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Andrology

Impact of Semen Characteristics on the Success of Intrauterine Insemination[1]

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***Purpose*:** To evaluate the influence of sperm characteristics on the outcome of infertility treatment using intrauterine insemination (IUI).
***Methods*:** Retrospective study of 431 infertility couples who underwent 1007 IUI treatment cycles from June 1999 to October 2002. Sperm parameters before and after preparation for IUI were evaluated and correlated with pregnancy outcome.
***Results*:** Clinical pregnancy occurred in 12% of cycles and 28% of patients. Initial sperm motility and processed forward progression were independently associated with pregnancy after IUI. The mean number of cycles per patient was 4.3. Although pregnancy rate per cycle did not differ from cycle to cycle, the cumulative pregnancy rate approached plateau after five cycles.
***Conclusions*:** Sperm motility is an independent factor influencing IUI-related pregnancy. A forward progression score of 3 to 4 in a processed specimen is necessary for IUI success. The number of IUI attempts per patient should be individualized depending upon the needs of patients.

KEY WORDS: Intrauterine insemination; pregnancy rate; sperm forward progression; sperm motility.

INTRODUCTION

Intrauterine insemination (IUI) is frequently used as a first line strategy in the treatment of infertile couples because of its relatively low cost and simplicity. It can be performed efficiently in the office and does not require sophisticated equipment. Studies have confirmed its efficacy for specific indications including unexplained infertility, endometriosis, male infertility, and anovulation (1–5). Predictive sperm parameters and threshold values with respect to semen characteristics for successful intrauterine insemination have been controversial. Although the World Health Organization's (WHO) reference values for semen analysis are often used to assess sperm quality, more often than not successful pregnancies with IUI have been achieved in subfertile couples with sperm parameters below reference values listed in the WHO manual (3,4).

Sperm processing techniques for IUI vary from laboratory to laboratory and even from patient to patient. Miller *et al.* reported significantly lower pregnancy rate for couples with less than 10 million processed total motile sperm (TMS) (6), whereas this same value of 10 million TMS before processing was also suggested as a threshold value (3). The gap between pre- and postprocessing, as measured by recovery, could be a major factor in patients with a borderline value for TMS count. In addition to sperm concentration and total count, other parameters such as motility, forward progression, and days of

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abstinence may also contribute to the success of IUI. In this retrospective analysis we attempted to evaluate relationships between IUI outcome and various parameters of sperm quality, before and after laboratory preparation.

MATERIALS AND METHODS

Patient Population

A total of 431 couples who underwent 1007 IUI treatment cycles from June 1999 to October 2002 in the Johns Hopkins Assisted Reproductive Technology (ART) program were included in the study. The study included only patients with fresh semen specimens for IUI and excluded those with retrograde ejaculation, cycles with frozen semen specimens, or donor specimens. All couples in the study were determined to be infertile on the basis of the clinical evaluation by the attending physician. All patients had at least a recent hysterosalpingogram and a semen analysis. Some patients also had diagnostic laparoscopy. Couples were considered eligible if the female had at least one patent fallopian tube and had a documented normal endometrial cavity. Couples with male-factor infertility were included, provided motile sperm were present in the ejaculate.

Semen Preparation by Density Gradient Separation

On the day of insemination, a semen specimen was collected in a sterile cup and was examined after 30-min liquefaction at 37°C. Initial volume, viscosity, motility, progression, and sperm counts were assessed according to WHO's guidelines (7). The semen samples were processed using density gradient separation procedure in order to remove seminal fluid and enhance sperm quality for IUI. Briefly, a maximum of 2 mL of a semen specimen was gently overlaid on a column consisting of 1 mL of 50% and 1 mL of 90% density gradient Isolate (Irvine Scientific, Santa Ana, CA) in a 15-mL conical tube (Falcon, Becton and Dickinson Labware, Franklin Lakes, NJ). If the specimen volume was greater than 2 mL, an additional column was used. After centrifugation at 300× g for 15 min at room temperature, the pellet(s) was suspended and combined in 5 mL of sperm-washing medium containing modified human tubal fluid medium (mHTF, Irvine Scientific, Santa Ana, CA) and 10% synthetic serum substitute (SSS, Irvine Scientific, Santa Ana, CA). Following centrifugation at 300× g for 8 min, the pellet was resuspended in 0.5 mL of wash medium. The sample was then assessed using a Makler chamber (Sefi-Medical instruments, Haifa, Israel) under a phase contrast microscope (Olympus, Japan) for concentration, motility, forward progression, and total motile sperm count.

If an initial analysis resulted in a TMS count of less than 5×10^6, a simple sperm wash procedure was employed in order to recover a maximum number of the sperm. In this procedure, rather than passing through a density gradient, the semen specimen was transferred into 15-mL sterile conical tubes and mixed with wash medium in a one to one dilution, followed by centrifugation at 300× g for 15 min at room temperature. The pellet was suspended in 5 mL of wash medium and processed as described above.

Ovulation Induction and Insemination

All female patients underwent ovarian stimulation with clomiphene citrate at a dose of 50-mg daily for 5 days beginning on either the 3rd or 5th day of the menstrual cycle. The stimulated cycles were monitored by pelvic ultrasound and serum LH levels. Insemination was performed either 24 h following a spontaneous serum LH surge (>25 mIU/mL) or 36–44 h after hCG administration (10,000 IU i.m) if the largest follicle had achieved a diameter of 23–24 mm and serum LH level was less than 25 mIU/mL. For insemination, the processed sperm was loaded into an Insemi-Cath catheter (Cook Ob/Gyn, Spencer, IA) and then gently deposited into the uterus.

Statistics

Statistical analyses were performed using commercially available software (SPSS Inc. Chicago, IL). Two-tailed unpaired t test was used to compare various variables as appropriate. Chi-square analysis was used for comparison of nominal data between pregnant and nonpregnant cycles and the effect of single parameters between groups. A binary logistic regression method was used to identify variables that may have an impact on pregnancy outcome after IUI using likelihood ratios. Variables selected for analysis in the regression model included sperm motility and sperm forward progression. A p value of <0.05 was considered statistically significant.

RESULTS

The mean age of males was 37.1 ± 13.3 years (Mean ± SD) and of females 35.2 ± 4.5 years (Mean ± SD) at the time of IUI.

Table I. Comparison of Sperm Parameters in Pregnant and Not-Pregnant Groups Before and After Processing with Density Gradient Separation

Sperm parameters	Nonpregnant (mean ± SD)	Pregnant (mean ± SD)	*p* value
Initial specimen			
Concentration (10^6/mL)	71.49 ± 52.56	80.97 ± 57.26	NS
Motility (%)	57.21 ± 18.56	61.27 ± 15.95	0.02
TMS (10^6)	125.93 ± 129.15	128.26 ± 124.10	NS
Processed specimen			
Concentration (10^6/mL)	92.83 ± 90.79	95.16 ± 88.37	NS
Motility (%)	78.85 ± 18.04	81.34 ± 15.33	NS
TMS (10^6)	35.11 ± 35.96	36.50 ± 34.92	NS

Note. TMS = total motile sperm.

Clinical pregnancy occurred in 121 (12%) of 1007 cycles. A comparison of semen parameters in pregnancy and nonpregnancy cycles is showed in Table I. Of the sperm characteristics studied, only sperm motility in the initial specimen differed between the pregnant and the nonpregnant groups ($p < 0.05$). The lowest inseminated TMS count resulting in pregnancy was 0.8×10^6. In our logistic regression model, initial sperm motility and processed forward progression were independently associated with pregnancy after IUI (Table II).

When comparisons between subgroups were performed (Table III), sperm motility in the initial semen specimen of >80% ($p = 0.02$) provided a favorable pregnancy rate (PR) of 17.6%. After processing for insemination, sperm concentration of $51–100 \times 10^6$/mL ($p = 0.019$, PR 16.5%), TMS of $11–100 \times 10^6$ ($p = 0.049$, PR 13.5%), and forward progression of 4 (equal to A in WHO manual) ($p = 0.02$, PR 13.3%) were optimal values for pregnancy.

With regard to sperm morphology by Kruger's strict criteria (8), the value from the semen analysis prior to that for the IUI itself was used since morphology on the specimen for IUI is not routinely evaluated in our laboratory. Morphology did not correlate with any of the factors measured in this study.

The mean number of cycles per patient was 4.3 and cumulative PR was 28% per patient (Fig. 1). The maximum number of cycles for a single patient was 13. Pregnancy rate per cycle did not differ when the rates were compared from cycle to cycle ($p > 0.05$). The cumulative PR reached plateau after the 5th cycle. Patients with 8–10 and 11–13 cycles were clustered because of the small number. Very few patients (11 out of 431) continued after 6 attempts. Of these 11 patients, 5 became pregnant in the 7th to 13th cycle attempt.

DISCUSSION

Intrauterine insemination has been used with variable success for the treatment of numerous indications in the infertile couples (2,9). Accordingly, several semen parameters have been evaluated as predictors of a successful outcome with IUI. This study sought to evaluate the effects of various sperm characteristics, before and after preparation, on IUI-related pregnancy. The study also attempted to determine the optimal ranges of semen parameters. The present study showed that initial sperm motility was significantly higher in specimens which resulted in pregnancy than in nonpregnancy cycles ($p < 0.05$). When the motility before semen preparation was greater than 80%, the pregnancy rate was 17.6% ($p = 0.02$) as compared to pregnancy rates of 7.8–12.2% when the motility was less than 80%.

Table II. Parameters Included in Logistic Regression Model and Their Impact on Pregnancy After IUI

Parameters	B	Exp(B)	95.0% confidence intervals for Exp(B)		*P*
			Lower	Upper	
Initial motility	0.013	1.013	1.002	1.024	0.023
Processed progression	0.448	1.565	1.055	2.319	0.026

Table III. Breakdown of Sperm Parameters in Relation to Pregnancy Rate Per Cycle

Semen parameters	No. of cycles	No. of pregnancies	Pregnancy rate per cycle (%)	*P* value (<0.05)
Initial specimen				
Concentration ($\times 10^6$/mL)				
<50	433	44	10.2	NS
51–100	321	42	13.1	NS
>100	253	35	13.3	NS
Motility (%)				
<40%	179	14	7.8	NS
40–80%	720	88	12.2	NS
>80%	108	19	17.6	0.02
Progression[a]				
1–2	118	9	7.6	NS
3	627	78	12.4	NS
4	262	34	13.0	NS
TMS ($\times 10^6$)				
<11	53	4	7.5	NS
11–100	525	64	12.2	NS
>100	429	53	12.4	NS
Processed specimen				
Concentration ($\times 10^6$/mL)				
<50	416	41	8.9	NS
51–100	237	39	16.5	0.019
>100	354	41	11.6	NS
Motility (%)				
<40	73	7	9.5	NS
40–80	233	28	12.0	NS
>80	697	86	12.3	NS
Progression				
1–2	29	0	0	NS
3	180	15	8.3	NS
4	798	106	13.3	0.02
TMS (10^6)				
<11	267	23	8.6	NS
11–100	681	92	13.5	0.049
>100	59	6	10.1	NS

Note. TMS = total motile sperm.
[a]Progression 4–1 = A–D (WHO).

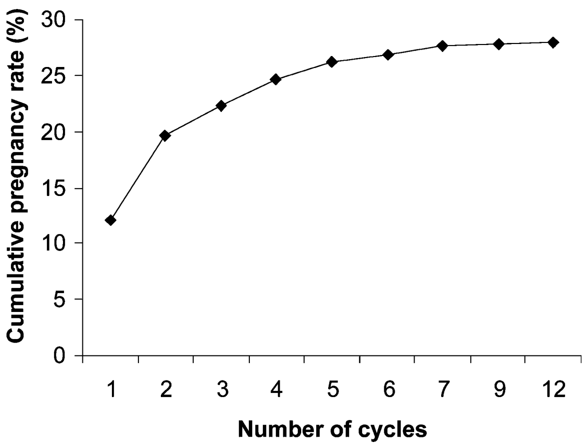

Fig. 1. Cumulative pregnancy rates by number of IUI attempts. For X axis, 9 = cycle number 8–10; 12 = cycle number 11–13.

Hendin *et al.* (10) reported that sperm motility was associated with successful IUI outcome, but this study referred to postwash motility rather than motility in the original semen specimen. In our study, overall postprocessing sperm motility did not differ in patients who conceived and in those who did not. In other words, although sperm motility is improved following sperm-processing procedures, occurrence of pregnancy does not associate with motility in postprocessed specimen. Sperm preparation techniques in these two studies, density gradient separation versus sperm wash, may be responsible for the difference. In a study comparing sperm morphology, motility, and concentration in fertile and infertile men in the original specimens, motility greater than 63% has been identified in the fertile group (11). This conclusion is similar to that in the current study in which an average motility of 61.27% was observed in the pregnancy group (Table I).

In addition to sperm motility, we found that forward progression (after processing) in the final inseminated specimen is another major factor associated with pregnancy as shown in our logistic regression model ($p = 0.026$). Progression 1 to 2 (motile, nonprogressive) did not result in any pregnancy, indicating that sperm motion at moderate to rapid progression is necessary for oocyte fertilization and pregnancy in patients undergoing IUI.

In this study, sperm concentration and total motile sperm count, either before or after processing, did not correlate with IUI outcome. Published reports have been controversial in this regard. Several studies identified no relationship between sperm count and outcome after IUI. For example, Francavilla *et al.* (12) reported that, in the absence of teratozoospermia and severe oligospermia, low total motile sperm count did not have an adverse effect on outcome after IUI. In other studies including couples with isolated male factor infertility, processed total motile sperm count was not associated with pregnancy (13,14) or live birth (10) after IUI. In contrast, Huang *et al.* (15) reported that the TMS count strongly correlated with success after IUI and Miller *et al.* (6) reported that processed TMS count was independently associated with fertility after IUI. The differences may be reflected to patient population, sperm preparation techniques, and/or analysis methodology. In our study, inseminated specimen of sperm concentration ranging between 51 and 100×10^6/mL ($p = 0.019$, PR 16.5%) and total motile sperm count ranging from 11 to 100×10^6 ($p = 0.049$, PR 13.5%) are associated with higher pregnancy rates. When the sperm concentration is greater that 100×10^6 and TMS count is higher than 100×10^6 in the inseminated specimen, the pregnancy rates fell to 11.6 and 10.1% respectively, indicating that too high sperm concentration may have negative impact on sperm motility and fertilizing ability. These findings may reflect that in an environment with a decreased proportion of medium, the sperm metabolic process may be influenced or sperm metabolic product may have impact on sperm quality. If this is the case, it may be advisable that specimen with higher sperm concentration ($>100^6$/mL) or higher TMS count ($>100^6$) should be diluted after preparation/before insemination or only portion of specimen should be used in preparation for IUI.

In the present study of 1007 cycles, pregnancies occurred almost uniformly over 1–13 repeated IUI cycles; although very few patients continued this treatment beyond six attempts. The cumulative pregnancy rate reached plateau after five attempts. Dickey *et al.* suggested that continuing CC-IUI for a fifth and sixth cycle might help to improve the outcome in older women, males with poor sperm quality, and women who respond to CC with fewer preovulatory follicles (5). Others have reported that the cumulative pregnancy rate plateaus by the ninth IUI cycle (16). As in most forms of infertility treatment, the success of IUI is largely influenced by the etiology of the infertility and the sperm quality. However, alternative options including in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) may enhance the chances of success, particularly in older women in whom the age factor becomes increasingly prominent and who cannot afford months or years of attempts with IUI. Social, economic, and religious reasons also play a role in the selection of treatment option. We believe that management must be individualized to the specific needs of a couple and a multitude of factors should be considered to optimize the chance of pregnancy.

CONCLUSION

The IUI data presented in this study demonstrate that sperm motility in the initial specimen is an independent factor affecting the success of IUI treatment. A forward progression score of 3 to 4 in the post-processing specimen may be necessary for a positive outcome following IUI. Processed sperm concentration between 51 and 100×10^6/mL and total motile sperm count ranging from 11 to 100×10^6 per insemination offer the best potential for success. Initial sperm concentration, forward progression, and total motile sperm count had no significant effect on IUI outcome. Number of IUI attempts per patient should be individualized to the needs of each couple based upon both male and female factors before proceeding to alternative means to treat infertility.

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