Postwash total motile sperm count: should it be included as a standard male infertility work up

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Introduction: *Pregnancy rates after intrauterine insemination (IUI) varies greatly. We aimed to identify pre and post processing semen analysis parameters that may be predictive of successful pregnancy in couples undergoing IUI.*

Materials and methods: *A retrospective study of the records of all couples underwent IUI for a 2 year period at our infertility center. Different characteristics of female subjects, pre and post processing semen parameters and treatment parameters were compared statistically.*

Results: *Thirty-two clinical pregnancies followed 526 IUI cycles in 294 couples, for a clinical pregnancy rate of 6.1% per cycle and a 10.9% per couple. The mean age of the women at IUI was 31.14 ± 6 years (range 19-45 years). Neither maternal age, body mass index, number of mature follicles, maximum day 3 follicle stimulating hormone level, presence or absence of previous children, number of previous miscarriages, nor prewash semen parameters had any impact on pregnancy rate post IUI. Postwash total motile sperm count (TMSC) (p = .027) and number of cycles (p = .042) were independent predictors of successful pregnancy after IUI.*

Conclusions: *A postwash TMSC of 5 million sperm or more is significantly associated with a high pregnancy rate. After ruling out medically or surgically correctable male factors that may contribute to infertility, we recommend including a pretreatment sperm processing during routine male fertility work up for proper patient counseling and direction to the suitable assisted reproduction technique.*

Key Words: infertility treatment, intrauterine insemination, sperm, sperm motility, total motile sperm count

Introduction

Intrauterine insemination (IUI) is a minimally invasive, less expensive and more acceptable infertility treatment compared with other complex assisted reproduction techniques such as in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI).[1,2] Therapeutic IUI using the husband's sperm is commonly performed for male factor infertility, cervical infertility, anovulation, endometriosis with a healthy fallopian tube, as well as to enhance the probability of conception in various unexplained infertility conditions.[3,4] The rationale for IUI therapy is to increase the gamete density at the site of fertilization in order to increase the probability of pregnancy.[3]

Pregnancy rates after IUI varies greatly from one study to another according to patient selection criteria, the presence of various infertility factors, methods of ovarian stimulation, number of cycles performed, different sperm parameters, and technique of preparation. Due to the heterogeneity of the sample

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patient population, it is difficult to indicate which sperm features have high fertilization potential after IUI.[5] However, several semen parameters have been shown to correlate with IUI outcome such as number of motile sperms and normal morphology.[6,7] Others found that no male factors correlate with the treatment outcome.[8]

Semen processing prior to insemination is necessary to remove prostaglandins, infectious agents, and antigenic proteins as well as removal of nonmotile spermatozoa, leukocytes, or immature germ cells. This may be an important factor in enhancing sperm quality by decreasing the release of lymphokines and/or cytokines and reducing the formation of free oxygen radicals after sperm preparation.[9]

We hypothesized that post processing parameters may be more predictive of success than preprocessed parameters which may be useful in counseling patients regarding their chance of success with intrauterine insemination or the choice of alternate methods of assisted reproduction such as IVF and ICSI. Hence, we tried to identify pre and post processing semen analysis parameters that may be predictive of successful pregnancy in couples undergoing IUI.

Material and methods

After our Institutional Review Board approval, records of all couples underwent IUI for a 2 year period at the infertility center in King Khalid University Hospital, King Saud University, Saudi Arabia were retrospectively reviewed.

All women had patent tubes documented by either hysterosalpingogram or laparoscopy with chromopertubation to be qualified for IUI treatment. After the female patient starts her prescribed gonadotropin stimulation medication injections, her ovulation was monitored by regular transvaginal ultrasound imaging examinations, where the mean diameters of the maturing follicles were accurately measured.

All couples were requested to abstain from intercourse for 2-7 days before IUI. Semen samples were produced by masturbation into sterile containers, and the volume of the ejaculate was recorded. Sperm concentration and motility were determined according to WHO criteria.[10] Total motile sperm count (TMSC) in the ejaculate was calculated by multiplying the ejaculate volume times the sperm concentration times the percentage of motile sperm in the sample. Total progressive motile sperm count (TPMSC) was calculated by multiplying TMSC times the percentage of A and B motility spermatozoa.

Semen processing was carried out using the discontinuous density gradients technique of the WHO laboratory manual 2010.[10] Semen specimens were centrifuged through two-layered density gradient (PureSperm 40/80 from Nidacon International-Sweden, Catalogue No.PSK-020), for 20 minutes, at 18000 rpm. The sperm supernatant were then washed twice with HEPES-buffered media (Quinn's Advantage Medium, with HEPES, from SAGE Company, Cat. No. 1023), supplemented with serum (Quinn's Advantage Serum Protein Substitute SPS from SAGE Company, Cat. No. 3010), where final sperm product was constituted within 0.5 mL of the same media. Post processing TMSC and TPMSC were calculated. Pre and post processing TMSC were further categorized into less than 5×10^6 or 5×10^6 and more. Similarly, pre and post processing TPMSC were dichotomized into less than 1×10^6 or 1×10^6 and more.

Intrauterine insemination was performed 36 hours after the injection of 5,000 IU β-hCG injection (Pregnyl, Merck, West Orange, NJ, USA), (Novarel, Ferring Pharmaceuticals, Inc., Tarrytown, NY, USA) or 250 mcg Ovidrel injection, (Merck-Serono Laboratories, Rockland, MD, USA). The insemination was performed in a sterile fashion, using a flexible plastic catheter with the patient in the dorsal lithotomy position. The patient should not assume a prone position for at least 10 minutes after the end of the insemination.

Data analysis

Descriptive statistics are presented as the mean (SD) and percent. For comparative statistics of the impact of the different demographics and clinical characteristics on the two pregnancy outcome groups (positive versus negative cases), categorical variables were compared using chi-square/ Fisher's exact tests and continuous variables were analyzed using Student's t-test.

To control for confounding variables and multiplicity, different parameters were evaluated by multiple logistic regression analysis with forward stepwise elimination using likelihood ratio to estimate the adjusted odds of a positive pregnancy test. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were estimated separately for each factor. A p value of less than 0.05 was considered significant for all tests performed using SPSS statistical software.

Results

Thirty-two clinical pregnancies followed 526 IUI cycles in 294 couples, for a clinical pregnancy rate of 6.1% per cycle and a 10.9% per couple with an average of 1.79 ± 0.94 IUI cycle per couple (range 1-5 cycles). The mean

TABLE 1. **Characteristics of female partners in pregnant and non pregnant couples after intrauterine insemination (IUI)**

	Pregnant	Non pregnant	p value	Test
Age (years)	30.4 ± 6	31.2 ± 6	.463	t-test
Body mass index	29.9 ± 5	30.1 ± 6	.859	t-test
Previous children			.621	chi-square
No	23 (11.5%)	177 (88.5%)		
Yes	9 (9.6%)	85 (90.4%)		
Miscarriage			0.88	chi-square
No miscarriage	19 (8.6%)	202 (91.4%)		
Twice miscarriages or less	11 (17.5%)	52 (82.5%)		
More than 2 miscarriages	2 (20%)	8 (80%)		
Number of IUI cycles	1.5 ± 0.8	1.8 ± 0.95	.042	t-test
Number of IUI cycles			1.00	fisher's exact
3 cycles or less	31 (11.1%)	249 (88.9%)		
More than 3 cycles	1 (7.1%)	13 (92.9%)		
Diameter of mature follicles	1.9 ± 1.1	1.8 ± 0.99	.454	t-test
Max day 3 FSH (mIU/mL)	6.12 ± 2.4	6.95 ± 2.98	.133	t-test

age of the women at IUI was 31.14 ± 6 years (range 19-45 years). Table 1 compares characteristics of female subjects who conceived to those who did not.

Maternal age, body mass index (BMI), number of mature follicles, maximum day 3 follicle stimulating hormone (FSH) level, presence or absence of previous children and the number of previous miscarriage had no impact on pregnancy rate post IUI. The number of IUI cycles was found to be an independent predictor of clinical pregnancy after IUI (p = .042). Most of the pregnancies (31/32, 96.9%) were encountered in patients who underwent three cycles or less.

Table 2 compares pre and postwash sperm motility parameters in pregnant and non pregnant couples after IUI. None had any impact on the pregnancy rate after IUI whether they were measured before or after processing apart from the postwash TMSC. Compared to a postwash TMSC of less than 5 X 10^6, more counts were significantly associated with a higher pregnancy rate (p = .027). On the other hand, comparing pre and postwash TMSC, 41 (14.4%) and 32 (11.9%) of males having prewash TMSC of more than 5 and 10 million respectively had a postwash TMSC less than 5 millions.

The impact of various female treatment and pre and post processing semen analysis parameters on pregnancy rate was further evaluated by multiple logistic regression analysis, Table 3. Number of IUI cycles and the postwash TMSC were significantly associated with and sustained an independent significant impact on a positive pregnancy test. Positive pregnancy was 7.9 times more with 5 X 10^6 or more postwash TMSC compared to a lower postwash TMSC (p = .045).

Discussion

Intrauterine insemination is generally attempted before proceeding to more expensive and invasive assisted reproductive techniques.[1] The choice of the most appropriate assisted reproductive treatment for the individual couple is often a difficult one. The aim is to achieve a live birth with the least invasive technology available, hence, it is imperative to identify which subfertile couples are likely to benefit from IUI and which are more likely to benefit from IVF or ICSI.[11]

The effectiveness of IUI depends on many factors, including semen quality.[12-14] However, the predictive sperm parameters and threshold values that are related to IUI success are controversial.[15-17]

Our results showed that postwash TMSC was an independent predictor of clinical pregnancy after IUI. A postwash TMSC of 5 X 10^6 or more was associated with 10.6% pregnancy/couple compared to a 0.3% (one pregnancy only) if postwash TMSC is less than 5 X 10^6 (p = .027).

The postwash TMC represents the total number of motile sperms that are present after preparation and are subsequently available for insemination in IUI. It has a unique value as a prognostic tool as it reflects both sperm concentration and motility, as well as the effects

TABLE 2. **Pre and postwash sperm motility parameters in pregnant and non-pregnant couples after intrauterine insemination (IUI)**

	Pregnancy	No pregnancy	p value	Test
Prewash TMSC (n X 10^6)	152 ± 185	104.6 ± 109	.165	t-test
Prewash TMSC (n X 10^6)			.608	fisher's exact
Less than 5 X 10^6	2 (8.3%)	22 (91.7%)		
5 X 10^6 or more	30 (11.1%)	240 (88.9%)		
Prewash TMSC (n X 10^6)			1.00	fisher's exact
Less than 10 X 10^6	0 (0%)	10 (100%)		
10 X 10^6 or more	32 (11.3%)	252 (88.7%)		
Prewash progressive motility (%)	59.4 ± 12	55.4 ± 14.8	.144	t-test
Prewash progressive motility (%)			.054	fisher's exact
Less than 40%	0 (0%)	28 (100%)		
40% or more	32 (12%)	234 (88%)		
Prewash TMPSC (n X 10^6)	96.4 ± 119	59.4 ± 73.96	.127	t-test
Prewash TMPSC (n X 10^6)			.604	fisher's exact
Less than 1 X 10^6	0 (0%)	9 (100%)		
1 X 10^6 and more	32 (11.2%)	253 (88.8%)		
Postwash TMSC (n X 10^6)	34.9 ± 32.4	23.6 ± 27.5	.066	t-test
Postwash TMSC (n X 10^6)			.027	chi-square
Less than 5 X 10^6	1 (2%)	49 (98%)		
5 X 10^6 or more	31 (12.7%)	213 (87.3%)		
Postwash progressive motility (%)	67 ± 19	67 ± 19	.957	t-test
Postwash progressive motility (%)			1.00	fisher's exact
Less than 40%	2 (10%)	18 (90%)		
40% or more	30 (10.9%)	244 (89.1%)		
Postwash TMPSC (n X 10^6)	25.6 ± 26.4	16.9 ± 20.9	.078	t-test
Postwash TMPSC (n X 10^6)			.232	fisher's exact
Less than 1 X 10^6	0 (0%)	16 (100%)		
1 X 10^6 and more	32 (11.5%)	246 (88.5%)		

TMSC = total motile sperm count; TMPSC = total motile progressive sperm count

of sperm processing.[11] Studies have demonstrated that motility is considered an important success factor for natural pregnancy as well as for IUI.[5,18,19]

Prewash TMSC did not attain any significant impact on pregnancy after IUI in our results whether entered as a continuous variable or dichotomized using the threshold values of 5 or 10 million sperm, Table 2. Furthermore, up to 14.4% of those who may be considered to have normal TMSC according to these threshold values had abnormal counts (less than 5

TABLE 3. **Significant independent predictors of pregnancy after intrauterine insemination (IUI)**

Variable	p value	Odds of pregnancy	95% confidence interval
Number of cycles	.027	.564	.340-.937
Postwash TMSC			
Less than 5 X 10^6	.045	1	1.048-59.767
5 X 10^6 or more		7.916	

TMSC = total motile sperm count

millions) in postwash TMSC estimation. As semen is processed prior to insemination, it would make sense that parameters of raw samples do not correlate with cycle fecundity as do processed specimens.[6,7,20,21]

In our results, postwash TMSC was not a significant predictor when entered as a continuous variable but when dichotomized using the threshold of 5 million motile sperm. Cut off for defining severe male factor infertility has varied from a study to study, but threshold values between 0.3 and 20 million postwash TMSC were reported.[11] In a meta-analysis of 16 studies, the cut off values with the greatest shift from pretest to posttest probability varied between 0.8 and 5 million processed motile sperm.[11]

The postwash total motile sperm count (TSMC) has been proposed as a test to help distinguish the couples who would benefit from IUI from the couples who would benefit more from IVF or ICSI.[11,22] Such information can be useful in counseling patients. The postwash TMC may be assessed during the routine fertility work up or at the actual time of insemination. In view of our results, we believe it is worthwhile to perform a pretreatment sperm preparation during standard fertility work up to evaluate the count of postwash total motile sperms. Whenever this count is high, we recommend attempting three cycles of IUI before more sophisticated assisted reproduction techniques.

Dinelli et al investigated the prognostic factors of pregnancy after IUI.[23] In their study, the pregnancy rate increased when the postwash TPMSC is greater than 1 million as compared to the pregnancy rate when it is fewer than 1 million. However, this association was not sustained in multivariate analysis. Postwash TMPSC had no impact on the pregnancy rate after IUI in our patients' cohort whether tested as a continuous variable or when dichotomized to more and less than the threshold of 1 million, Table 2.

Our results also showed that number of IUI cycles was an independent predictor of clinical pregnancy after IUI. The mean number of IUI cycles was significantly less in the pregnant group compared to non pregnant one (p = .042). In the present study, 31 clinical pregnancies had been achieved (96.1%) by the end of the third cycle, Table 1. Similarly, Plosker and Amato[24] advised infertile couples to receive IVF after three unsuccessful IUI. In cases of unexplained infertility, Aboulghar et al[25] found a cumulative pregnancy rate of 39.2% after three cycles and 48.5% after six cycles in a study of 1,112 IUI cycles (16.4% per cycle).

Our study is limited by being retrospective with the inherent limitations of a case-controlled study design. Also, we did not analyze the impact of sperm morphology, which has been reported to have an independent impact on pregnancy rates after IUI.[26,27]

Our observation that postwash TMSC is an independent predictor of positive pregnancy after IUI is important in counseling the infertile couple regarding their chance of success and directing them to the proper assisted reproduction technique. We recommend inclusion of pretreatment sperm processing during routine fertility work up after ruling out medically or surgically correctable male factors that may contribute to infertility. We also suggest that assessment of postwash TMSC during the routine male fertility work up may be helpful to identify males with normal unprocessed semen analysis and unexplained infertility. Our study also opens the door for future research to evaluate the threshold value of postwash TMSC needed for natural pregnancy.

Conclusions

Postwash total motile sperm count and number of IUI cycles are independent predictors of clinical pregnancy after IUI. Postwash TMSC is a useful prognostic predictor of successful IUI. A postwash TMSC of 5 million sperm or more is significantly associated with a high pregnancy rate. This is of significance in counseling the patients and to help distinguish the couples who would benefit from IUI from the couples who should be directed to alternate methods of assisted reproduction. We recommend including a pretreatment sperm processing during routine fertility work up to evaluate the count of motile sperms. Attempting IUI as the initial treatment is recommended for couples with a postwash TMSC of 5 million sperm or more, after ruling out medically or surgically correctable male factors that may contribute to infertility. □

References

1. Dodson WC, Haney AF. Controlled ovarian hyperstimulation and intrauterine insemination for treatment of infertility. *Fertil Steril* 1991;55(3):457-467.
2. de Araújo LFP, de Araújo Filho E, Fácio CL et al. Efficacy of sperm motility after processing and incubation to predict pregnancy after intrauterine insemination in normospermic individuals. *Reprod Biol Endocrinol* 2013;11:101.
3. Allen NC, Herbert CM, Maxson WS, Rogers BJ, Diamond MP, Wentz AC. Intrauterine insemination: a critical review. *Fertil Steril* 1985;44(5):569-580.
4. Marchetti C, Dewailly D. Intrauterine insemination: indications and methods. *Rev Prat* 2006;56(5):500-506.

5. Shulman A, Hauser R, Lipitz S et al. Sperm motility is a major determinant of pregnancy outcome following intrauterine insemination. *J Assist Reprod Genet* 1998;15(6):381-385.
6. Wainer R, Merlet F, Bailly M, Lombroso R, Camus E, Bisson JP. Prognostic sperm factors in intra-uterine insemination with partner's sperm. *Contracept Fertil Sex* 1996;24(12):897-903.
7. Horvath PM, Bohrer M, Shelden RM, Kemmann E. The relationship of sperm parameters to cycle fecundity in superovulated women undergoing intrauterine insemination. *Fertil Steril* 1989;52(2):288-294.
8. Farimani M, Amiri I. Analysis of prognostic factors for successful outcome in patients undergoning intrauterine insemination. *Acta Med Iran* 2007;45(2):101-106.
9. Aitken RJ, Clarkson JS. Significance of reactive oxygen species and antioxidants in defining the efficacy of sperm preparation techniques. *J Androl* 1988; 9(6):367-376.
10. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th ed. Switzerland: WHO press. 2010: pp 7-168.
11. van Weert JM, Repping S, Van Voorhis BJ, van der Veen F, Bossuyt PMM, Mol BWJ. Performance of the postwash total motile sperm count as a predictor of pregnancy at the time of intrauterine insemination: a meta-analysis. *Fertil Steril* 2004;82(3): 612-620.
12. Campana A, Sakkas D, Stalberg A et al. Intrauterine insemination: evaluation of the results according to the woman's age, sperm quality, total sperm count per insemination and life table analysis. *Hum Reprod* 1996;11(4):732-736.
13. Khalil MR, Rasmussen PE, Erb K, Laursen SB, Rex S, Westergaard LG. Intrauterine insemination with donor semen. An evaluation of prognostic factors based on a review of 1131 cycles. *Acta Obstet Gynecol Scand* 2001;80(4):342-348.
14. Montanaro Gauci M, Kruger TF, Coetzee K, Smith K, Van Der Merwe JP, Lombard CJ. Stepwise regression analysis to study male and female factors impacting on pregnancy rate in an intrauterine insemination programme. *Andrologia* 2001;33(3): 135-141.
15. Merviel P, Heraud MH, Grenier N, Lourdel E, Sanguinet P, Copin H. Predictive factors for pregnancy after intrauterine insemination (IUI): an analysis of 1038 cycles and a review of the literature. *Fertil Steril* 2010;93(1):79-88.
16. Francavilla F, Romano R, Santucci R, Poccia G. Effect of sperm morphology and motile sperm count on outcome of intrauterine insemination in oligozoospermia and/or asthenozoospermia. *Fertil Steril* 1990;53(5):892-897.
17. Van Voorhis BJ, Barnett M, Sparks AE, Syrop CH, Rosenthal G, Dawson J. Effect of the total motile sperm count on the efficacy and cost-effectiveness of intrauterine insemination and in vitro fertilization. *Fertil Steril* 2001;75(4):661-668.
18. Huang HY, Lee CL, Lai YM et al. The impact of the total motile sperm count on the success of intrauterine insemination with husband's spermatozoa. *J Assist Reprod Genet* 1996;13(1):56-63.
19. Brasch JG, Rawlins R, Tarchala S, Radwanska E. The relationship between total motile sperm count and the success of intrauterine insemination. *Fertil Steril* 1994;62(1):150-154.
20. Branigan EF, Estes MA, Muller CH. Advanced semen analysis: a simple screening test to predict intrauterine insemination success. *Fertil Steril* 1999;71(3):547-551.
21. Zhang E, Tao X, Xing W, Cai L, Zhang B. Effect of sperm count on success of intrauterine insemination in couples diagnosed with male factor infertility. *Mater Sociomed* 2014;26(5):321-323.
22. Arny M, Quagliarello J. Semen quality before and after processing by a swim-up method: relationship to outcome of intrauterine insemination. *Fertil Steril* 1987;48(4):643-648.
23. Dinelli L, Courbière B, Achard V et al. Prognosis factors of pregnancy after intrauterine insemination with the husband's sperm: conclusions of an analysis of 2,019 cycles. *Fertil Steril* 2014; 101(4):994-1000.
24. Plosker SM, Jacobson W, Amato P. Predicting and optimizing success in an intra-uterine insemination programme. *Hum Reprod* 1994;9(11):2014-2021.
25. Aboulghar M, Mansour R, Serour G, Abdrazek A, Amin Y, Rhodes C. Controlled ovarian hyperstimulation and intrauterine insemination for treatment of unexplained infertility should be limited to a maximum of three trials. *Fertil Steril* 2001;75(1):88-91.
26. Badawy A, Elnashar A, Eltotongy M. Effect of sperm morphology and number on success of intrauterine insemination. *Fertil Steril* 2009;91(3):777-781.
27. Sun Y, Li B, Fan LQ et al. Does sperm morphology affect the outcome of intrauterine insemination in patients with normal sperm concentration and motility? *Andrologia* 2012;44(5):299-304.