive tract may also alter gene expressions during embryo development (Cooper and Moley, 2008).

Tobacco cigarettes not only cause potential fertility risks for women who smoke, but also pose an environmental hazard for non-smokers, resulting in a negative fertility outcome (Huang et al., 2009; Neal et al., 2005). In a study by Zenzes and Reed (1998), environmental exposure to cigarette smoke by female non-smokers was enough to detect cotinine, a nicotine metabolite found in cigarettes, in the serum suggesting its pervasiveness in the environment. These toxic compounds decrease the optimal quality for an oocyte, affecting several processes necessary for normal maturation, ovulation and fertilization. In a study with female mice exposed to chronic cigarette smoke and cigarette smoke condensates for 4 weeks, significantly fewer oocytes developed to the blastocyst stage ($P < 0.05$; Huang

et al., 2009). Therefore, the present study concludes that there is substantial evidence to implicate the role of tobacco smoke in adverse reproductive function and fertility outcomes.

Environmental pollutants, plasticizers and heavy metals

Overall, oocyte development is a three-stage process with its corresponding windows of vulnerability: fetal development, birth and puberty. In all these processes, female gametes may be disturbed by exogenous substances that alter its normal growth and development.

Environmental pollutants, plasticizers and heavy metals have been linked to several adverse effects on the female reproductive system in various animal studies. The general population is exposed ubiquitously to numerous environmental factors, including polychlorinated biphenyls, polycyclic aromatic hydrocarbons, pesticides and pesticide metabolites, to some extent, either directly as an occupational exposure or indirectly. Research in this area remains undeveloped and there are sparse and inconsistent data. Evidence to link environmental factors to compromised fertility is weak (Younglai et al., 2005). However, the absence of conclusive evidence does not suggest a nonexistence of negative effects on the developmental competence of oocytes or early embryo by the numerous environmental factors that modern society produces.

Pesticides

Pesticides are used in agriculture to control insects and weeds and in public health to protect humans from several types of diseases. There are few studies that have examined the effects of environmental pesticides and pollutants on the female reproductive tract but pesticides have been proved detrimental to the female reproductive tract (Bretveld et al., 2006) and pesticides and pollutants have been shown to interfere with the female sex hormones that regulate the ovarian cycle (Cooper et al., 2005; Farr et al., 2004). These chemicals disrupt the hormonal function in one or more of the seven mechanisms that promote healthy function of the reproductive system: hormone synthesis, hormone release and storage, hormone transport and clearance, hormone receptor recognition and binding, hormone post-receptor activation, thyroid function and central nervous system function (Bretveld et al., 2006). It is possible that pesticides cause hormonal dysfunction by one of the following modes of action: direct damage of cellular structures, interference of biochemical pathways necessary for normal cell function and biotransformation resulting in toxic metabolites (Bretveld et al., 2006). The exact details of the mechanisms that cause hormonal disruption are not known for many pesticides. Some pesticides, such as lindane, atrazine and mancozeb, are known to have hormonal and ovotoxic properties, delaying ovulation and altering the oestrous cycle in animal models (Farr et al., 2004; Guillette and Moore, 2006; Kumar, 2004). In particular, atrazine has been shown to decrease LH concentrations, resulting in anovulation (Bretveld et al., 2006). Another popular pesticide, polychlorinated biphenyl 126, has been

associated with follicle destruction and altered oocyte maturation and blastocyst development (Younglai et al., 2005). Alternatively, dichlorodiphenyltrichloroethane, a pesticide known to be an endocrine disruptor, is still commonly used in Africa, India and Mexico to control mosquito infestation and there are still no reports of decreases in fertility (Attaran and Maharaj, 2000).

Bisphenol A, phthalates and other plasticizers

Bisphenol A (BPA), phthalates and other plasticizers are ubiquitous as components of polycarbonate plastics, resins lining food/beverage containers and additives in a variety of consumer products (Calafat et al., 2005). Several effects of these oestrogenic disruptors on the female reproductive system have been well characterized in animal models (Chen et al., 2007; Eichenlaub-Ritter et al., 2008; Flaws et al., 1994; Susiarjo et al., 2007). BPA exposure affects early meiotic events in the oogenesis, such as synapsis and recombination, increasing oocyte loss and creating chromosomally abnormal eggs (Eichenlaub-Ritter et al., 2008; Hunt et al., 2003; Susiarjo et al., 2007). BPA also produces progressive proliferative lesion of the oviduct and more severe pathologies of the uterus including adenomyosis, leiomyoma and atypical hyperplasia in murine model (Newbold et al., 2007). Similarly, BPA exposure to mouse oocytes have profound effects on oocyte growth and maturation that lead to increased risks of aneuploidy even at low doses and in the short term (Hunt et al., 2003). Furthermore, Susiarjo et al. (2007) exposed pregnant female mice to BPA and observed an increase risk in mis-segregation during the meiotic prophase of oocyte development which subsequently translated into aneuploid oocytes and embryos. These observations in animal models can be used to develop a hypothesis for additional testing as well as determining their relevance to the fertility of female humans.

Heavy metals

Heavy metal exposure may also have severe effects on the female reproductive system. Despite available research data regarding the effects of heavy metals on the structure and function of the ovary and fetal development, more research is needed for conclusive evidence (Kumar, 2004).

Irgens et al. (1998) studied the associations between occupational lead exposure and reproductive outcome and observed that offspring of lead-exposed mothers had an increased risk of low birthweight and neural tube defects. Others (Factor-Litvak et al., 1991; Recknor et al., 1997) have suggested that pregnancies occurring despite an increased heavy metal load are at greater risk of low birthweight as well as preterm births. Thorlacius-Ussing et al. (1985) reported on the effect of mercury stored in the anterior pituitary of exposed rats, which in turn might affect the production of gonadotrophins and overall reproductive function (Thorlacius-Ussing et al., 1985). Various in-vitro and in-vivo studies have reported an accumulation of heavy metals in the ovary itself (Paksy et al., 1997, 2001). In Paksy et al. (2001), the concentration of lead in the follicular fluid of 23 women (0.056 $\mu\text{mol/l}$) was higher than the serum con-

centration but was not high enough to affect progesterone secretion by the ovaries. Alternatively, when $\geq 1600 \mu\text{mol/l}$ was placed with human granulosa cells *in vitro*, a significant decrease in progesterone and detachment of cells from each other was observed (Paksy et al., 2001). In a more recent study of nine women exposed to 0–21 $\mu\text{g/kg}$ lead, the mean lead concentrations in the follicular fluid of the non-pregnant group were double those of the pregnant group ($P = 0.0059$; Silberstein et al., 2006). These studies suggest heavy metals may have an effect on female reproductive system, damaging the ovary and the hormonal production and release process.

Furthermore, the effects of combined exposure to lead and cadmium on granulosa cells of female rats have also been studied (Nampoothiri et al., 2007). Both metals accumulated in the ovary after metal exposure and caused a decrease in reduced glutathione content along with elevated lipid peroxidation in all groups. The authors concluded that toxic metals disturbed membrane integrity of cells via ROS, thereby decreasing gonadotrophin binding and leading to reproductive dysfunction in receptor binding, steroidogenesis and hormone production.

Effects of fluids *in vivo* versus *in vitro* on gamete and embryo integrity

Preimplantation embryos are vulnerable to environmental conditions that may affect pre- and post-natal future growth and development. Some studies have shown that cultured mouse embryos, after transfer, result in reduced fetal growth as compared with in-vivo counterparts (Biggers et al., 1965). In humans, a number of studies have shown an increase in preterm delivery, low birthweight and perinatal mortality in singleton pregnancies following assisted reproduction technology compared with regular natural conception (Doyle et al., 1992; Hansen et al., 2002; Olivennes et al., 2002; Schieve et al., 2002). A recent study has demonstrated that mouse embryo culture conditions following embryo transfer result in altered behaviour of the offspring (Ecker et al., 2004). Culture media has also been linked to a higher exposure of ROS to embryos due to the differing oxygen tension *in vitro* as compared with *in vivo* (Agarwal et al., 2006; Martin-Romero et al., 2008). Lower oxygen concentrations of 5% as compared with 20% oxygen in culture media produced a higher number of good-quality preimplantation human embryos; these embryos exhibited faster cleavage, no fragmentation, an expanded blastocoel, a cohesive trophectoderm and an oval-shaped inner cell mass (Kovacic and Vlasisavljevic, 2008; Waldenstrom et al., 2009). In a prospective study using 988 sibling human oocytes, Kovacic and Vlasisavljevic (2008) compared 5% and 20% oxygen on fertilization rates and embryo development: no change was found with respect to fertilization rates, which suggests oxygen concentrations have no effect, but a notable 31% of embryos under low oxygen tension developed into optimal-quality blastocysts compared with 14.6% of embryos cultured in a higher oxygen environment ($P = 0.001$). In response to culture conditions, an increase in hydrogen peroxide production and attendant risk from ROS have previously been described (Johnson and Nasr-Esfahani, 1994; Nasr-Esfahani et al., 1990).

The overproduction of extracellular ROS due to higher levels of oxygen exposure during IVF culture could create suboptimal culture conditions and lead to altered gene expressions and impaired energy production that may harm oocyte and embryonic development (Agarwal et al., 2006; Harvey et al., 2002; Martin-Romero et al., 2008).

Alternatively, the COC within the in-vivo environment, once picked up by the Fallopian tubes during ovulation, resides in a micro-environment bathed with essential nutrients and growth factors. The number of spermatozoa reaching the site of fertilization is also very low: approximately 250 spermatozoa make their way to the COC and the zona pellucida of the oocyte (Williams et al., 1993). However, in in-vitro culture, 10,000–100,000 spermatozoa aggregate to the COC in a volume of 50–500 μ l. This removes most of the somatic cells associated with the oocyte. Moreover, spermatozoa with lost energy reservoir settle in the vicinity and increase the oxidative stress around the female gamete. It is not understood whether it is the embryo's role to bring the cumulus cells still attached to it during its transit from the Fallopian tube to the uterus during the growth phase.

Co-culture, or the placement of human and nonhuman live cells alongside the embryo in media culture, is a way of improving the developing embryo's environment. Oviduct epithelial cell co-culture promotes IVF in human, bovine and porcine species. Several studies indicate that cumulus co-culture or embryo transfer with cumulus cells may enhance clinical pregnancy rate (Gabler et al., 2008; Lin et al., 2009; Parikh et al., 2006).

Microfluidic platforms that enable embryo culture in precisely defined, submicrolitre volumes (5–500 nl) are being investigated by several groups (Melin et al., 2009). Their use with embryo culture *in vitro* is a tempting approach that may reduce some of the limitations of traditional microdrop culture on embryo growth and may enhance the research into gamete and embryo physiology. These devices may lead to better in-vitro embryo development and quality by more closely mimicking the in-vivo growth conditions (Heo et al., 2010; Krisher and Wheeler, 2010).

Bean et al. (2002) found that mouse embryos were most prone to nondisjunction during the first two cleavage divisions, whether *in vitro* or *in vivo*, and that there was a significantly lower incidence of mosaicism under low oxygen tension, which suggests that a small change in culture media can result in significant changes to the genetic quality of the early embryo especially in situations where there is a predisposing risk for nondisjunction. An excellent review of culture media and its effects on embryo developmental competence (Lane and Gardner, 2007) suggests that manufactured culture media (i.e. DM1/2/3, G1/G2 media, KSOM and Quinns Advantage) for either system, sequential or monoculture, are designed to allow for embryo growth to the blastocyst stage and are sufficient. Implantation and pregnancy rates are likely influenced by supporting factors such as contact supplies, oil overlay, temperature and pH as well as the level of quality control and assurance of the laboratory environment (Lane and Gardner, 2007; Pool, 2005). For example, deviations of media pH from the acceptable range of pH 7.0–7.4, such as any small fluctuation exceeding pH 7.45, may lead to oocyte fragmentation (Butler et al., 1988). Also, oocyte culture maintained at

37°C and then cooled down to room temperature in as little as 10 min has been observed to exhibit disruption to the spindle structure, including a reduction in spindle size, a disorganization of microtubules within the spindle itself or a complete lack of microtubules (Pickering et al., 1990).

In another observation by Sjöblom et al. (2005), a lack of cytokine in culture media of murine embryos reveals lower fetal growth, a more rapid compensatory growth after birth, increased body mass as adults and greater fat deposits in the abdomen compared with controls. The features are noted to be quite similar to those found in offspring of women who were undernourished during their pregnancies (Roberts, 2005). On the other hand, a study by Caperton et al. (2007) demonstrated no link between de-novo point mutations and assisted reproduction treatment in surviving infants, thus suggesting that culture media is unlikely the cause of point mutations (Caperton et al., 2007; Rosenwaks and Bendikson, 2007). Consequently, in-vitro and in-vivo studies support the idea that an embryo's environment is critical for its future and the parallel analysis of in-vivo and in-vitro models is required to understand those mechanisms (Fleming et al., 2004).

Some of the mechanisms that could affect the potential early embryo development are related to epigenetic events (Lucifero et al., 2004). Observations from murine studies suggest that in-vitro conditions cultivate abnormal epigenetic modifications and subsequently affect gene expression and fetal development (Maher, 2005; Mann et al., 2004). It is known that gametogenesis and early development are decisive periods for the acquisition and maintenance of genomic imprints (Lucifero et al., 2004). The methylation pattern of imprinted genes has been shown to be modified in a gene-specific manner in response to embryo culture conditions, indicating effects of the environment on the preservation of gene methylation profile (Doherty et al., 2000). Recently, there has been considerable interest in the epigenetic effects of assisted reproduction technology on the preimplantation embryo. Hormonal stimulation, egg retrieval, IVF, intra-cytoplasmic sperm injection, micro-manipulation of gametes, preimplantation genetic diagnosis (or screening) and in-vitro maturation of oocytes have all been implicated and may have long-term effects on patterns of gene expression (Katari et al., 2009). In a recent comparison of more than 700 genes between children conceived from IVF procedures and children conceived naturally, the genes of IVF children revealed a small but significantly different level of DNA methylation than their counterparts; either the environmental stress of the assisted reproduction treatment or a characteristic of the infertile patient population served by assisted reproduction technology is perhaps the reason (Katari et al., 2009).

More interestingly, Market-Velker et al. (2010) recently demonstrated that high levels of ovarian stimulation in mouse models is associated with disruption of DNA methylation – previously thought to occur only in maternal alleles – in both maternal and paternal alleles of the embryos. This is the first study to evaluate the effects of high and low levels of ovarian stimulation on DNA methylation at the imprinted loci *Snrpn*, *Peg3*, *Kcnq1ot1* and *H19* and the study implicated these loci as causal factors of imprint disorders, Angelman and Beckwith–Wiedemann syndromes. The study evaluated two different dosages of hormones – 6.25 IU

(low) and 10 IU (high) – and noted that all four imprinted genes investigated displayed a dose-dependent response. In embryos from spontaneously ovulating female patients, the normal response towards the genes *Snrpn*, *Peg3* and *Kcnq1ot1* is paternal expression and maternal methylation. However, at the high-hormone dosage, a significant number of embryos displayed no methylation on the maternal alleles of *Snrpn*, *Peg3* and *Kcnq1ot1* as compared with the low-hormone dosage. Similarly, *H19* is normally maternally expressed and paternally methylated but results demonstrated an increase in maternal methylation and more surprisingly, a loss of methylation in the paternal *H19* allele with the high-hormone dosage (Market-Velker et al., 2010). Hyperovulation is believed to disrupt methylation mechanisms during oogenesis by either the unintended rescue of oocytes that were destined for the atresia pathway or the induction of ovulation in premature oocytes before proper or complete imprints can be acquired (Baerwald et al., 2009; Ludwig et al., 2005; Market-Velker et al., 2010; Paoloni-Giacobino and Chaillet, 2004; Van der Auwera and D’Hooghe, 2001). Furthermore, Market-Velker et al. (2010) suggest that events during oocyte maturation may be regulating genomic imprinting of paternal alleles in the embryo, but the exact mechanism is unknown. In summary, Market-Velker et al. (2010) postulated that hyperovulation most likely has dual effects during oogenesis, acting to disrupt the acquisition of imprints in the growing oocyte and causing molecular changes that disrupt maternal-effect gene products subsequently required for genomic imprint maintenance during preimplantation development.

However, the associations between the causal mechanisms relating embryo environmental sensitivity and abnormal post-natal development are unlikely to reside completely at the epigenetic level (Fleming et al., 2004). Other important variables include the maternal nutrient supply gained at an early stage of development and, of course, the quality of the oocyte (Sutton et al., 2003; Thomas et al., 2003). In addition, the ionic and nutrient composition of the oviduct and uterus is extremely important (Leese et al., 2001). Energy substrate concentrations may fluctuate between human oviduct and uterine fluids (Gardner et al., 1996). Also, nutrient concentrations are relatively low compared with those routinely contained by culture media (Leese, 2003). The implications of impaired embryonic metabolic activity on fetal and post-natal development are potentially serious. As a result, the mechanisms and consequences involved must be explored further (Fleming et al., 2004).

Conclusions

A growing body of literature shows a wide variety of substances may adversely affect the female reproductive system, impairing oocyte and embryo developmental competence. However, the evidence for some of the adverse effects on fertility is incomplete and knowledge is still fairly limited. Although knowledge of the effects of individual products is expanding, the reality is more complex. Single exposure does not occur and very few studies address the consequences for female fertility of simultaneous, complex exposure to compounds such as food additives, toxicants,

contaminants, outdoor and indoor air pollutants, endocrine disruptors and hazardous substances. A clear side effect for the lack of a broad picture of complex exposures is an underestimation of the consequences of exposing the population to a wide variety of products. Finally, the oocyte and embryo represent a developmental window during which susceptibility to environmental circumstances is prevalent. Careful handling of human embryos and lower dosage of hormones for ovarian stimulation will probably reduce the potential for adverse consequences. In addition, investigations on the influence of female health and environmental or lifestyle exposures/preventions on oocyte and embryo development could be more important, or at least just as important, than the study of multiple ovarian stimulations with gonadotrophins prior to an IVF treatment.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2010.11.009.

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