
Threshold Levels of Sperm Parameters Impacting on Pregnancy Rate in an Intrauterine Insemination Programme

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Abstract: Objectives. Intrauterine insemination (IUI) is frequently used as a first line strategy in the treatment of male and unexplained infertility. Threshold levels of sperm parameters associated with IUI success are controversial. The aim of this study was to evaluate the influence of sperm parameters on the outcome of IUI. Study Design. A prospective observational study. Patients and methods. This study included 295 IUI cycles. All IUI cycles were preceded by ovarian superovulation with clomiphene citrate 50 mg tablets orally twice daily for 5 days starting on the second day of menses and recombinant FSH 150 units IM on the 6th, 8th, and 10th day. Cycles were monitored by transvaginal ultrasound. The IUI was performed with a catheter 36 ± 4 hours after hCG injection. Sperm parameters before and after semen treatment for IUI were evaluated and correlated with pregnancy outcome. Results. A total of 29 clinical pregnancies were obtained, for a pregnancy rate per cycle of 9.83%. No pregnancy was obtained when less than one million spermatozoa were inseminated ($p = 0.022$). A statically significant increase in pregnancy rate was observed when normal sperm morphology was $> 20\%$ before semen treatment ($p = 0.01$) and $> 25\%$ after semen treatment ($p = 0.034$). NTSI (number of typical spermatozoa inseminated) and NTMSI (number of typical and mobile spermatozoa inseminated) significantly influence the clinical pregnancy rate. A 65% decrease in pregnancy rate was observed when the NTSI $< 2 \times 10^6$ compared to the NTSI $\geq 2 \times 10^6$ (5.16% vs 15%, $p = 0.004$). Similarly, a 54% decrease in pregnancy rate was observed when the NTMS $< 10^6$ compared to the NTMS $\geq 10^6$ (6.12% vs 13.5%, $p = 0.026$). Conclusion. IUI used for treating male factor infertility seems to have little chance of success when NMSI $< 1 \times 10^6$, NTSI < 2 million, NTMSI < 1 million. If these thresholds cannot be obtained, IVF should be recommended.

Keywords: Intrauterine Insemination, Sperm Parameters, Morphology, Motility, Pregnancy Rate

1. Introduction

Intrauterine insemination (IUI) is the simplest, least invasive and least expensive of the Assistance Reproductive Technologies (ART). Cervical infertility, male and unexplained infertility are still the major indications of this procedure [1]. However, the success rates of this technique are still low, suggesting that IUI should be retained only when it is medically indicated and biologically feasible.

There are certain variables that are currently known to be predictive of IUI success, most of which relate to the female partner, no male factors were found to correlate with the treatment outcome. There is only one accepted male semen analysis parameter, either from the pre or post processing analysis, which has been shown to be predictive of IUI

outcome. That parameter total motile sperm count [2]. Data from the literature are contradictory and do not allow to identify clearly if other sperm parameters before or after semen treatment are associated with IUI success and which minimum threshold values should be accepted to use in daily practice in order to be able to establish a real guide of good practices for insemination [3].

The evaluation of our practices was therefore necessary so as to offerable patients an adapted and personalized management. The aim of our study was to determine the impact of the sperm parameters on the success of IUI.

2. Patients and Methods

From January 2006 to December 2009, 295 couples (women younger than 40 years of age), all at their first

attempt, completed 295 IUI cycles for male factor infertility in our department of biology and reproductive medicine. Institutional Review Board approval was obtained for this study. Before resorting to IUI, all women were subjected to testing for tubal patency by hysterosalpingography, and evaluating the pelvic anatomy by transvaginal ultrasonography. Laparoscopy was done when there was a possibility of pelvic adhesions or endometriosis in the HSG or transvaginal ultrasonography. The assessment of the ovarian reserve was performed in all patients by a hormonal dosage done on day 3 of the cycle (FSH, LH, E2) associated to an ultrasound antral follicles count. Men had at least two semen analyses and microbiological tests before any treatment. All couples were tested for hepatitis B virus and hepatitis C virus before they were offered the IUI trial. Additional testing depended on any abnormalities observed.

Normal semen analyses were defined by the threshold values of the World Health Organization [4](concentration 20×10^6 /mL, total count 40×10^6 , progressive motility 50%, and typical morphology 30%). IUI was indicated only if at least 10^6 progressive motile spermatozoa were selected during a migration survival test. All semen samples were collected in the laboratory after 3 to 5 days of sexual abstinence. After liquefaction for 30 minutes at room temperature, volume, pH, sperm count, and progressive motility "a + b" were evaluated according to the WHO standard criteria. 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 ($\times 4,000$ magnifications). The motility was graded progressive, nonprogressive, or immotile. Motile sperm were selected by a swim-up procedure. In all cases, the motile sperm fraction was washed twice by centrifugation, and the sperm pellet was suspended in 0.35 mL of Earle's balanced salt solution (Sigma-Aldrich Co., Ayrshire, United Kingdom) as a capacitating medium in all patients. Sperm were then counted, and progressive motility assessed. To analyze sperm morphology, smears were prepared from the whole ejaculated fraction and from the motile selected one. The staining procedure was carried out according to the Spermac kit manufacturer's guidelines (Stain Enterprises, Onderstepoort, South Africa). The sperm smear was allowed to air-dry before being fixed in the formalin solution (fixative I) provided in the Spermac kit for 5

minutes at room temperature (23°C). Each slide was flooded with stain solution A for 2 minutes and then rinsed off with water. Each slide was then flooded with stain solution B and C each for 1 minute successively. The percentages of morphologically normal spermatozoa and of various sperm abnormalities were evaluated on 100 sperm at $\times 1,000$ magnification according to criteria of David and al. [5] modified by Jouannet and al. [6]

Ovarian stimulation was performed in all the patients with clomiphene citrate (Clomid®, Sanofi-Aventis, France) 100 mg / d from 2nd to 6th day of the cycle and recombinant FSH (GonalF®, Serono, France) 75 or 150 units on the 6th, 8th, and 10th day. Cycles were monitored by transvaginal ultrasound for the mean follicular volume and thickness of the endometrium on days 10, 12, and 14 of the cycle. Human chorionic gonadotropin 5,000 IU injection was given to induce ovulation when at least one follicle measured 18 mm or more. The IUI was performed with a catheter (Frydmancatheter®(CCD, France) 36 ± 5 hours after hCG injection. All patients received 400 mg/day of dydrogesterone (Utrogestan®, International Besins, France) after insemination till the day of hCG testing. Serum hCG was determined 2 weeks after hCG injection for diagnosis of pregnancy. The outcome measure was the occurrence of clinical pregnancy, diagnosed by the visualization of fetal cardiac activity on ultrasound scan.

Data are expressed as the mean \pm SD or percentages. The χ^2 -test, Fisher's exact test, and Student's *t*-test were used for statistical analysis with SPSS software, version 15.0 for Windows (SPSS, Inc, Chicago, IL, USA). $P < .05$ was considered statistically significant.

3. Results

The mean age of the women at IUI was 33.82 ± 5.074 years (range 23–40 years). According to the WHO criteria [4], various sperm abnormalities were encountered such as isolated oligospermia in 18 patients (6%), isolated asthenospermia in 12 patients (4%), isolated teratospermia in 65 patients (22%), oligoasthenospermia in 9 patients (3%), oligoteratospermia in 6 patients (2%), asthenoteratospermia in 36 patients (12%), and oligoasthenoteratospermia in 40 patients (13%).

Twenty-nine clinical pregnancies followed 295 IUI cycles, for a clinical pregnancy rate of 9.83 %. Spontaneous early abortions occurred in 11 pregnancies (3.7%), and twin pregnancies occurred in 4 patients (1.3%).

Table 1. Sperm parameters values observed in both pregnant and non-pregnant groups before and after semen treatment.

	Before semen treatment			After semen treatment		
	Non pregnant (n=266)	Pregnant (n=29)	P	Non pregnant (n=266)	Pregnant (n=29)	P
Concentration (106/ml)	68.16 \pm 18.1	53.45 \pm 17.8	.288	24.26 \pm 10.5	25.59 \pm 11.2	.732
Motility « a » (%)	13.44 \pm 6.3	16.21 \pm 7.2	.132	36.02 \pm 8.3	41.38 \pm 9.1	.111
Motility « b » (%)	29.52 \pm 15.4	30.52 \pm 14.6	.592	35.17 \pm 10.3	34.66 \pm 10.5	.806
Motility « a+b » (%)	42.96 \pm 20.6	46.72 \pm 19.8	.178	73.12 \pm 18.6	76.03 \pm 19.1	.121
Typical form (%)	17.98 \pm 7.4	24.93 \pm 8.2	.001	22.15 \pm 8.6	29.76 \pm 7.7	.005

Table 2. Pregnancy rate correlated to sperm parameters values before and after semen treatment.

	Before semen treatment			P	After semen treatment			P
	%	cycles	Pregnancy n (%)		%	cycles	Pregnancy n (%)	
Motility « a » (%)	<15	149	10 (6.7)	.052	<35	128	9 (7.03)	.111
	≥15	146	19 (13.01)		≥35	167	20 (11.97)	
Motility « a+b » (%)	<50	170	15 (8.82)	.314	<75	129	12 (9.3)	.474
	≥50	125	14 (11.2)		≥75	166	17 (10.2)	
Typical form (%)	<20	167	10 (5.9)	.010	<25	154	10 (6.4)	.034
	≥20	128	19 (14.84)		≥25	141	19 (13.4)	

Table 3. Comparison of NMSI, NTSI and NTMSI between pregnant and non-pregnant groups.

	Non pregnant (n=266)	Pregnant (n=29)	P
NMSI (106)	5.3	5.45	.877
NTSI (106)	3.42	3.45	.977
NTMSI (106)	1.65	1.34	.471

NMSI: number of motile spermatozoa inseminated; NTSI: number of typical spermatozoa inseminated;

NTMSI: number of typical and motile spermatozoa inseminated.

Table 4. Pregnancy rate correlated to NMSI, NTSI, NTMSI.

		cycles	Pregnancy n (%)	P
NMSI (106)	<1	35	0 (0)	.022
	1≤N<5	119	14 (11.76)	.980
	5≤N<10	99	11 (11.11)	
	N≥10	42	4 (9.52)	
NTSI (106)	<2	155	8 (5.16)	.004a
	≥2	140	21 (15)	
NTMSI (106)	<1	147	10 (6.12)	.026b
	≥1	148	19 (13.5)	

NMSI: number of motile spermatozoa inseminated; NTSI: number of typical spermatozoa inseminated; NTMSI: number of typical and motile spermatozoa inseminated; a a 65% decrease in pregnancy rate; b a 54% decrease in pregnancy rate.

Table 1 shows no significant difference in: total sperm concentration, motility 'a', 'b' and "a + b" before and after sperm treatment, between patients who achieved pregnancy and those who failed. Only normal sperm morphology was significantly associated to pregnancy rate (p=0.001 before sperm treatment and 0.005 after)

Table 2 shows a statically significant increase in pregnancy rate when normal sperm morphology was > 20% before semen treatment (p = 0.01) and > 25% after semen treatment (p = 0.034).

Comparison of NMSI (number of motile spermatozoa inseminated), NTSI (number of typical spermatozoa inseminated) and NTMSI (number of typical and motile spermatozoa inseminated) between pregnant and non-pregnant groups showed no significant differences (table 3).

No pregnancy was obtained when less than one million spermatozoa were inseminated (p = 0.022). Above one million, there is no statistical difference in pregnancy rates between subgroups $1 \leq \text{NMSI} < 5$; $5 \leq \text{NMSI} < 10$ and $\text{NMSI} \geq 10$. Beyond 10 million, our results showed a slight decline (though not significant).

NTSI and NTMS significantly influence the occurrence of clinical pregnancy. A 65% decrease in pregnancy rate was observed when the NTSI < 2 10^6 compared to the NTSI ≥ 2

10^6 (5.16% vs 15%, p = 0.004). Similarly, a 54% decrease in pregnancy rate was observed when the NTMS < 10^6 compared to the NTMS $\geq 10^6$ (6.12% vs 13.5%, p = 0.026). (Table 4)

4. Discussion

There is good evidence in literature that intrauterine insemination (IUI) is the best first line treatment and most cost-effective procedure for moderate male factor subfertility. It seems very difficult to identify individual semen parameters predicting the likelihood of pregnancy after IUI. This can be explained by a lack of standardization of semen analysis, but many other methodological variables may also influence IUI success rates such as the patient selection, type of ovarian stimulation and number of inseminations per cycle [7].

The majority of studies attempting to find which sperm characteristics correlated better with cycle outcome included multiple female infertility problems as well. Hence, the results might be biased by the other infertility etiologies that were treated in parallel. In this study, only the male factor problems were recruited to make the differences in sperm parameters more significant. Twenty-nine clinical pregnancies were achieved after 295 IUIs (9.83%). In a review of 17 papers about IUI with ovarian stimulation by hMG, Wainer et al. [8] reported 274 pregnancies in 2,223 IUI cycles, for a pregnancy rate per cycle of 12.33%.

It has previously been demonstrated that, the number of motile spermatozoa inseminated (NMSI) and sperm morphology are the most valuable sperm parameters to Predict IUI outcome. The minimum recommended number of motile sperm after semen treatment in various reports varies from 0.8 to 10×10^6 [9, 10, 11 and 12]. In the study of Badaway and al [13] the pregnancy rate per cycle was significantly lower in the subgroups with number of motile sperm $< 5 \times 10^6$ (5.55% vs. 24.8%). This was in agreement with Francavilla and al. [14], who found a significant increase in success rate when the total motile sperm count exceeded 5×10^6 . Van Weert and al [15] in a meta-analysis of 16 studies reported a cut-off of NSMI between 0.8 and 5 million. In this study, the number of motile sperm inseminated (NMSI) was not significantly different in the cycles resulting in pregnancy compared to the failed cycles. But, when (NMSI) was $< 1 \times 10^6$, no pregnancy was obtained in our study (p < 0.05). However, the low number of attempts with less than 1 million NMSI generated a sampling bias limiting the relevance of this parameter. Beyond 1 million,

the NSMI does not seem to influence pregnancy rate significantly. But, when NSMI exceeded 10 million, our results showed a slight non-significant decrease in pregnancy rate, which could be related to excessive production of free radicals by the spermatozoa. Most authors recommend IVF when the number of motile sperm is $<1 \times 10^6$ [11, 12, 16 and 17]. Some authors suggested using the total number of motile spermatozoa of the initial sperm count (before semen treatment) as the criterion for choosing between IUI and IVF, and have recommended threshold values ranging from 5 to 10×10^6 [18, 19]. In other studies, total sperm motility before semen treatment was mentioned with a cut-off level between 30 and 50% [20, 21, 22 and 23]. The usefulness of this criterion, however, is limited by the variability of quality from one ejaculation to another, as well as the variable results of sperm preparations. Our study shows no significant difference in sperm motility before sperm treatment, between patients who achieved pregnancy and those who failed.

Sperm morphology using strict criteria is well known as one of the best predictors of IVF outcome [24, 25 and 26]. Sperm morphology has also turned out to be a good predictive parameter in IUI. In a meta-analysis, a significant improvement in pregnancy rates above the 4% threshold for strict criteria was described [15]. Universally, the most common accepted classification system used for sperm morphology is the WHO criteria [20]. The threshold of spermatozoa with normal morphology below which IVF is recommended varies widely in the literature from 4% to 50% [27, 28, 29 and 30]. The present analysis supported these results (thresholds of 20% in the initial sperm and 25% after semen treatment).

However, the implication of sperm morphology alone for choice and outcome of IUI is controversial. Many retrospective and prospective studies showed that sperm morphology alone, before or after preparation, did not help to predict IUI results [31, 32 and 33]. The evaluation of number of motile sperm in conjunction with sperm morphology might represent an important deliberation for clinical decision making. According to Wainer and al [8] For a normal morphology sperm rate $<30\%$, the clinical pregnancy rate was 4.62% when the number of motile sperm inseminated (NMSI) was $<5 \times 10^6$ and 9.45% when the NMSI was $>5 \times 10^6$, and this difference was statistically significant, suggesting a partial compensation of quality by the amount of spermatozoa. The study of Badawy and al [13] showed that above 5 million motile spermatozoa inseminated, the clinical pregnancy rate per cycle was 5.3% when the normal morphology sperm rate (NTSI) rate $<30\%$ and 18.42% when $>30\%$. Our study supported these findings and showed that the NTSI and the NTMSI significantly influence the pregnancy rate in IUI. Thus, when the NTSI was less than 2 million, IUI proved to be an inefficient ART indication with a pregnancy rate of only 5.16%. In contrast, when the NTSI was greater than or equal to 2 million pregnancy rate increases to 15% ($p = 0.04$).

Similarly, when the NTMSI was less than 1 million, the success rate of IUI was 6.12%. This rate increased to 13.5%

when the NTMSI was greater than or equal to 1 million ($p = 0.026$).

5. Conclusion

In view of these results, IUI used for treating male factor infertility seems to have little chance of success when NMSI $<1 \times 10^6$, NTSI <2 million, NTMSI <1 million. If these thresholds cannot be obtained, IVF should be recommended. In this study 295 inseminations cycles were evaluated, it is possible that the sample size may not be enough to detect statistical significance for some parameters with small differences expected in the groups. Further prospective trials will help elucidate the literature. Furthermore, these trials should control for confounding parameters.

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