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Impact of Total Progressive Motile Spermatozoa Concentration Following Pellet Swim-up versus Double Density Gradient Centrifugation on Intrauterine Insemination Outcome: A Retrospective Cohort Study

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ABSTRACT

Introduction: Intrauterine Insemination (IUI) is a widely used treatment modality for infertile couples; however, the factors determining successful outcomes have been extensively studied. The prewash Total Motile Sperm Count (TMSC) has been shown to be a poor predictor of IUI outcomes and data regarding postwash Total Progressive Motile Spermatozoa Concentration (TPMSC) have remained inconsistent, with proposed values varying widely across studies.

Aim: To evaluate the impact of TPMSC following sperm preparation by pellet and swim-up (PSU) and Double Density Gradient Centrifugation (DDGC) on the IUI outcomes.

Materials and Methods: This retrospective cohort study included 490 patients who underwent IUI at an Assisted Reproductive Technology (ART) Clinic, Chennai, Tamil Nadu, India between January 2015 and January 2025. After considering the inclusion and exclusion criteria, the study cohort was broadly categorised based on the sperm preparation method (PSU: n=363, DDGC: n=127) and were then divided into four groups - Group I: TPMSC <5 x10^6/mL, Group II: TPMSC 5-9x10^6/mL, Group III: TPMSC 10-20x10^6/mL, Group IV: TPMSC >20x10^6/mL. The primary outcome was positive pregnancy rate, assessed and compared across TPMSC categories, while secondary outcomes included biochemical pregnancy, clinical pregnancy, ectopic pregnancy, and miscarriage rates. Statistical analysis was performed using IBM Statistical Package for the Social Sciences (SPSS) software, with categorical data evaluated using the Chi-square test.

Results: A significant increase in pregnancy rate was observed in the group with TPMSC >20 x10^6/mL (24.3%, p-value <0.001) when the sample was prepared by PSU, compared to lower TPMSC categories. In contrast, no such association was observed with the DDGC technique (p-value=0.154). There was no significant difference in biochemical, ectopic, clinical pregnancy and miscarriage rates among the groups.

Conclusion: The overall clinical pregnancy rate was 15.1% in the PSU group and 15% in the DDGC group. However, no association was observed between TPMSC and secondary outcomes in either the PSU or DDGC groups.

Keywords: Infertility, Sperm preparation, Clinical pregnancy rate

INTRODUCTION

Infertility is a condition affecting the male or female reproductive system, characterised by the inability to conceive after at least 12 months of regular, unprotected sexual intercourse [1]. The incidence of infertility among reproductive age couples ranges from 8-12% worldwide. Male factor solely contributes to around 20% of couples with infertility and as a contributing factor in 30-40% of couples experiencing infertility [2]. Male factor infertility may result from sexual dysfunction or abnormal semen parameters [3]. Semen analysis performed according to World Health Organisation (WHO) guidelines, is the primary step in diagnosing abnormalities in semen causing male infertility. Sperm concentration, total sperm count, motility and the percentage of morphologically normal sperms are the key parameters used to evaluate semen quality [4].

The IUI is a treatment method in Assisted Reproductive Technology (ART) involving deposition of a prepared spermatozoa suspension, usually 0.4-0.5 mL containing the maximum number of motile spermatozoa into the uterine cavity to increase the chance of fertilisation. IUI, being minimally invasive and cost-effective, helps in reducing the economic burden and is thus considered a first-line treatment option for fertile couples with unexplained infertility or mild to moderate male factor infertility [5]. Sperm preparation for IUI involves isolation of spermatozoa from seminal plasma to obtain a sample enriched with progressively motile, morphologically normal sperm cells, that are free from debris and non germ cells [4]. Sperm preparation can be performed by any of the following techniques: a) Direct Swim-up (DSU), b) Pellet and Swim-up (PSU) and c) Double Density Gradient Centrifugation (DDGC) [6].

Identification of factors predicting the success of IUI cycles has been the focus of several studies, with key determinants including the age of the female partner, duration and type of infertility, ovarian stimulation regime, number of treatment cycles, timing of insemination post trigger and number of mature follicles at the time of insemination [7,8]. Semen parameters obtained after semen analysis alone, or in combination with other clinical factors may also help in predicting the IUI outcomes [7,9].

The literature has widely used TMSC to predict IUI outcomes. It is a key quantitative parameter in semen analysis that reflects the total number of motile spermatozoa in an ejaculate and is widely used to predict fertility outcomes. However, TMSC measured in neat semen samples during routine analysis has been shown to be a poor predictor of IUI success rates [10]. Moreover, prewash TMSC in

most studies were from semen analyses performed at various time points, rather than on the actual day of IUI [10,11]. Postwash TMSC has also been extensively studied and used to predict IUI outcomes [11], but it includes both progressive and non progressive motile sperms, whereas successful IUI depends primarily on spermatozoa with forward progressive motility capable of reaching and fertilising the oocyte.

Recent research focuses on the postwash TPMSC, which represents the concentration of progressively motile spermatozoa per millilitre of prepared sample. By isolating this biologically relevant subpopulation, TPMSC more accurately reflects the functional sperm available for fertilisation [12]. Consequently, recent research has shifted toward TPMSC as it correlates more closely with IUI outcomes [13,14], justifying its use as the primary sperm parameter in the present study.

Despite the recognised importance of post-preparation sperm parameters in IUI success, there remains a lack of consensus regarding clinically relevant TPMSC values and limited comparative data on how TPMSC relates to pregnancy outcomes across different sperm preparation techniques. This single-centre study therefore, aimed to address this gap by evaluating postwash TPMSC measured on the day of insemination and examining its relationship with IUI outcomes, measured in terms of positive pregnancy rates, thereby providing novel evidence on a more functionally relevant sperm parameter for clinical decision-making.

MATERIALS AND METHODS

This retrospective cohort study was conducted at the Department of Reproductive Medicine and Andrology (ART Clinic affiliated with a tertiary care centre and university teaching hospital), Chettinad Fertility Services, Chettinad Super Speciality Hospital, Chennai, Tamil Nadu, India. Case records from 490 patients were included, who underwent IUI between January 2015 and January 2025 and data analysis was done from September 2025 to November 2025. After obtaining approval from the Institutional Human Ethics Committee, CARE (IHEC-I/4128/25), the study was conducted in accordance with the ethical standards of the Helsinki Declaration of 1975 and its later amendments.

Inclusion criteria: Age of the male partner: 23-40 years, age of the female partner: 21-35 years, collection of complete ejaculate by masturbation, controlled ovarian stimulation followed by single IUI and sperm preparation for insemination carried out either by pellet and swim-up, or double density gradient centrifugation method.

Exclusion criteria: Patients with unilateral tubal occlusion and other associated conditions like fibroids, endometritis, salpingitis and hydrosalpinx were excluded from the study. Use of frozen semen samples, methods of semen collection other than masturbation, donor insemination and leukocytospermia were also excluded.

Study Procedure

The female partner underwent ovarian stimulation using clomiphene citrate, letrozole or gonadotropins from day 2 or 3 of the menstrual cycle for five days, followed by monitoring of follicular growth using transvaginal ultrasonography. The cycle was cancelled if more than two dominant follicles developed. When the dominant follicle reached approximately 20mm in diameter, ovulation was triggered using either urinary or recombinant hCG injections, as both have been shown to provide similar pregnancy outcomes. IUI with 0.4-0.5mL of prepared sperm suspension was performed around 0- 36 hours post-hCG trigger.

On the day of IUI, the semen sample collected by masturbation was analysed according to WHO guidelines [4] and sperm preparation was done by either PSU or DDGC method. The PSU method involves centrifugation of semen sample to form a pellet, which is then overlaid with sperm washing media and motile sperms swim into the upper layer for collection. In contrast, DDGC involves layering of semen sample over 80% and 40% gradient media and centrifuging to obtain a pellet enriched with motile spermatozoa, which is free from debris and non sperm cells [4].

The study cohort was categorised into four subgroups based on the obtained TPMSC:

- **Group I:** TPMSC <5x10^6/mL
- **Group II:** TPMSC 5-9x10^6/mL
- **Group III:** TPMSC 10-20x10^6/mL
- **Group IV:** TPMSC >20x10^6/mL

The lower TPMSC value of <5 x10^6/mL was selected because pregnancy rates decline markedly below this level [15], while the upper value was set at >20x10^6/mL due to the absence of a clearly defined maximum limit to achieve a pregnancy, in the literature [12].

Outcome measures were recorded for all IUI cycles. The primary outcome was positive pregnancy rate, while secondary outcomes included biochemical pregnancy, clinical pregnancy, ectopic pregnancy, and miscarriage rates. All outcomes were analysed in relation to TPMSC obtained using the PSU or DDGC methods.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS software version 30.0 (IBM SPSS, Statistical Package for Social Science), with categorical data evaluated by the Chi-square test and Fisher's exact test. A p-value of <0.05 was considered statistically significant.

RESULTS

The total study population included 490 patients, of which semen samples from 363 patients were prepared using the PSU method and 127 patients using the DDGC method. Considering that the age of the female partner is a major determinant of pregnancy in an IUI cycle, there was no significant difference in age (p-value=0.181) among the four TPMSC groups, as shown in [Table/Fig-1].

TPMSC Groups	Mean age±SD (in years)	p-value
Group I (n=43)	27.39±2.96	0.181
Group II (n=51)	28.50±2.72	
Group III (n=108)	28.22±2.78	
Group IV (n=288)	28.4±3.25	

[Table/Fig-1]: Mean and standard deviation of maternal age across TPMSC groups.

The distribution of the study population based on the concentration of total progressive motile spermatozoa obtained after preparation using pellet and swim-up, and double density gradient centrifugation methods is shown in [Table/Fig-2].

PSU (n=363)		DDGC (n=127)	
Group I	n= 41	Group I	n=2
Group II	n= 41	Group II	n=10
Group III	n= 88	Group III	n=20
Group IV	n=193	Group IV	n=95

[Table/Fig-2]: Distribution of study population by TPMSC groups in PSU and DDGC methods

i) Impact of TPMSC on IUI Outcomes: PSU Method

Primary outcome (Positive Pregnancy Rates): The overall success rate of IUI cycles performed with spermatozoa prepared using the pellet and swim-up method was 55 pregnancies out of 363 cycles (15.1%). A progressive increase in pregnancy rates was observed with increasing TPMSC values as depicted in [Table/Fig-3]. Statistical analysis revealed a significant positive association between TPMSC and pregnancy rates (p-value <0.001), with Group IV showing higher pregnancy rates than all other groups, indicating that TPMSC has a significant positive impact on IUI outcomes with the PSU method.

PSU- Group	Positive pregnancy	Percentage
Group I (n=41)	1	2.4%
Group II (n=41)	0	0%
Group III (n=88)	7	7.9%
Group IV (n=193)	47	24.3%

[Table/Fig-3]: IUI outcome in the groups following PSU method.
Pearson Chi-square: p-value <0.001

To assess whether higher TPMSC values were associated with improved outcomes, Group IV (>20×10^6/mL) was compared with all other groups using the Chi-square test. Group IV showed significantly higher pregnancy rates than Group I (p-value <0.001), Group II (p-value <0.001), and Group III (p-value <0.001), while Group III was not significantly better than Group I (p-value=0.197), as shown in [Table/Fig-4]. Based on these findings, TPMSC >20×10^6/mL was associated with significantly higher pregnancy rates in samples prepared by PSU method. However, pregnancies occurred at TPMSC as low as 4×10^6/mL, indicating that lower values reduce but do not eliminate the chances of pregnancy.

Comparison groups	Pregnancy rates	p-value
Group IV vs Group I	24.3% vs 2.4%	<0.001
Group IV vs Group II	24.3% vs 0%	<0.001
Group IV vs Group III	24.3% vs 7.9%	<0.001
Group III vs Group I	7.9% vs 2.4%	0.197

[Table/Fig-4]: Comparison of pregnancy rates between TPMSC groups following PSU Method.
Pearson Chi-square: p-value <0.001

Secondary Outcome

Group 2 was excluded from statistical analysis of secondary outcomes, as no pregnancies were achieved in this group. Among the participants with positive pregnancy tests, clinical pregnancy rates were high across all TPMSC groups (Group I: 100%, Group III: 100%, Group IV: 85.1%), with no statistically significant differences observed between groups (p-value=0.564). Although there was an increase in clinical pregnancy rates, no statistically significant differences were observed in biochemical pregnancy (p-value=0.796), ectopic pregnancy (p-value=1.000) or miscarriage rates (p-value=1.000) among the groups, as shown in [Table/Fig-5].

Outcome	Group I (n=1)	Group III (n=7)	Group IV (n=47)	p-value
Biochemical pregnancy	0	0	5 (10.6%)	0.796
Clinical pregnancy	1 (100%)	7 (100%)	40 (85.1%)	0.564
Ectopic pregnancy	0	0	2 (4.3%)	1.000
Miscarriage*	0	1/7 (14.3%)	6/40 (15%)	1.000

[Table/Fig-5]: Secondary outcomes in PSU groups with positive pregnancies.
*Miscarriage rate calculated from clinical pregnancies
Fisher's exact test

ii) Impact of TPMSC on IUI Outcomes: DDGC Method

Primary outcome (Positive Pregnancy Rates): The overall success rate of the IUI cycles performed using spermatozoa prepared by the DDGC method was 19 pregnancies out of 127 cycles (15%). The positive pregnancy rates were 10% in Group II (TPMSC 5-9 x10^6/mL) and 18.9% in Group IV (TPMSC >20x10^6/mL) while, no pregnancies were achieved in Group I and III as depicted in [Table/Fig-6]. Although Group IV demonstrated a relatively higher pregnancy rate, this difference was not statistically significant (p-value=0.154), suggesting that TPMSC did not have a significant influence on IUI outcomes in the DDGC subgroup. This lack of statistical significance may be attributable to the smaller sample sizes within the individual groups.

To assess whether TPMSC was associated with IUI outcomes using the DDGC preparation method, pairwise comparisons across TPMSC groups were performed using the Chi-square test. No statistically significant differences in pregnancy rates were observed between the groups (p-value=0.154). Although the highest pregnancy rate occurred in Group IV, pregnancies were inconsistently distributed across TPMSC categories, with occurrences in Group II but not in Groups I or III. Given the lack of statistical significance, small group sizes, and low overall pregnancy numbers, TPMSC did not demonstrate a reliable association with pregnancy outcomes in semen samples prepared using DDGC method.

DDGC - Group	Positive pregnancy	Percentage
Group I (n=2)	0	0%
Group II (n=10)	1	10%
Group III (n=20)	0	0%
Group IV (n=95)	18	18.9%

[Table/Fig-6]: IUI outcome in the groups following DDGC method
Pearson Chi-square: p=0.154

Secondary Outcome

Groups I and III were excluded from the statistical analysis of secondary outcomes, as no pregnancies were achieved. As no ectopic pregnancies were observed in any of the four groups, this variable was also excluded from analysis. Among the participants with positive pregnancy tests, clinical pregnancy rates were 100% in Group II and 88.9% in Group IV, with no statistically significant difference observed between groups (p-value=1.000). On comparing these Groups, no statistically significant differences were observed in biochemical pregnancy rates (p-value=1.000) or miscarriage rates (p-value=1.000), as shown in [Table/Fig-7].

Outcome	Group II (n=1)	Group IV (n=18)	p-value
Biochemical pregnancy	0	2 (11.1%)	1.000
Clinical pregnancy	1 (100%)	16 (88.9%)	1.000
Miscarriage*	0	2/16 (12.5%)	1.000

[Table/Fig-7]: Secondary outcomes in DDGC groups with positive pregnancies
*Miscarriage rate calculated from clinical pregnancies.
Fisher's exact test

DISCUSSION

The overall clinical pregnancy rate was 15.1% in the PSU group and 15% in the DDGC group, which is at par with the average IUI outcome reported in literature (9-11%) [16,17]. This reflects the importance of optimised patient selection, controlled ovarian stimulation, and the choice of sperm preparation technique employed based on the semen parameters. Previous studies have attempted to identify clinical variables that could serve as reliable predictors of successful IUI outcomes, with particular attention to TMSC assessed both during routine semen analysis and after sperm preparation [18,19]. The literature on pre- and postwash TMSC is heterogeneous, with Mankus EB et al., [10] reporting no association between prewash sperm parameters and pregnancy outcomes, whereas Ok EK et al., [12] found a significant correlation, particularly with postwash TMSC.

The shift toward TPMSC as a more refined predictor represents an evolution in understanding of sperm function. Unlike TMSC, which includes all motile sperm (both progressive and non progressive), TPMSC specifically quantifies sperm with forward progressive movement that are most capable of traversing the female reproductive tract and achieving fertilisation. A study by Tan O et al., has demonstrated that postwash TPMSC provides superior predictive value compared to traditional TMSC measurements [20].

Recent research has established various TPMSC values. Inceoglu C et al., demonstrated significantly higher chemical pregnancy rates in couples with TPMSC above 5×10^6/mL and observed no pregnancies in the group with TPMSC below 5×10^6/mL [15].

Ok EK et al., reported a linear increase in pregnancy rates when TPMSC exceeded 10×10^6/mL [12]. Lin H et al., found correlations between postprocessing TPMSC and live birth rates, concluding that pregnancy rates were higher when the TPMSC was 22.32×10^6/mL [21].

In the present study, TPMSC after sperm preparation was specifically assessed, measured on the day of IUI, to overcome inherent variability in semen samples analysed at unrelated time points. The findings also demonstrated that TPMSC has differential predictive value depending on the sperm preparation method employed, a distinction not systematically addressed in previous literature. Following sperm preparation by the PSU method, a significant increase in pregnancy rates was observed in Group IV (TPMSC $>20\times10^6$/mL; p-value <0.001). This finding supports the hypothesis that higher postwash TPMSC enhances fertilisation potential by improving the probability of sperm–oocyte interaction [21]. In contrast, no association was observed between TPMSC and IUI outcomes in the double density gradient centrifugation (DDGC) group (p-value=0.154). The lack of statistical significance may be attributed to the smaller sample size in this subgroup, but it may also reflect inherent differences between the two preparation techniques. The biological rationale for this finding was multifactorial. PSU selectively recovers highly motile spermatozoa [22], whereas DDGC may yield a broader population with variable motility [23]. This could explain the superior performance of PSU in achieving higher pregnancy rates in this study. Although there was a significant association between TPMSC and pregnancy rates in the PSU preparation method (p-value <0.001), no statistical significance was observed in secondary outcomes including biochemical pregnancy rates, clinical pregnancy rates, ectopic pregnancy rates, or miscarriage rates among Groups III and IV. Similarly, in the DDGC method, no statistically significant differences were observed in secondary outcomes including biochemical pregnancy rates, clinical pregnancy rates, or miscarriage rates between TPMSC groups. The study also showed similar overall pregnancy rates between methods (PSU: 15.1% vs DDGC: 15%), though TPMSC was a significantly better predictor of outcomes with PSU.

While multiple studies have reported a postwash TMSC value of 10×10^6/ mL [12,24], current data demonstrated successful pregnancies at TPMSC values as low as 4×10^6/mL, indicating that low TPMSC does not always preclude the possibility of pregnancy. This study found that pregnancy rates were significantly higher when TPMSC $>20\times10^6$/mL in samples prepared using PSU method, although pregnancies still occurred at lower values. In contrast, pregnancy outcomes were inconsistently distributed across TPMSC ranges in samples prepared using DDGC method. These results highlight the method-dependent predictive value of TPMSC and underscore that the choice of sperm preparation technique critically influences the IUI outcomes.

Limitation(s)

A limitation of this study was its retrospective design, which may introduce selection and information bias. A potential challenge in using postwash TPMSC for patient counselling is that sperm preparation was not routinely included in standard semen analysis, therefore TPMSC can only be determined on the day of the IUI procedure. Future prospective studies with larger sample size are warranted to validate these findings and to define clinically relevant TPMSC predictive values for identifying improved clinical outcomes.

CONCLUSION(S)

According to this study, similar overall pregnancy rate of 15.1% in the PSU group and 15% in the DDGC group was observed. TPMSC obtained after PSU preparation demonstrated a significant association with the pregnancy rate, with higher TPMSC values ($>20\times10^6$/mL) showing significantly improved outcomes (p-value <0.001). However, no association was observed with DDGC, possibly due to methodological differences or smaller subgroup size. This study demonstrated that the association between TPMSC and pregnancy outcomes depends on the choice of sperm preparation method. Further prospective, multi-centre studies are required to establish validated TPMSC predictive values and to optimise semen preparation protocols for improving IUI outcomes.

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