Risk factors for multiple gestation in women undergoing intrauterine insemination with ovarian stimulation

Eleonora B. Pasqualotto, M.D.,*† Tommaso Falcone, M.D.,*† Jeffrey M. Goldberg, M.D.,*† Christina Petrauskis,* David R. Nelson, M.S.,‡ and Ashok Agarwal, Ph.D.*§

The Cleveland Clinic Foundation, Cleveland, Ohio

Objective: To identify whether sperm characteristics after washing and/or ovulation induction cycle characteristics can predict the occurrence of multiple conception in patients undergoing ovarian stimulation and IUI.

Design: Retrospective study.

Setting: A gynecology clinic and an andrology laboratory at a tertiary care facility.

Patient(s): One hundred patients with single pregnancies and 22 patients with multiple pregnancies.

Intervention(s): Patients underwent ovarian stimulation and IUI with their partner’s sperm.

Main Outcome Measure(s): Relation of patient characteristics, ovarian stimulation, and sperm characteristics after washing to the occurrence of multiple pregnancy.

Result(s): The mean serum E₂ level on the day of hCG injection was significantly higher in the multiple conception group, but the number of follicles was not. The total sperm count, total motile sperm count, and sperm motility after washing did not differ between the groups. However, couples with multiple pregnancies had sperm with a significantly higher amplitude of lateral head movement than couples with single pregnancies. A peak E₂ level of >583 pg/mL on the day of hCG injection and sperm with an ALH of >4 μm after washing predicted the occurrence of multiple pregnancy.

Conclusion(s): Sperm with an amplitude of lateral head movement of >4 μm and a peak E₂ level of >583 pg/mL are significant risk factors for multiple pregnancy in patients undergoing IUI. (Fertil Steril 1999;72: 613–8. ©1999 by American Society for Reproductive Medicine.)

Key Words: Multiple pregnancy, intrauterine insemination, ovarian stimulation, computer-assisted semen analysis

Ovarian stimulation improves the cycle fecundity rate in part by increasing the number of follicles available for fertilization and correcting subtle, unpredictable ovulatory dysfunction. Combined with IUI, ovarian stimulation is recommended for many causes of infertility, as long as at least one fallopian tube is patent (1–3).

The two main complications associated with this kind of treatment are ovarian hyperstimulation syndrome and multiple pregnancy (4–8). More than one third of multiple pregnancies in this country are associated with the new reproductive technologies (9). The rate of spontaneous occurrence of a twin pregnancy has been estimated to range from 1%–1.35% and that of a triplet pregnancy from 0.01%–0.017% (7, 10). However, with ovulation induction followed by IUI, the incidence of multiple pregnancy increases, ranging from 7.5%–29% per couple (4–6, 8, 11, 12).

The maternal risks associated with multifetal pregnancy are well documented. These complications include hypertension, preterm labor and delivery, postpartum hemorrhage, anemia, and thrombophlebitis. The primary fetal and neonatal complications of multifetal pregnancy are the sequelae of preterm delivery; the increased likelihood of intrauterine growth restriction, malpresentation, and cord accidents also are cause for concern (13, 14). These all are associated with significant emotional and
monetary costs. In view of these problems, reducing the incidence of multiple pregnancy without affecting the treatment cycle fecundity rate is desirable.

Many variables have been suggested as possible predictors of multiple gestation, including the maximum serum E2 concentration (6, 7, 11, 15), number of preovulatory-sized follicles observed sonographically (5–7, 11, 15, 16), and total number of motile sperm inseminated (12). However, other studies have failed to find any clinical variable that could predict reliably the occurrence of multiple pregnancy (4, 8).

The purpose of our study was to determine whether the characteristics of the sperm after washing or of the ovulation induction cycle are associated with the occurrence of multiple conception in patients undergoing ovarian stimulation and IUI.

MATERIALS AND METHODS

Study Design

The medical records of 162 consecutively seen pregnant women who underwent ovarian stimulation and IUI with their partner’s sperm at the Cleveland Clinic Foundation from January 1993 through December 1997 were reviewed in this retrospective study. Couples with ectopic pregnancies, spontaneous abortions, and medical records with incomplete data were excluded from the analysis (n = 40). Institutional review board approval was obtained for this study.

Patient Evaluation

All couples had experienced at least 1 year of primary or secondary infertility with their present partner and had completed a basic workup that included a medical history and physical examination, two or more semen analyses, and a hysterosalpingogram and/or laparoscopic examination that demonstrated at least one patent tube. All the women had regular menstrual cycles with midluteal progesterone levels demonstrated at least one patent tube. All the women had regular menstrual cycles with midluteal progesterone levels of ≥10 ng/mL and/or an in-phase endometrial biopsy either spontaneously or while receiving clomiphene citrate (CC).

Patients who received CC (44/122, 36%) were not monitored. In these patients, the insemination was timed using commercially available LH kits. Of the 44 patients who were treated with CC, 19 were treated empirically. Superovulation was performed using hMG, followed by the injection of hCG (10,000 IU) to trigger ovulation. For the patients who received hMG (78/122, 64%), a standard dose of two ampules of gonadotropins was administered for 4 days, starting on day 3, 4, or 5 of the menstrual cycle. The dose was adjusted based on transvaginal ultrasonographic findings and serial serum E2 levels. Sixty-three patients conceived single pregnancies (63%) and 15 patients conceived multiple pregnancies (68%) after undergoing hMG-stimulated cycles. Only the cycle that resulted in conception was used in the analysis.

The variables examined were patient clinical characteristics (age and indication for IUI), treatment aspects of the conception cycle (type of ovarian stimulation, maximum serum E2 level on the day of hCG administration, number of days of hMG stimulation, number and size of the follicles developed), and semen characteristics after processing for IUI.

Sperm Preparation

Semen was collected by masturbation after 2–3 days of sexual abstinence. After liquefaction, semen analysis was performed by both manual semen analysis and computer-assisted semen analysis (CASA) techniques. A high degree of correlation between CASA and manual sperm counts ($r^2 = 1$, slope 1) and motility ($r^2 = 0.97$, slope 0.97) in fresh specimens established the accuracy of CASA measurements. The reproducibility of the findings of the semen analyzer was determined with the use of a calibration videotape recording. Intra-assay variation of <10% was seen in CASA sperm counts and motility. The rejection criterion was a value of >2 SDs. The Endtz test (myeloperoxidase staining) was performed on all specimens in which the undifferentiated round cell concentration was >10 6/mL to identify the presence of granulocytes (17).

Specimens were prepared using PerWash (Irvine Scientific, Santa Ana, CA), a suspension of coated silica particles used to prepare a density gradient for centrifugation. Aliquots of up to 3 mL of liquefied semen were placed on the upper phase of the bilayered PerWash in a sterile conical centrifuge tube. Specimens were centrifuged for 20 minutes at 600 × g. The supernatant was removed and the pellet was resuspended in 2 mL of human tubal fluid medium (Irvine Scientific). The specimen then was centrifuged for 7 minutes at 600 × g and the supernatant was removed again. The final pellet was resuspended in a volume of 0.4 mL of human tubal fluid medium and a semen analysis was performed.

Only semen characteristics after washing were analyzed in this study: sperm concentration, total sperm count, number of leukocytes, total motile sperm count, and six sperm motion characteristics (percent motility, curvilinear velocity [the total distance traveled by a given spermatozoon divided by the total time elapsed], straight-line velocity [the straight-line distance from the beginning of the sperm track divided by time], average path velocity, linearity [the departure of the sperm track from a straight line], and amplitude of lateral head movement [ALH, the mean width of sperm head oscillation]).

The complex sperm motion characteristics were assessed using a computer-assisted semen analyzer (IVOS; Hamilton-Thorne Research, Beverly, MA). The parameter settings used were as follows: frames acquired, 30; frame rate, 30 Hz; minimum contrast, 90; minimum cell size, 5 pixels; nonmotile head size, 5 pixels; nonmotile brightness (head intensity), 90; low path velocity, 25 μm/s; slow cells motile, yes; and threshold straightness, 80.
Insemination Procedure

A single IUI was performed approximately 36 hours after hCG administration or the day after the LH kit showed positive results using a flexible plastic catheter with the patient in the lithotomy position. Only clinical pregnancies were considered, which were defined by the visualization of fetal cardiac activity on vaginal ultrasonography.

Statistical Analysis

Patients with single and multiple pregnancies were compared using Kruskal-Wallis tests for continuous variables, such as sperm motility, or Fisher’s exact tests for binary variables, such as diagnosis. Subsequent stepwise multivariate logistic regression analyses were performed to determine further those risk factors that predicted multiple pregnancy. Receiver operating characteristic curves were calculated to determine the cutoff values of continuous variables that predicted multiple pregnancy. The cutoff values were selected to maximize sensitivity and specificity. All summary statistics were presented as means ± SE, and \( P < .05 \) was considered statistically significant. All calculations were performed with SAS version 6.12 (SAS Institute, Cary, NC). This study had 90% power to detect a difference of 12% motility and \( 24 \times 10^9/\text{mL} \) total motile sperm.

RESULTS

Of 162 pregnant patients identified, 122 met the inclusion criteria. There were 22 patients with multiple pregnancies, representing an overall rate of 14%. Of these 22 pregnancies, 15 (68%) were the result of hMG-stimulated cycles and 7 (32%) of CC-stimulated cycles. Eighteen (82%) were twin gestations and 4 (18%) were triplet gestations. All the triplet gestations were induced with hMG. The clinical characteristics of the single and multiple gestation groups are shown in Table 1. These two groups did not differ significantly in age or indication for IUI, although patients who had multiple pregnancies tended to be younger.

In Table 2, the stimulation characteristics of the cycle that resulted in conception are compared between the single and multiple pregnancy groups in patients who received hMG stimulation. Patients in both groups received similar amounts of hMG for comparable intervals. The mean serum E2 level on the day of hCG injection was significantly higher in the multiple pregnancy group (879 ± 102.24 pg/mL) than in the single pregnancy group (610.89 ± 45.38 pg/mL) (\( P = .01 \)). No statistically significant differences were found in the number or size of the follicles achieved on the day of hCG injection.

A comparison of semen characteristics after washing between the single and multiple gestation groups is presented in Table 3. There were no differences in the semen chara-
Seminal characteristics after washing between patients who received CC or hMG; therefore, they were considered as a single group. The total sperm count, total motile sperm count, and percent motility after washing did not differ between the groups. However, the multiple pregnancy group had a significantly higher postwash sperm curvilinear velocity ($P = .04$), linearity ($P < .001$), and ALH ($P = .001$).

Using a stepwise multivariate logistic regression analysis and receiver operating characteristic curves, two variables were identified as significant risk factors for multiple pregnancy (Fig. 1): the peak serum E2 level on the day of hCG injection was significant at a cutoff value of 583 pg/mL (odds ratio = 4.8, 95% confidence interval = 1.1–19.5, $P = .03$) and postwash ALH was significant at a cutoff value of 4 µm (odds ratio = 8.4, 95% confidence interval = 2.0–34.6, $P = .003$). Table 4 summarizes the multiple pregnancy rates based on these two risk factors. Of 38 patients who did not have either risk factor, none had a multiple pregnancy.

**DISCUSSION**

The combination of IUI with ovarian stimulation is a widely accepted therapy for many types of infertility. Various hormonal treatment protocols have been used for ovarian stimulation, including CC and hMG, alone or in combination (18). Currently, because most patients undergoing IUI also receive ovarian stimulation, the main concern is not the efficacy of the treatment but the prevention of its complications (6).

One of the most serious risk factors associated with ovarian stimulation and IUI is multiple pregnancy, especially high-order pregnancy (triplet or more), because it is associated with many maternal and fetal complications (9, 19). Although some consider twin gestation a beneficial treatment outcome for an infertile couple, twin pregnancies do have a greater risk for adverse outcomes compared with single pregnancies (20).

We found no statistically significant difference in the indications for IUI or the age of the women between the two groups. Neither the amount of hMG used per cycle nor the duration of ovarian stimulation was significantly related to multiple pregnancy. However, the peak E2 level on the day of hCG injection was a significant factor. Patients with multiple pregnancies had higher E2 levels than patients with single pregnancies; an E2 level of >583 pg/mL predicted the occurrence of a multiple pregnancy.

Our finding is in agreement with the findings of other investigators, who have reported a correlation between peak E2 levels and multiple pregnancy (5, 6, 11). Farhi et al. (6) suggested that the difference between treatment cycles that resulted in a single conception and those that resulted in a multiple conception was not the mode of treatment but the magnitude of ovarian response. Navot et al. (5) found that the rate of multiple pregnancy doubled with a preovulatory E2 level of 500–1,500 pg/mL compared with a level of <500 pg/mL.

Sperm concentration, motility, total count, and morphology have been found to be significantly related to overall pregnancy rates after IUI (5, 21–26). The risk of multiple pregnancy was reported to increase significantly when the number of total motile sperm inseminated at IUI was $>20 \times 10^6$/mL (12). However, others have found no correlation between the number of total motile sperm inseminated and the rate of multiple pregnancy (4, 11).

Our study found that the total sperm count, total motile sperm count, and sperm motility after washing were not correlated with the rate of multiple pregnancy. However, ALH was the only sperm motion characteristic found to be significantly correlated with multiple pregnancy in a multivariate analysis. Moreover, we found that an ALH of $>4 \mu m$
was a good indicator of the occurrence of multiple pregnancy.

The CASA technology reports sperm kinematics (i.e., the movement of spermatozoa in time and space) from reconstructed sperm tracks (27). Sperm motion characteristics, specifically ALH and linearity, have been reported to be related to sperm fertilizing capacity (27–30). These sperm characteristics have been shown to correlate with sperm–zona pellucida interaction and sperm–oocyte fusion, and with the establishment of pregnancy in vivo (27, 29). Computer-assisted semen analysis has the potential to provide more incisive measurements of sperm function than traditional sperm population average values.

In a multivariate discriminant analysis of a cohort of men with oligospermia, Aitken (28) reported that the percentage of spermatozoa that had an ALH of <10 μm was negatively correlated with the rate of spontaneous pregnancy. In this study, which included only men with oligospermia in couples who were observed for 4.5 years using natural intercourse, ALH was the most significant predictor of infertility (28). A low level of ALH was found to be negatively correlated with pregnancy when cryopreserved donor sperm were used for insemination (31, 32).

Monitoring of the ovarian response has been used to maximize pregnancy rates and reduce the risk of multiple gestation and ovarian hyperstimulation (7, 8). Using ultrasound, Fink et al. (33) could not demonstrate more than one mature follicle of 16–25 mm in diameter in any of the multiple pregnancies in their study, so they concluded that ultrasound imaging was not useful in predicting the occurrence of multiple pregnancy. Haning et al. (34) found that the best predictor of multiple pregnancy was the number of follicles with a diameter of >10 mm; however, their findings were not statistically significant. Navot et al. (5) also showed a significant correlation between the number of intermediate-sized follicles (15–17 mm) and the number of fetuses. Other studies also support this finding (6, 11, 16).

Our investigation identified an ALH after washing of >4 μm and an E2 level on the day of hCG administration of >583 pg/mL as risk factors for multiple pregnancy. In addition, patients with an ALH of >4 μm had a higher risk of multiple pregnancy than patients with an ALH of <4 μm, regardless of the E2 level on the day of hCG administration (Table 4). Because ovarian stimulation usually results in more than one follicle, this finding suggests that the most important variable associated with multiple pregnancy is the ALH after washing, which is a functional characteristic of spermatozoa. Moreover, in our study, the number and size of the follicles were not associated with the occurrence of multiple gestation. This suggests that the E2 level on the day of hCG administration is a better indicator of the risk of multiple pregnancy than the number or size of the follicles as determined ultrasonographically.

We conclude that the ability of the ovaries to respond to exogenous stimulation is one of the decisive factors in the occurrence of multiple pregnancy and that monitoring the ovarian response by the serum E2 level was better for predicting multiple pregnancy than was monitoring the number

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**Table 4**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total no. of pregnancies</th>
<th>No. of multiple pregnancies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALH &lt;4 μm, E2 &lt;583 pg/mL</td>
<td>38</td>
<td>0 (0)</td>
</tr>
<tr>
<td>ALH &lt;4 μm, E2 &gt;583 pg/mL</td>
<td>25</td>
<td>7 (28)</td>
</tr>
<tr>
<td>ALH &gt;4 μm, E2 &lt;583 pg/mL</td>
<td>7</td>
<td>4 (57)</td>
</tr>
<tr>
<td>ALH &gt;4 μm, E2 &gt;583 pg/mL</td>
<td>7</td>
<td>3 (43)</td>
</tr>
</tbody>
</table>

Note: ALH = amplitude of lateral head movement; E2 = serum E2 level on the day of hCG administration.
and size of the follicles by ultrasound. The total sperm count, total motile sperm count, and sperm motility after washing were not correlated with the occurrence of multiple pregnancy. The probability of multiple pregnancy was related primarily to the functional characteristics of the sperm when multiple oocytes were ovulated in response to ovarian stimulation therapy.

Acknowledgment. The authors thank Ms. Robin Verdi for secretarial help.

References