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<td><strong>Author(s)</strong></td>
<td>Makkar, G; Ng, EHY; Yeung, WSB; Ho, PC</td>
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Prognostic factors for successful outcome in patients undergoing controlled ovarian stimulation and intrauterine insemination

Objective. To determine the prognostic factors associated with successful outcome following controlled ovarian stimulation and intrauterine insemination.

Design. Retrospective analysis.

Setting. University-based assisted reproductive technology centre, Hong Kong.

Patients and methods. Patients included 292 couples undergoing 600 treatment cycles, following a standard protocol of human menopausal gonadotrophin injections. Multiple logistic regression analysis was performed to determine which demographic and sperm parameters gave the maximum discrimination to predict pregnancy.

Results. One hundred and eleven pregnancies resulted from treatment. The pregnancy rates were 18.5% per cycle and 37.9% per couple. The age of the women was significantly lower for pregnant cycles, and the serum oestradiol levels and number of follicles greater than 16 mm in diameter were significantly higher, compared with non-pregnant cycles. The sperm concentration and number of motile spermatozoa were also significantly increased in pregnant cycles. Pregnancy rate was significantly increased when the raw semen sample contained 20 million/mL or more spermatozoa, normal forms comprised 7% or more, and when the number of motile spermatozoa in inseminated samples was 1 million or greater.

Conclusion. Using multiple logistic regression analysis, age of the women and serum oestradiol level had the maximum power to predict pregnancy following ovarian stimulation and intrauterine insemination.

Introduction

Intrauterine insemination (IUI) in conjunction with controlled ovarian stimulation (COS) is usually offered to infertile couples if the woman has patent fallopian tubes, prior to other assisted reproductive methods. Controlled ovarian stimulation may correct subtle problems of ovulation, increase the number of oocytes available...
for fertilisation, and enhance the accuracy of timing of insemination. The rationale for performing IUI is that a higher number of motile spermatozoa with normal forms can be inseminated around the time of ovulation to the uterine cavity, closer to the site of fertilisation. Moreover, sperm preparation removes leukocytes and dead and moribund spermatozoa from the semen sample. These can generate free oxygen radicals and reduce the functional capacity of intact spermatozoa.\(^1\)\(^2\)

The aim of this retrospective analysis was to determine prognostic factors for successful outcome following COS/IUI.

**Patients and methods**

Six hundred COS/IUI cycles for 292 couples completed between February 1993 and August 2001 at the Department of Obstetrics and Gynaecology, Queen Mary Hospital, were analysed retrospectively. All women in this study had a diagnosis of infertility for more than 2 years, regular menstrual cycles, bilateral patent fallopian tubes shown on diagnostic laparoscopy, and no contra-indications for pregnancy. Patients with multiple causes of infertility or anovulation were excluded from the study.

All patients underwent COS using human menopausal gonadotrophin (HMG). On the second hour of the treatment cycle, the serum oestradiol (E2) level was checked and baseline transvaginal scanning was performed. If E2 level was less than 220 pmol/L, and there was no ovarian cyst evident on the scan, treatment of 150 IU of HMG (Pergonal; Serono, Aubonne, Switzerland) was given intramuscularly daily from day 3 onwards. The ovarian response was regularly assessed using both transvaginal scanning and serum E2 levels. Ten thousand international units of human chorionic gonadotrophin (HCG) [Profasi; Serono, Aubonne, Switzerland] were given when the leading follicle was greater than 18 mm in diameter, and there were no more than three follicles of greater than 16 mm in diameter. Patients with an excessive response were counselled to have the cycle cancelled because of the increased risk of multiple pregnancy.

The husband was asked to submit a semen sample in a sterile plastic container about 2 hours before the IUI procedure, after an abstinence of 2 to 3 days. The sample was allowed to liquefy completely at room temperature, usually occurring within 30 minutes. After liquefaction, sperm preparation was completed by a discontinuous density gradient centrifugation method, using Percoll (Pharmacia, Uppsala, Sweden) or Isolate (Irvine Scientific, Santa Ana, US) sperm separation media.\(^3\) The pellet obtained after centrifugation was washed twice with Earl’s balanced salt solution (EBSS; Sigma, St Louis, US), supplemented with 0.35% Plasmanate (PPF; Bayer Corporation, Elkhart, US) or 8% patient’s serum. The resulting sperm pellet after washing was overlaid with the same medium, adjusting the final volume to 0.3 mL to 0.5 mL.

Semen analysis was performed according to World Health Organization (WHO) guidelines,\(^4\) both before and after sperm preparation. Concentration, progressive motility, and normal morphology were evaluated after staining by Diff-Quick method.\(^5\) A thin and well-spread smear was air-dried on a clean glass slide at room temperature. The slides were fixed in Diff-Quick fixative (1.8 mg/L triaryl methane in methyl-alcohol) for 15 seconds, stained in Diff-Quick solution 1 (1 g/L xanthen in sodium acide-preserved buffer) for 10 seconds, and finally in Diff-Quick solution 2 (0.625 g/L azure A and 0.625 g/L methylene blue in buffer) for 10 seconds. Morphology was assessed by counting 100 spermatozoa using WHO criteria.\(^4\)

Intrauterine insemination was performed once, 38 hours after HCG using a Tomcat catheter (Monojec, St Louis, US) as described previously.\(^5\)\(^7\) The patient was asked to rest in the supine position for 15 minutes after the procedure, and thereafter to resume her routine activities. The luteal phase was supported by two further doses of 1500 IU HCG on day 5 and day 10, after the ovulatory HCG injection. Serum E2 and progesterone levels were also checked on day 10 after the ovulatory HCG injection. Pregnancy testing was performed on day 20 after the ovulatory HCG injection, and if positive, a pelvic ultrasound was arranged to confirm the presence of an intrauterine pregnancy and to determine the number of gestational sacs. In vitro fertilisation treatment was advised if the patient was not pregnant after three treatment cycles.

**Statistical analysis**

Only clinical pregnancies were considered in this retrospective analysis. A clinical pregnancy was defined as the presence of an intrauterine gestational sac(s) on scanning, or products of conception on histological examination in cases of miscarriage. Biochemical pregnancies were excluded from the analysis. The number of motile spermatozoa (in millions) was obtained by multiplying semen volume and the concentration and percentage of progressive motile spermatozoa. Recovery rate (%) was calculated by dividing the total motile spermatozoa in the prepared sample by that of the raw sample.

Data were expressed as median and range. Comparison of various characteristics in pregnant and non-pregnant cycles was carried out using the Mann-Whitney U test. Multiple logistic regression analysis was performed to determine which demographic and sperm parameters gave maximum discrimination to predict pregnancy. Statistical analysis was performed using the Statistical Package for the Social Sciences (Windows version 10; SPSS Inc., Chicago, US). Two-tailed P<0.05 values were considered statistically significant.

**Results**

One hundred and eleven pregnancies were achieved, and the pregnancy rate (PR) was 18.5% per treatment cycle, and
37.9% per couple. The PR per cycle was 14.3% (25/175) for couples with male-factor infertility, and 20.2% (86/425) for other causes, including mild endometriosis and unexplained infertility. The multiple PR (twins and triplets) for other causes, including mild endometriosis and unexplained infertility, was 22.6%. There were 12 (10.8%) clinical abortions, and 37.9% per couple. The PR per cycle was 14.3% (25/175) for couples with male-factor infertility, and 20.2% (86/425) for other causes, including mild endometriosis and unexplained infertility. The multiple PR (twins and triplets) for other causes, including mild endometriosis and unexplained infertility, was 22.6%. There were 12 (10.8%) clinical abortions, and 37.9% per couple. The PR per cycle was 14.3% (25/175) for couples with male-factor infertility, and 20.2% (86/425) for other causes, including mild endometriosis and unexplained infertility. The multiple PR (twins and triplets) for other causes, including mild endometriosis and unexplained infertility, was 22.6%. There were 12 (10.8%) clinical abortions, and 37.9% per couple. The PR per cycle was 14.3% (25/175) for couples with male-factor infertility, and 20.2% (86/425) for other causes, including mild endometriosis and unexplained infertility. The multiple PR (twins and triplets) for other causes, including mild endometriosis and unexplained infertility, was 22.6%. There were 12 (10.8%) clinical abortions, and 37.9% per couple. The PR per cycle was 14.3% (25/175) for couples with male-factor infertility, and 20.2% (86/425) for other causes, including mild endometriosis and unexplained infertility. The multiple PR (twins and triplets) for other causes, including mild endometriosis and unexplained infertility, was 22.6%. There were 12 (10.8%) clinical abortions, and 37.9% per couple. The PR per cycle was 14.3% (25/175) for couples with male-factor infertility, and 20.2% (86/425) for other causes, including mild endometriosis and unexplained infertility. The multiple PR (twins and triplets) for other causes, including mild endometriosis and unexplained infertility, was 22.6%. There were 12 (10.8%) clinical abortions, and 37.9% per couple. The PR per cycle was 14.3% (25/175) for couples with male-factor infertility, and 20.2% (86/425) for other causes, including mild endometriosis and unexplained infertility. The multiple PR (twins and triplets) for other causes, including mild endometriosis and unexplained infertility, was 22.6%. There were 12 (10.8%) clinical abortions, and 37.9% per couple. The PR per cycle was 14.3% (25/175) for couples with male-factor infertility, and 20.2% (86/425) for other causes, including mild endometriosis and unexplained infertility. The multiple PR (twins and triplets) for other causes, including mild endometriosis and unexplained infertility, was 22.6%. There were 12 (10.8%) clinical abortions, and

37.9% per couple. The PR per cycle was 14.3% (25/175) for couples with male-factor infertility, and 20.2% (86/425) for other causes, including mild endometriosis and unexplained infertility. The multiple PR (twins and triplets) was 22.6%. There were 12 (10.8%) clinical abortions, and two (1.8%) ectopic pregnancies. A significantly higher PR was noted in the first (19.9%) and second (21.1%) cycles than in the third cycle (12.1%). None of the patients experienced moderate or severe ovarian hyperstimulation syndrome.

Table 1 summaries demographic data and ovarian response data. The median age of women was significantly lower in pregnant cycles than in non-pregnant cycles (33.0 years vs 34.0 years, P=0.03, Mann-Whitney U test). The median E2 level and the median number of follicles greater than 16 mm in diameter on the ovulatory HCG day were significantly higher in pregnant cycles compared to non-pregnant cycles.

The volume of semen, percentage of motile spermatozoa in the raw and inseminated samples, and the recovery rate of motile spermatozoa were similar in pregnant and non-pregnant cycles (Table 2). The sperm concentration, the percentage of normal spermatozoa, and the number of motile spermatozoa were significantly higher in pregnant than non-pregnant cycles in both raw and inseminated semen samples.

<table>
<thead>
<tr>
<th>Causes of infertility</th>
<th>Pregnant cycles* (n=111)</th>
<th>Non-pregnant cycles* (n=489)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male factor</td>
<td>25</td>
<td>150</td>
</tr>
<tr>
<td>Endometriosis; unexplained</td>
<td>86</td>
<td>339</td>
</tr>
<tr>
<td>Age of women (years)</td>
<td>33.0 (25.0-37.0)</td>
<td>34.0 (23.0-45.0)</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>3.0 (2.0-13.0)</td>
<td>4.0 (2.0-13.0)</td>
</tr>
<tr>
<td>Ampoules of human menopausal</td>
<td>14 (6-46)</td>
<td>14 (5-88)</td>
</tr>
<tr>
<td>No. of follicles &gt;16 mm in diameter</td>
<td>2 (1-5)</td>
<td>1 (0-5)</td>
</tr>
<tr>
<td>Oestradiol level on ovulatory human chorionic gonadotrophin (pmol/L)</td>
<td>2770 (59-11134)</td>
<td>1788 (70-12132)</td>
</tr>
<tr>
<td>Size of the largest follicle (mm)</td>
<td>18.7 (16.0-25.0)</td>
<td>19.0 (16.0-28.0)</td>
</tr>
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</table>

Table 2. Comparison of spermatozoa parameters in raw and inseminated semen between pregnant and non-pregnant cycles

<table>
<thead>
<tr>
<th></th>
<th>Raw semen</th>
<th>Prepared semen</th>
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<tr>
<td></td>
<td>Pregnant cycles*</td>
<td>Non-pregnant cycles*</td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>3.2 (0.3-11.0)</td>
<td>3.2 (0.3-12.0)</td>
</tr>
<tr>
<td>Concentration (million/mL)</td>
<td>75.0 (1.6-1040.0)</td>
<td>63.5 (3.0-500.0)</td>
</tr>
<tr>
<td>Progressive motility (%)</td>
<td>52.0 (5.0-87.0)</td>
<td>51.0 (0.6-87.0)</td>
</tr>
<tr>
<td>No. of motile spermatozoa (million)</td>
<td>116.6 (0.2-1154.0)</td>
<td>93.7 (0.6-1083.0)</td>
</tr>
<tr>
<td>Normal morphology using WHO criteria (%)</td>
<td>20.0 (3.0-54.0)</td>
<td>18.0 (0.7-6.0)</td>
</tr>
<tr>
<td>Recovery rate (%)</td>
<td>-</td>
<td>-</td>
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Table 3. Factors of prognostic significance on multiple logistic regression analysis

<table>
<thead>
<tr>
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<th>Standard discriminant function coefficient β</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Age of women</td>
<td>-0.098</td>
<td>0.009</td>
</tr>
<tr>
<td>Oestradiol level on human chorionic gonadotrophin day</td>
<td>0.000</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Normal spermatozoa (raw sample)</td>
<td>0.026</td>
<td>0.011</td>
</tr>
<tr>
<td>Concentration (inseminated sample)</td>
<td>0.003</td>
<td>0.039</td>
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A significantly higher PR was achieved when the sperm concentration in the raw semen sample was 20 million/mL or greater (19.7% vs 9.2%, P<0.05, Chi squared test), or the percentage of normal morphology was 7% or greater (19.4% vs 10.3%, P<0.05, Chi squared test). When the number of motile spermatozoa in inseminated samples was 1 million or greater, PR was also significantly increased (19.4% vs 3.7%, P<0.05, Chi squared test). The percentage of progressive motility in both raw and inseminated samples did not affect PRs, even when it was as low as 10%.

Using logistic regression analysis, the serum E2 level on the day of HCG was the most discriminating variable to predict pregnancy following IUI (P<0.001) [Table 3]. The age of women showed a negative correlation with pregnancy (standard discriminant function coefficient β = −0.098, P=0.009). The log concentration of spermatozoa in the
inseminated samples demonstrated a significant correlation with the outcome of insemination (P<0.05). The percentage of normal forms, and the concentration of spermatozoa in raw semen samples were other significant semen parameters predictive of pregnancy (P<0.05).

Discussion

Controlled ovarian stimulation/IUI treatment is a less expensive, less stressful, and less invasive treatment compared with other assisted reproduction techniques, such as in vitro fertilisation treatment. Consequently, it is usually the first-line treatment offered to patients with patent fallopian tubes and varying causes of infertility. Three stimulated cycles have been reported as necessary to optimise the success of IUI.9,9 Our study showed a significantly higher PR in the first and second cycles, compared with the third cycle. Burr et al10 have also reported a sharp decrease in PR from the first cycle to the third cycle. A reasonable chance of conception, however, is present even in the third cycle. Tomlinson et al14 reported a success rate in the third cycle of 14%, in keeping with this study’s findings. Other studies have also reported a higher PR in the first cycle, and that the majority of pregnancies are established within three to four cycles. It seems then that COS/IUI is most effective in the first three treatment cycles.

A higher number of follicles with a mean diameter of greater than 16 mm were present in pregnant compared to non-pregnant cycles. This was reflected in higher serum E2 level in pregnant cycles. A better PR has been reported in cycles with three follicles of greater than 16 mm in diameter,12 as well as in cycles with two follicles of greater than 16 mm in diameter.9 The risk of multiple pregnancy should be taken into consideration with respect to multi-follicular development, however.

This study found that the age of the women was a significant discriminating factor predictive of pregnancy. No pregnancy was achieved in women older than 38 years. Similar reports in the literature have noted that women older than 40 years had a poor success rate after IUI with ovarian stimulation treatment.9,11-14 These findings are in keeping with other previous studies documenting a decline in female fecundity with donor spermatozoa.15-18 This decline has been suggested to be a result of decreased oocyte quality,19,20 higher rates of chromosomal abnormalities,24 and/or reduced uterine receptivity.22,23 Extensive counselling should be offered to women who are older than 38 to 40 years before they proceed to COS/IUI treatment. However, there have been some studies showing that the success of IUI does not decline with increasing female age.11,24

The influence of sperm parameters in predicting the results of assisted reproductive techniques is a matter of debate. In IUI, conventional semen parameters have been reported to have no power to discriminate between pregnant and non-pregnant ejaculates in either donor insemination or IUI programmes.13 Controversially, conventional andrological diagnosis has been said to be essential for IVF or insemination therapy.26

Sperm concentration and the number of motile spermatozoa in both raw and inseminated semen samples were significantly higher in pregnant than non-pregnant cycles in our study. Sperm concentration, progressive motility, and the number of motile spermatozoa have been reported to be significantly correlated with PR in IUI,27 in donor insemination cycles,18,28 and with the time of conception.29 Similarly, Bielsa et al30 reported that the number of motile spermatozoa inseminated showed the best association with fertility outcome (P<0.003). Conversely, no correlation was demonstrated between PR and inseminated number of motile spermatozoa by Burr et al,40 and by Horvath et al27 when the concentration of spermatozoa was between 1 and 30 million.

The chances of achieving pregnancy were reported to be significantly increased when the raw semen sample contained 20 million/mL or more spermatozoa, below which the relationship between fertilisation capacity and sperm density was said to be less predictable.31 A similar threshold limit of approximately one million progressive motile spermatozoa for reasonable IUI success has previously been reported.27,32,33 However, pregnancies have been achieved with IUI with less than one million progressive motile spermatozoa in washed samples during natural cycles,8 with 0.8 million motile spermatozoa or more,44 and with as low as 0.2 million motile spermatozoa.10 Notwithstanding, the fact that the number of motile spermatozoa used for insemination influences the outcome of IUI, highlights the importance of using washing procedures that result in good levels of spermatozoa recovery.1

The percentage of normal morphology in raw and inseminated semen samples was significantly higher in pregnant compared to non-pregnant cycles. Moreover, the proportion of normal spermatozoa in the raw semen was shown to predict successful outcome. The percentage of normal morphology of raw and ‘post swim-up’ semen has also been found to correlate with in vitro fertilisation rates,20,35-37 and with higher PRs in IUI10,38 and donor insemination programmes.39 The threshold value for normal morphology in raw semen was 7%, below which PRs were significantly reduced. It has been suggested that the minimum percentage of normal sperm in raw semen for IUI cycles should be 10%.10

Conclusion

Age of the women and E2 level had maximum power to predict pregnancy following COS and insemination, with lower age of women and higher E2 level correlating positively with successful outcome. An increased number of follicles of greater than 16 mm in diameter were present in pregnant cycles compared with non-pregnant cycles. In addition, sperm concentration and the number of motile
spermatozoa were also significantly increased in pregnant cycles. The PR was significantly increased when the raw semen sample contained 20 million/mL or more spermatozoa, normal forms comprised 7% or greater, and when the number of motile spermatozoa in inseminated samples was 1 million or greater.

References