

# Predictive Factors For Intrauterine Insemination Success: Analysis Of Semen Parameters Affecting Outcome

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## Abstract

**Objectives:** The aim of this study was to determine factors influencing the pregnancy rate following intrauterine insemination (IUI) and to evaluate sperm parameters affecting outcome.

**Patients and methods:** A retrospective analysis of 223 IUI cycles with ovarian stimulation by clomiphene citrate and/or gonadotropins was performed. Clinical pregnancy rates were analyzed according to all variables such as the woman's age, the number of the attempt, the spermogram characteristics, the sperm morphology after preparation, and the total motile spermatozoa count.

**Results:** A total of 20 clinical pregnancies were obtained for a pregnancy rate per cycle (PR) of 9.2%. For patients who are under the age of 37 the pregnancy rate per cycle was 11.17% and no pregnancies occurred for the other patients who are above the age of 37 ( $p < 0.0001$ ). Three significant semen variables influencing pregnancy rates were identified by multiple logistic regression analysis which are rapid progressive motility in the whole sperm (a), the percentage of typical spermatozoa, and the total motile sperm count in the selected sperm. Intrauterine insemination with  $a \geq 10\%$  increased the PR from 3% to 11.5% ( $p = 0.017$ ). The clinical PR per couple decreased from 12.5% to 0% if teratozoospermia was higher than  $> 80\%$ ; ( $P < 0.0001$ ). The PR was significantly higher with total motile spermatozoa count inseminated  $\geq 5 \times 10^6$  compared to only  $< 5 \times 10^6$  inseminated (11.8% vs. 4.6%).

**Discussion and conclusions:** Homologous IUI achieves the best results with  $\geq 10\%$  flashing motility, percentage of typical forms in the selected sperm  $> 20\%$  and a high number of total motile spermatozoa inseminated  $\geq 5 \times 10^6$ . These thresholds determined in our study should help to optimize the IUI outcome.

## INTRODUCTION

IUI is widely used for treating infertility among couples because it is a simple, inexpensive, and acceptable assisted reproductive technique (ART) [1]. The indications for IUI include female factors such as cervical mucus abnormalities, ovulatory disturbances, endometriosis with a healthy fallopian tube and non-severe male factor infertility as well as unexplained infertility. IUI is attempted to before proceeding to more expensive and invasive ART such as conventional in vitro fertilization (IVF) or intracytoplasmic sperm injection.

Pregnancy rates after IUI differ from one study to another according to patient selection criteria, to the presence of various infertility factors, to the woman age, to methods of ovarian stimulation, to number of cycles performed, and to different sperm parameters and technique of preparation. Several semen parameters have been shown to correlate with

IUI outcome such as the number of motile sperm and normal morphology especially after semen preparation [2, 3]. In this retrospective study, we will try to evaluate the results of IUI with ovarian stimulation and determine the predictive semen variables assessed after sperm preparation which contribute to the success of treatment. These data would be valuable to improve sub fertility treatment and achieve the best pregnancy rate of IUI therapy among couples.

## MATERIALS AND METHODS

A total of 176 couples received 223 Cycles of IUI during a one year period from 2010 to 2011. This treatment using the standard IUI technique with partner's spermatozoa was performed at our center. Institutional review board approval was not required for this observational study because patients were treated according to standard and customary clinical practice. These couples under study had at least one year of infertility. They also had undergone extensive

infertility evaluation prior to IUI consisting of at least two recent standard semen analysis, semen analysis after sperm preparation test notably the total motile spermatozoa count and sperm morphology after selection (TMS), serum hormone assays on the third day of the menstrual cycle (FSH, LH, E), prolactin and thyroid hormone concentrations, monitoring of ovulation by ultrasound and mid-luteal progesterone, a post coital test performed in the pre-ovulatory phase, and hysterosalpingography. All couples were tested for hepatitis B virus, hepatitis C and HIV virus before they were offered the IUI trial. Normal sperm was defined by the threshold values of the World Health Organization (WHO) (concentration  $20 \times 10^6$ /ml, progressive motility 30% and typical morphology 30%) [4]. Couples with husband's progressively motile sperm count after preparation  $< 1 \times 10^6$ /ml in the basic infertility evaluation, were not enrolled in IUI treatment.

- Sperm preparation:

All semen were produced by masturbation in the laboratory the day of the attempt after 3 to 5 days of sexual abstinence and collected in sterile containers. After complete liquefaction for 30 minutes at room temperature, each sample was analyzed for conventional semen parameters according to WHO guidelines. Sperm concentration was performed with a hemocytometer on two separate preparations of the semen sample (dilution 1/20 in Ringer's solution). Sperm motility was determined by assessing at least five microscopic fields to classify at least 200 spermatozoa ( $\times 4.000$  magnification). The motility was graded in to fleshing motility (a), progressive (a+b) or absence of mobility. Sperm morphology was analyzed according to the Shorr staining procedure. The percentage of morphologically normal spermatozoae and of various sperm abnormalities were evaluated on 100 sperm at 1.000 x magnification according to the criteria of David et al [5] edited by Jouannet et al [6]. The standard centrifugation in a discontinuous density gradient column 90% and 45% (Puresperm®, Nidacon international, Goteborg, Sweden) technique was used for preparation. The final pelled was resuspended in 0.3 to 0.5 ml of a capaciting medium (Ferticult®, Beernem, Belgium). Sperm morphology was reevaluated from the motile selected sperm and the TMS count was obtained by multiplying the total sperm count by the prewash percentage of mobility.

- Ovarian stimulation and timing of insemination:

All women in the study underwent ovarian stimulation using Clomiphene Citrate (serpafar 50 mg, Faran laboratories) and/ or recombinant FSH (Gonal F, Sereno, Boulogne, France). For clomiphene citrate-stimulated cycles, 100 mg clomiphene citrate was given between day 3 and day 7 followed by 150UI of gonadotropins added by day 9. Cycles were monitored by transvaginal ultrasonography for the mean follicular volume and thickness of the endometrium on days 9 to 13 of the cycle. Human chorionic gonadotropin 5000UI injection or ovitrelle® 250ug was administrated to induce ovulation when at least one follicle measured was  $> 16$ mm.

- Insemination procedure and pregnancy detection:

Intrauterine insemination was performed using an intrauterine catheter (the Frydman series from CCD, Paris, France) placed in the center of the uterine cavity and the sperm preparation was injected slowly. If this catheter was unable to pass the cervix, a hard catheter was used (the TDT series from CCD). All patients received 400 mg/day of micronized progesterone (vaginal or rectal suppository). Plasma  $\beta$ HCG levels were routinely measured two weeks after IUI. Clinical pregnancy was defined as transvaginal ultrasonographic visualization of intrauterine gestational sac(s).

- Statistical analysis: The statistical data analysis were done by using the R2.2.1 logical foundation for statically computing (Vienna, Austria). Multivariate logistic regression analysis was used to explore the association between PR and independent variables and to identify significant variables that contribute to the success of IUI treatment. The differences were considered to be statistically significant if  $P < 0.05$  at a confidence interval of 95%.

## RESULTS

Female age ranged from 22 to 40 years (mean: 33.26). The majority of them experienced infertility duration of 3 years maximum (66%). 60% of them had primary infertility. The overall PR per cycle was 9.2 % (20/223) (table 1). The pregnancy rates according to sperm parameters are summarized in table 2.

Our results showed a significant decline in pregnancy rate related to female age ( $p < 0.001$ ). However, no significant difference was evident in patients younger than 37 years, and no pregnancy occurred in the limited sample of women who were  $\geq 37$  years. Nevertheless, a significant difference was

found only when comparing pregnancy rates per cycle in the <37 and  $\geq 37$  age groups. Twenty pregnancies out of 179 cycles (PR=11.17%) occurred in the <37 age group. The PR declined to 0 pregnancies out of 44 cycles among older women ( $p<0.001$ )

If we analyze the relationship between pregnancy rates and standard semen parameters, we will find that there was an overall significant difference in pregnancy rate per cycle in relation to flashing motility. The highest PR per cycle (11.5%) was observed among those couples with a flashing motility of  $\geq 10\%$  versus 3% when flashing motility is  $<10\%$  ( $p=0.017$ ). However, there was not any significant difference in relation to total progressive motility or total sperm concentration among pregnant and non-pregnant study population.

For a normal sperm morphology  $<30\%$ , the clinical pregnancy rate per cycle decreased from 13.7% to 10.3% and 0% if teratozoospermia was higher than the abnormal form threshold (respectively  $<70\%$ , 70-80% and  $>80\%$ ,  $p<0.0001$ ). Regarding final total motile sperm number inseminated, the pregnancy rate per cycle was significantly reduced in the group of only  $<5 \times 10^6$  motile spermatozoa inseminated compared to the other group of  $\geq 5 \times 10^6$  ( $p=0.03$ ). The pregnancy rate per cycle was only of 4.6% with number of motile sperm  $<5 \times 10^6$ . However, 16 pregnancies were reported with a number of motile sperm  $\geq 5 \times 10^6$  in 136 cycles accounting for a 11.8% PR per cycle ( $p=0.03$ ). Nevertheless, there was no obvious advantage in increasing the total motile sperm count inseminated from 5-10  $\times 10^6$  to  $>10 \times 10^6$  as the pregnancy rate per cycle remained unchangeable (12.7%,  $p>0.05$ ).

However, no evident relations were found between PR and other variables as the rank of the attempt or the number of preovulatory follicles or the basal FSH. The average number of IUI cycles was 1.3 per couple and there was no statistically significant association between PR and the number of cycles ( $p=0.34$ ). The average number of preovulatory follicles ( $>16$ mm in diameter) was  $1.3 \pm 0.62$ . In cycles with a single preovulatory follicle, the pregnancy rate was not significantly lower compared to cycles with 2 or more follicles (7.1% vs 9.6%,  $p>0.05$ ). In fact, we did not find any significant difference in pregnancies according to the basal FSH concentration. The average value of serum concentration was  $7.1 \pm 2.8$  UI/mU. The ongoing pregnancy rate per couple was 9.9% with an FSH level of 7.5 UI/mU and 9.5% with an FSH level above 7.5 UI/mU ( $p>0.05$ ).

In our study, logistic regression analysis revealed four predictive variables regarding pregnancy which are the female age ( $p=0.001$ ), the typical forms of spermatozoa ( $p<0.0001$ ), the flashing motility ( $p=0.017$ ), and the number of final motile sperm inseminated ( $p=0.03$ ).

## DISCUSSION

IUI is often suggested to infertile couples where the woman should have at least one permeable fallopian tube and the man should have a partially modified sperm. The pregnancy rate depends on sperm parameters and female factors. Unfortunately, the majority of studies attempting to find which sperm characteristics correlated better with IUI cycle outcome included multiple female infertility problems as well. Most studies found progressive motility [7, 8, 9] and/or the total motile sperm count [10] to be the best predictors of pregnancy after sperm processing. Some others reports related pregnancy outcome to post processing total sperm count [9, 10, 11] and sperm morphology [2, 12]. Hence, the result might be biased by the other infertility etiologies that were treated in parallel. In this study, only the male factor problems or unexplained infertility without an evident female factor were recruited to make the differences in sperm parameters more significant.

Twenty clinical pregnancies were achieved after 223 IUI cycles (9%) within averagely 1.3 attempts. The reported pregnancy rates achieved by IUI have usually varied between 8% and 22% [13, 14]. In the present study, the success of rate of IUI with partner's semen decreased with increasing age, which is in agreement with the results of many studies [15, 16, 17, 18, 19, 20] and no pregnancies occurred with woman aged more than 37 years. The most important selection criterion for suitability of couples for treatment by IUI is the sperm quality. No relation between sperm count or progressive motility and IUI outcome was found in this study but flashing's motility significantly influenced the ongoing pregnancy rate at 11.5% for the over or equal to 10% versus 0% for the under 10% ( $p<0.017$ ). The clinical PR per couple decreased from 12.5% to 0% if teratozoospermia was higher than the abnormal form threshold ( $>80\%$ ;  $P<0.0001$ ). Regarding the total motile spermatozoa inseminated, the clinical PR per couple was 4.6% when TMS was lower than 5 million and was significantly higher (11.8%) when this number was above 5 million ( $p=0.03$ ).

The age related decline in female fecundity has been well documented in every aspect of natural and artificial

reproduction [21, 22, 23], particularly, in women undergoing IUI. In our study, the woman's age significantly influenced the ongoing pregnancy rate, at 11.17% for the under 37 s versus 0% for the over 37s. No pregnancies occurred above the age of 37 years with number of final motile sperm  $<5 \times 10^6$  or  $\geq 5 \times 10^6$ . The female age seems to be an independent factor in predicting pregnancy after IUI. Alternative options including IVF and ICSI may enhance the chances of success in the case of older women in whom the age factor becomes increasingly prominent.

Fewer reports have shown an evident correlation between progressive or flashing motility in whole sperm and PR [7, 8, 9, 20, 24, 25]. Therefore, in our study, the best results were obtained with flashing motility that is  $\geq 10\%$ , PR per cycle achieved 11.5% versus 3% if (a) is  $<10\%$ . This finding was in agreement with Haim et al [24] who found threshold of 10% for (a) in the whole sperm as a predictive value on IUI outcome.

Many retrospective and prospective studies showed that sperm morphology influences the pregnancy rate. For instance, Burr et al [26] noted a drop in the pregnancy rate from 18.2% to 4.3% per cycle when teratozoospermia reaches 90%. According to Belaish-Allart et al. [27], a teratozoospermia rate over 80% plays against IUI. Our results show the same threshold of teratozoospermia (80%) as the PR decreases from 13.7% to 10.3% if the degree of teratospermia is respectively  $<70\%$  and between 70-80% and no pregnancies occurred among patients with more than 80% of atypical forms. Wainer et al [28] analyzed 2564 IUIs and reported that the pregnancy rates were not significantly influenced by teratospermia as long as more than 5 million motile spermatozoa were available for insemination. However, we didn't find any significant difference between couples with a number of motile sperm inseminated  $<$  or  $\geq 5 \times 10^6$  if normal morphology was either  $\geq 30\%$  or  $<30\%$ . As a result, we recommended a minimum of 20% of typical forms in the selected sperm.

The minimum recommended number of motile sperm after preparation in various reports varies from 0.8 to  $10 \times 10^6$  [10, 29, 30, 31]. Couples with  $<1 \times 10^6$  were referred directly to IVF treatment and eliminated from our study as recommended by some authors [7, 10, 11, 29, 30, 32, 33]. The pregnancy rate per cycle (4.6%) was significantly lower in the subgroups with a number of motile sperm  $<5 \times 10^6$  vs. a PR of 11.8% in the second group. Nonetheless, there was no significant increase in the pregnancy rate above the threshold

of  $10 \times 10^6$  (12.7% versus 11.8% in group 2). This was in agreement with Badawy et al [2] and Francavilla et al [12], Wainer et al [29] who found a significant increase in success rate when the total motile sperm count exceeded  $5 \times 10^6$  and in disagreement with the finding of some authors [31, 34, 35, 36] who showed that an average post-wash total motile sperm count of  $10 \times 10^6$  as useful threshold value for IUI success.

IUI used for treating male factor infertility seems to have little chance of success when the woman is older than 37, when the number of motile sperm inseminated is  $<5 \times 10^6$ , or when the normal sperm morphology is  $<20\%$ , and flashing motility is  $<10\%$ . Therefore, we conclude that many couples with male factor infertility, as demonstrated throughout this study, can have the chance to become pregnant using IUI.

## CONCLUSIONS

According to these study, we recommended a cutoff point for number of motile sperm inseminated after sperm preparation as  $5 \times 10^6$ , typical forms as 20% after preparation, and flashing motility as 10% in the whole sperm. They may be used to distinguish between those couples with male infertility factors who could become pregnant with IUI and therefore, should undergo IUI before IVF and other couples should be referred directly to IVF treatment in the presence of  $<5 \times 10^6$  motile sperm.

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**Table 1**

Intrauterine pregnancy rate according to different variables

	No of cycles	Pregnancy rate/cycle	P value
Age (years)			
<37	179	11.6%	
≥ 37	44	0%	P<0.001
Type of infertility			
Primary	134	10.4%	
secondary	89	6.7%	P=0.19
Infertility duration(years)			
1-2	78	10.1%	
2-3	75	9.3%	
>3	70	7.2%	P=0.69
Cycle number(rang)			
1cycle	143	10.7%	
2cycles	60	12.5%	
≥ 3 cycles	20	13.3%	P=0.34
Preovulatory follicle number≥16mm			
1follicle	56	7.1%	
≥2follicles	167	9.6%	P=0.27
Basal FSH level(UI/ml)			
3-5.7	78	8.9%	P=1.3
5.8-7.5	82	9.9%	
7.5-10	63	9.5%	P=0.94

**Table 2**

Pregnancy rate according to sperm parameters

Sperm variable	No of cycles	Pregnancy rate/cycle	P value
Sperm concentration(x10 <sup>6</sup> )			
<10	36	5.6%	
10-20	45	11.1%	NS
>20	142	9.1%	NS
Sperm progressive motility(%)			
<30	29	6.9%	NS
30-40	111	9%	
>40	83	9.6%	NS
Sperm fleshing motility(%)			
<10	67	3%	
≥10	156	11.5%	0.017
Percentage of sperm With normal forms after preparation	143		
<20	63	0%	
20-30	58	10.3%	<0.0001
>30	102	13.7	<0.0001
Final total motile sperm Count inseminated(x10 <sup>6</sup> )			
<5	87	4.6%	0.27
5-10	65	10.7%	0.029
>10	71	12.7%	0.021
Note : NS =not significant			

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