CLINICAL ASSISTED REPRODUCTION

Relationship of Total Motile Sperm Count and Percentage Motile Sperm to Successful Pregnancy Rates Following Intrauterine Insemination[1]

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Submitted: January 26, 1999
Accepted: May 4, 1999

Purpose: *This study sought (i) to investigate the relationship between postwash total motile sperm count and postwash percentage motile sperm in predicting successful intrauterine insemination and (ii) to determine the minimal postwash total motile sperm count required to achieve pregnancy with intrauterine insemination.*

Methods: *Five hundred four women, who underwent 1636 intrauterine insemination cycles with their partner's sperm for infertility treatment from 1993 through 1995, were included in this retrospective study. All patient charts were reviewed for age, infertility etiology, ovarian stimulation regimens, semen characteristics, and treatment outcome. To determine the relationship between total motile sperm count and intrauterine insemination outcome, patients were grouped as (1) less than 0.5 million, (2) 0.5 to 1 million, (3) 1 to 5 million, (4) greater than 5 million, and (5) greater than 20 million.*

Results: *Similar live birth rates (per cycle) were seen among the postwash total motile sperm count groups: group 1, 3.5%; group 2, 2.4%; group 3, 7.0%; group 4, 6.9%; and group 5, 7.0% ($P = 0.37$). However, regardless of the postwash total motile sperm count, the postwash motility predicted intrauterine insemination success at a cutoff value of 40%.*

Conclusions: *The percentage of postwash sperm motility, and not the postwash total motile sperm count, can predict successful intrauterine insemination outcome. Such information can be useful in counseling patients regarding their chance of success with intrauterine insemination and in determining when alternate methods of assisted reproduction may be a better approach.*

KEY WORDS: male infertility; intrauterine insemination; sperm; sperm motility; total motile sperm count.

INTRODUCTION

In the past decade intrauterine insemination (IUI) with the partner's sperm has been increasingly used to treat male-factor infertility as well as other infertility conditions. The overall success of IUI in recent studies ranges from 5.6 to 16.1% per cycle (1–4). A positive correlation between the pregnancy rate and the total number of motile sperm (total motile sperm count; TMSC) inseminated has been found in some studies (5–9), but others have reported no such correlation (1,2,10–12). While, in theory, there must be a lower limit to the TMSC, below which IUI is no longer efficacious, it is unclear what this limit is and when

[1] Poster presentation at the 1999 Annual Meeting of the American Society of Andrology, Louisville, KY, April 10–13 (Abstract 94).
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in vitro fertilization (IVF) should be advised. Some clinicians have been counseling patients with a postwash total motile sperm count below 1 million, to consider alternative therapies such as artificial insemination with frozen donor spermatozoa or IVF (5). No lower limit for TMSC inseminated has been established to date. Some investigators have found a positive correlation between postwash sperm motility and pregnancy success (5,11,13).

Considering the costs and benefits of different methods of assisted reproduction, IUI is the least invasive and, generally, the least expensive method (14,15). Therefore, knowing which semen characteristics predict IUI success can help physicians counsel patients concerning their chance for success with IUI versus more invasive and expensive assisted reproductive procedures.

The goals of the present study were (a) to investigate the relationship between postwash total motile sperm count and postwash percentage motile sperm with successful IUI (live birth rate) and (b) to determine if there is a lower limit above which total motile sperm count can predict IUI success.

MATERIALS AND METHODS

Study Design

The medical records of all women who underwent IUI with their partners' sperm at The Cleveland Clinic Foundation from January 1993 through December 1995 were reviewed in this retrospective study. These were the first 3 years during which data collection at our institution was standardized for the variables under investigation. All couples with complete data recorded were included for analysis. This study was approved by the Institutional Review Board.

Patient Evaluation

All couples had at least 1 year of primary or secondary infertility with their present partner, and they had completed a basic workup that included medical history, physical examination, and at least two semen analyses. All patients had at least unilateral tubal patency as demonstrated by hysterosalpingogram, chromopertubation at laparoscopy, or both. All patients with suspected female-factor infertility, due to pelvic disease (endometriosis, adhesions), were evaluated laparoscopically. Ovulation was evaluated by basal body temperature, mid-luteal phase progesterone, or endometrium biopsy. Patients with and without ovarian stimulation were included in this study. In patients receiving human menopausal gonadotropin (hMG), ovarian monitoring was performed by transvaginal ultrasonography and serial estradiol (E_2) determinations. All patient charts were reviewed for age, infertility etiology, ovarian stimulation regimens, number of treatment cycles, semen characteristics, and treatment outcome. Patients were divided into five groups according to their postwash TMSC: (1) $< 0.5 \times 10^6$, (2) $0.5–1 \times 10^6$. (3) $1–5 \times 10^6$, (4) $>5 \times 10^6$, and (5) $>20 \times 10^6$. We selected these cutpoints based on the findings from other studies (3,8,9,16).

Sperm Preparation

Semen was collected by masturbation after 2 to 3 days of sexual abstinence. After liquefaction, semen analysis was performed by both manual and computer-assisted semen analysis. Complex sperm motion characteristics were assessed using a computer-assisted semen analyzer (Motion Analysis VP-50; Motion Analysis Corporation, Santa Rosa, CA). The Endtz test (myeloperoxidase staining) was performed on all specimens in which the undifferentiated round cell concentration was greater than 1×10^6/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 (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 min at 600*g*. The supernatant was removed and the pellet was resuspended in 2 ml of human tubal fluid (HTF) medium (Irvine Scientific, Santa Ana, CA). The specimen was then centrifuged for 7 min at 600*g*, following which the supernatant was removed again. The final pellet was resuspended in a volume of 0.4 ml HTF, and semen analysis was performed. Prewash and postwash semen characteristics analyzed included specimen volume, number of leukocytes, sperm concentration, total sperm count, total motile sperm count, and six sperm motion characteristics (percentage motility, curvilinear velocity, straight-line velocity, average path velocity, linearity, and amplitude of lateral head movement).

Insemination Procedure

Intrauterine insemination was performed using a flexible plastic catheter with the patient in the lithot-

Table I. Clinical Data in Patients with or Without Live Births Undergoing Intrauterine Insemination[a]

Variable	Patients with live births (n = 104)	Patients without live births (n = 400)	P^b
Age (mean yr ± SE)	36.8 ± 0.4	37.8 ± 0.1	0.02
Primary infertility	57.8	59.5	0.09
Ovarian stimulation	79	67	0.01
Diagnostic classification			
Idiopathic	31.7	28.8	0.61
Male factor	19.2	17.8	0.67
Female factor	55.1	59.7	0.36

[a] All data are expressed as a percentage unless otherwise indicated.
[b] $P < 0.05$ was considered significant.

omy position under sterile conditions. Single insemination was performed in 1618 IUI cycles and double insemination was performed on successive days in 18 cycles. Only clinical pregnancies were considered, which were defined by visualization of the gestational sac on vaginal ultrasonography. Successful pregnancies were defined as live births.

Statistical Analysis

Variables associated with live births following IUI were analyzed using repeated measures logistic regression with generalized estimating equation (GEE) techniques. Cycles of IUI that failed to result in pregnancy or that resulted in spontaneous abortions were considered unsuccessful. An initial (not including semen characteristics) stepwise GEE analysis was performed to determine which variables were significant risk factors for successful pregnancy. Semen characteristics and the significant risk factors were included in this analysis to determine its relationship to successful pregnancy; in addition to "per-cycle" analyses, patients were classified based on their average postwash percentage motility and postwash total motile sperm count, and per-couple success rates were calculated with Kaplan–Meier estimates. All summary statistics are presented as mean ± standard error, and values were considered statistically significant at $P < 0.05$. All calculations were performed with SAS version 6.12 (SAS Institute, Cary, NC).

RESULTS

Five hundred thirty-three couples underwent 1728 IUI cycles using sperm from their partners. Of these, we included 504 couples (1636 cycles) for analysis, as we had a complete record of their data. These couples underwent an average of 3.2 ± 0.1 cycles of IUI. Pregnancy occurred in 132 patients (26.2%), and live births in 104 (20.6%). The pregnancy and live birth rates per cycle were 9.1 and 6.1%, respectively, for the study population. Indications for IUI were male-factor infertility in 245 cycles (15.0%); female-factor infertility, which included cervical factor, pelvic disease (endometriosis, adhesions), and ovulatory dysfunction, in 901 cycles (55.1%); and idiopathic infertility in 420 cycles (25.7%). The mean female age was 37.6 ± 0.2 years, and ovarian stimulation was used in 1108 (67.7%) cycles.

Table I compares clinical data between patients with and patients without live births. In the successful pregnancy group, women were significantly younger (P = 0.02) and were more likely to have undergone ovarian stimulation (P = 0.01). After adjusting for age and ovarian stimulation, TMSC and percentage motile

Table II. Comparison of Total Motile Sperm Count and Percentage Motile Sperm Between Successful and Unsuccessful Pregnancy Groups[a]

Characteristic	Patients with live births (n = 104)	Patients without live births (n = 400)	P^b
Ejaculated volume (ml)	3.16 ± 0.22	3.3 ± 0.2	0.82
Concentration (10^6/ml)	55.26 ± 5.05	54.3 ± 1.4	0.92
Prewash			
Total sperm count (10^6)	151.9 ± 17.3	137.2 ± 3.5	0.42
Motility (%)	55.6 ± 1.9	54.2 ± 0.7	0.45
Postwash			
Total sperm count (10^6)	33.4 ± 4.3	37.1 ± 1.3	0.33
Total motile sperm count (10^6)	28 ± 4	29.7 ± 1.1	0.37
Motility (%)	79.2 ± 1.5	75 ± 0.6	0.02[c]

[a] Values are mean ± SE.
[b] Results from multivariate analyses when all significant variables are included.
[c] $P < 0.05$ was considered significant.

Table III. Comparison of Successful Pregnancy Rates at Different Cutoff Values of Postwash Total Motile Sperm Count

Postwash TMSC group	Total number of cycles	Successful pregnancy cycles (%)	P^a
≥20 million	626	47 (7%)	0.93
<20 million	963	62 (6.4%)	
≥5 million	1254	86 (7%)	0.62
<5 million	382	23 (6%)	
≥1 million	1538	106 (7%)	0.17
<1 million	98	3 (3%)	
≥0.5 million	1579	107 (7%)	0.43
<0.5 million	57	2 (4%)	

[a] By the Wald chi-square test.

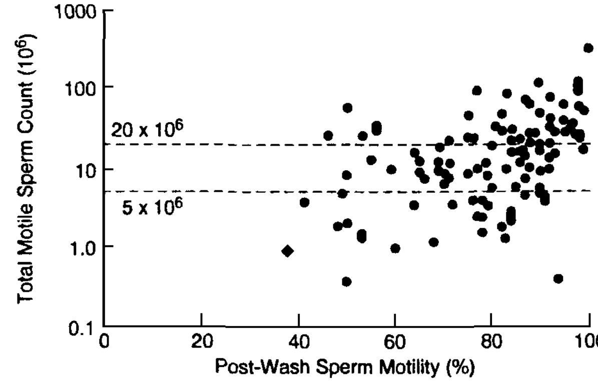

Fig. 1. Distribution of successful pregnancies according to postwash percentage motility and total motile sperm count. (◆) The single successful pregnancy occurring with less than 40% motility.

sperm were compared between successful and unsuccessful pregnancy groups (Table II). The postwash TMSC did not correlate with IUI success. Indeed, the only significant predictor of IUI success (live births) was a higher postwash sperm motility ($P = 0.01$). We found no difference in successful pregnancy rates using a cutoff of 20 million, 5 million, 1 million, or 0.5 million TMSC inseminated (Table III). Thus, patients were analyzed in groups with postwash TMSC between each of the original cutpoints, as follows: (1) less than 0.5 million, (2) 0.5 to 1 million, (3) 1 to 5 million, (4) more than 5 million, and (5) more than 20 million. This showed no significant difference in successful pregnancy rates among the groups (Table IV). The lowest postwash TMSC resulting in a pregnancy was 0.4 million.

Figure 1 shows the distribution of successful pregnancy rates according to postwash motility. Of 89 patients with less than 40% postwash motility, only 1 patient (1.1%) had a successful pregnancy (with 39% postwash motility); in contrast, 103 successful pregnancies occurred in 415 patients (24.8%) with greater than 40% motility. The successful pregnancy rate per cycle was 7% for inseminates with sperm motility greater than 40%, whereas it was only 1% when motility was less than 40%. A postwash sperm motility of >40% appeared to be a threshold for successful IUI, and higher postwash percentage motilities did not significantly increase the successful IUI rates (Fig. 2).

Figure 3 illustrates the per-couple success rates over the first five cycles based on average postwash percentage motility. The couples with 0 to 20 and 20 to 40% average postwash motility had less success during the first five cycles than to those with greater than 40%. No such trend was observed based on TMSC, for example, the highest five-cycle success rate was among the 0.5–1 million TMSC group (Fig. 4).

Table IV. Comparison of Successful Pregnancy Rates According to the Postwash Total Motile Sperm Count

Postwash TMSC group*	Number of cycles (n)	Successful pregnancies, n (rate/cycle)
$<0.5 \times 10^6$	57	2 (3.5%)
$0.5\text{–}1 \times 10^6$	41	1 (2.4%)
$1\text{–}5 \times 10^6$	284	20 (7.0%)
$>5 \times 10^6$	1254	86 (6.9%)
$\geq 20 \times 10^6$	626	47 (7.0%)

* $P = 0.37$ comparing all five groups by GEE.

DISCUSSION

Intrauterine insemination with the partner's sperm is used for a variety of indications, with varying pregnancy rates (1–4). After natural intercourse, a 5× to

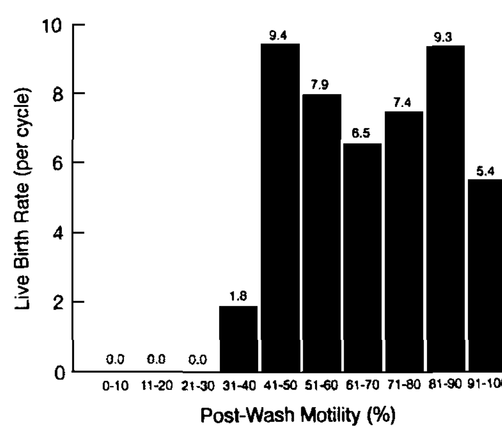

Fig. 2. Relationship between postwash percentage motility and successful pregnancy rate per cycle.

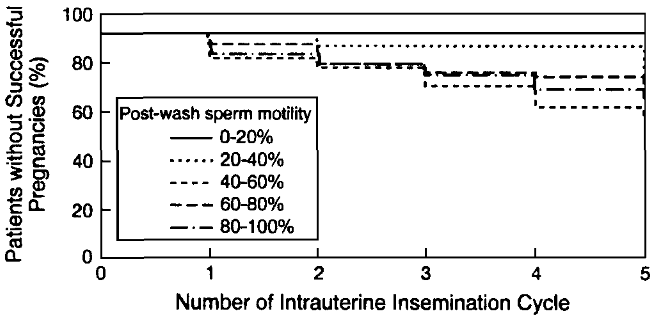

Fig. 3. Kaplan–Meier estimates of per-couple cumulative success rates based on average post wash percentage motility.

6× reduction in sperm number (from ejaculated to the site of fertilization) occurs along the length of the female reproductive tract (18). Consequently, the rationale for IUI therapy is to increase the gamete density at the site of fertilization in order to increase the probability of pregnancy. Previous studies showed that pregnancy rates increase significantly as the total motile sperm count increases, but reports of the cutoff value below which IUI is no longer successful vary greatly (5–9). Berg *et al.* (1997) showed that the total motile sperm threshold necessary for conception after IUI was 0.8 million (3). In contrast, Huang *et al.* (1996) found that the success rate significantly increased when the total motile sperm count was greater than 5 million (9). Pregnancy rates were reported to improve significantly when the total motile sperm count inseminated was greater than 1 million, with optimal pregnancy rates with insemination of more than 10 million (5). Brash *et al.* (1994) also determined that the number of conceptions increased as the total motile sperm count increased, however, the relationship became statistically significant only when the TMSC exceeded 20 million sperm (8). Other authors, in a review study, concluded that pregnancy was unlikely with insemination of fewer than 400,000 motile sperm and the optimal pregnancy rates occurred with insemination of 15 million motile sperm (16).

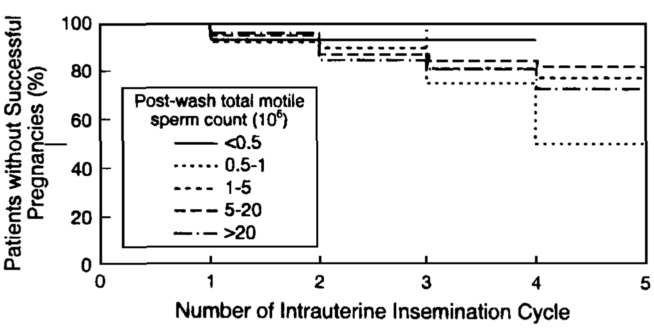

Fig. 4. Kaplan–Meier estimates of per-couple cumulative success rates based on postwash total motile sperm count.

In the present study, after adjusting for all confounding factors (age and ovarian stimulation), we investigated the relationship of two postwash variables (postwash total motile sperm count and percentage motile sperm) to successful pregnancy rates with IUI in 504 patients. Although there was a trend toward increased successful pregnancy rates when the postwash TMSC was greater than 1 million (Table IV), this relationship was not statistically significant. Furthermore, there was no significant difference in successful pregnancy rates even at 20 million sperm inseminated. Therefore, we could not identify any cutoff value for this sperm characteristic which could predict successful pregnancy. A similar lack of significant correlation between TMSC and pregnancy rate was reported by others (1,2,10–12,19). Very low pregnancy rates have been reported following IUI when fewer than 1 million TMSC were inseminated (20–23). In our study, only three patients achieved pregnancy with a postwash TMSC of <1 million sperm, with the lowest count being 0.4 million.

Our study had 90% power to detect whether patients with a postwash TMSC of 20 million had 1.1 times greater odds of IUI success than patients with less than 20 million and 90% power to detect if patients with a postwash TMSC greater than 1 million had 3.3 times greater odds of IUI success than those with less than 1 million. We found that the percentage postwash sperm motility was a good predictor of successful pregnancy. This is in agreement with others (5,11,13,24). In our study, live birth rates increased significantly when the postwash motility was greater than 40%, and only one successful pregnancy occurred below this value. Similar results were reported by Ombelet *et al.* (1996), who found a pregnancy rate of 8.6% per cycle when the postwash sperm motility was less than 40% and a per-cycle pregnancy rate of 14.5% when the percentage motility was greater than 40% ($P < 0.05$) (11). Arny *et al.* (1987) found that after sperm preparation using the swim-up method, only motility was a significant marker for discriminating between patients who would (post-swim-up motility, >78.7%) and patients who would not (motility, <78.7%) conceive (24). Horvath *et al.* (1989) demonstrated that pregnancy was unlikely if there was less than 40% motility in the post-swim-up specimen; among 42 pregnancies, only 2 occurred with a post-swim-up motility of less than 40%. In their study, however, sperm concentration (>1 million) and total motile sperm count (1 to 10 million) correlated more highly with cycle fecundity than post-swim-up percentage motility (5). Another study demonstrated a clinical pregnancy rate of 13.4% per cycle when the initial sperm motility was 30% or higher before preparation, compared to a rate of 0% when the motility was lower than 30% (25). Other investigators, however, found no correlation between overall postwash percentage motility and treatment outcome, but velocity and linearity were found to be correlated with IUI outcome (19,26).

We observed a positive correlation between the postwash TMSC and successful pregnancy rates, but TMSC failed to predict successful IUI outcome. However, a postwash percentage motility greater than 40% was a good predictor of successful outcome. Of particular interest is that although the postwash total motile sperm count did not correlate with successful pregnancy, the percentage of motile sperm did correlate. Thus, even in the setting of a high postwash TMSC, if the percentage of nonmotile sperm is greater than the percentage of motile sperm, the live birth rate will still be low. This may be due to the damaging effects of oxygen free radicals released from these nonmotile sperm, leukocytes, and immature germ cells. Numerous studies have documented reactive oxygen species production from sperm, with higher levels being produced from abnormal sperm (27,28). Improved sperm processing techniques might increase fertilization or pregnancy rates for couples with a poor postwash motility (removing nonmotile and abnormal sperm), and exogenous motility stimulants, such as pentoxifylline, may also be of benefit in therapeutic semen processing (29–31).

Some investigators have reported a correlation between successful pregnancy and sperm morphology alone or in combination with other sperm characteristics (2,8,32,33). Burr *et al.* suggest that sperm morphology rather than motility is a more sensitive guide to IUI outcome and have found no correlation between the number of spermatozoa inseminated and pregnancy (2). A limitation of our retrospective study is that we did not evaluate sperm morphology; it is possible that the postwash TMSC in association with sperm morphology could have significant power to predict whether IUI will be successful. Further studies are necessary to address this issue.

In conclusion, the percentage postwash motile sperm can predict the success of IUI. Such data will be useful in helping physicians counsel patients with adequate postwash percentage motility (>40%) to proceed with IUI as the initial assisted reproductive procedure, even with a low postwash total motile sperm count.

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