JSTMU Journal of Shifa Tameer-e-Millat University

https://j.stmu.edu.pk

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ORIGINAL ARTICLE

Post-wash total motile sperm count a useful predictor in the decision to perform IVF/ICSI in patients with non-male factor infertility

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Author`s Contribution

[1] Data analysis, interpretation, hand script drafting
[2] Hand script synthesis
[3] Data collection
[4] Manuscript preview and proofreading

Article Info.

Conflict of interest: Nil
Funding Sources: Nil

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Cite this article as: *Qureshi SM, Kafeel S,Bibi R, Mohmand J. Post-wash total motile sperm count a useful predictor in the decision to perform IVF/ICSI in patients with non-male factor infertility. JSTMU. 2020; 3(2):99-106.*

A B S T R A C T

Introduction: The unrestricted use of intracytoplasmic sperm injection (ICSI) for non-male factor infertility is associated with adverse outcomes. Post-wash total motile sperm count (PW-TMSC) offers prognostic value to assess sperm quality and aid in the decision to perform in vitro fertilization (IVF) or ICSI.

Objectives: The aim of this study was to identify the effect of PW-TMSC on fertilization rates in patients undergoing IVF cycles exclusively with non-male factor infertility. It also aimed to identify whether unnecessary ICSI could be avoided in such cases, thus maximizing optimal outcomes.

Materials & Methods: We retrospectively analyzed age, semen volume, prewash TMSC, and PW-TMSC in 68 conventional IVF cycles of infertile couples with non-male factor infertility. Clinical characteristics including female age, number of follicles, level of estradiol on trigger day, mature cumulus-oocyte complexes (COCs) collected, were also included.

Results: Incidence of <30% fertilization was significantly higher in the 4-<10 Million group compared with the ≥20 Million post-wash TMSC group (P<0.001). Furthermore, Receiver operating characteristics (ROC) analysis revealed post-wash TMSC as a significant predictor (P<0.05) of total failed fertilization (TFF) and of ≥30% fertilization (P<0.05) with area under curve (AUC) of 0. 79 and 0.77, respectively, with a deemed cutoff of 10.89 Million.

Conclusion: Post-wash TMSC is a good predictor of fertilization; it can help in avoiding potentially low or even total fertilization failure (TFF). A cut-off point of 10.89 Million or less should warrant the use of ICSI.

Keywords: *In vitro fertilization, fertilization rate, post wash sperm, total motile sperm count*

Introduction

Intracytoplasmic sperm injection (ICSI) procedure was a breakthrough technique introduced in 1992, in order to overcome male factor infertility and to improve fertilization outcomes in couples with fertilization failure in prior In Vitro Fertilization (IVF) cycles.[1,2] The benefits of utilizing ICSI for male factor infertility is well established.[3] In recent years ICSI has become increasingly popular, being encouraged by practitioners. Its use has broadened to include indications other than male factor infertility, such as poor oocyte quality, low oocyte yield, advanced maternal age, unexplained infertility, and even for routine use in all assisted reproductive technology (ART) cycles,

regardless of etiology.[4] The basis for utilizing ICSI for non-male factor indications is to prevent TFF and to maximize fertilization rates. However, there are potential risks associated with ICSI such as asynchrony in sperm chromosome decondensation,[5] oocyte degeneration, which is particularly higher in patients with fragile oocytes, the plausibility of injecting sperm with DNA anomalies,[6] lower implantation rates than conventional insemination in cases of non-male factor infertility,[7] risk of fetal malformations and chromosomal abnormalities.[5] Although the safety and efficacy of ICSI for male factor infertility has been evaluated.[8] Yet, the impact of unrestricted use of ICSI and associated risks for non-male factor infertility is still not fully understood. A recent study highlighted a strong association of ICSI with autism when used in the absence of male factor infertility.[9]

Currently, the decision to perform IVF or ICSI is mostly experience-based.[10] The total motile sperm count (TMSC) has been identified as a useful way to express semen quality; it is a combination of the ejaculate volume, sperm concentration (million sperm per ml), and motility percentage.[11] The use of TMSC in the native semen specimen has been proven to be of prognostic value in couples undergoing intrauterine insemination (IUI) cycles.[12] And according to 2010 classification of the World Health Organization (WHO), TMSC is considered of superior value in predicting the success of IVF cycles.[13] Several studies have suggested that TMSC following sperm preparation or post-wash, essentially by density gradient centrifugation method offers a more robust selection parameter for assessing semen quality. It reflects spermatozoa with high motility moreover, bearing normal morphology which fundamentally is associated with fertilization capacity.[14,15] The use of post-wash TMSC has been identified as a useful parameter to predict pregnancy in IUI cycles.[16] Moreover, post-wash TMSC parameter is a reproducible predictor of total failed fertilization (TFF) in conventional IVF cycles.[17] It is also used as a tool to assess sperm quality and aid in the decision to perform either IVF or ICSI.[10] However, the prognostic value of post-wash TMSC on fertilization rates in couples particularly of non-male factor infertility, undergoing IVF cycles is not yet well established. This study aimed to identify the effect of post-wash TMSC on fertilization rates in patients undergoing IVF cycles exclusively with non-male factor infertility. It is also intended to identify whether unnecessary ICSI could be avoided in such cases while ensuring that the simplest, most cost-effective and most successful treatment is offered to the patient.

Methods

Patient Selection

We retrospectively evaluated all cycles of IVF conducted during a 22-month period of time at a local fertility clinic for patients with no apparent indication for ICSI. This was confirmed by two prior semen analysis conducted as part of an initial workup. Semen was diagnosed as normozoospermic when having a sperm concentration of ≥20 Million /ml, motility ≥50%, and normal morphology ≥14%, as per the criteria of WHO 1999.[18] Sperm concentration was determined by Neubauer hemocytometer. The motility was evaluated by assessing at least 200 spermatozoa and expressed as a percent of motile sperms. Sperm morphology was assessed by Papanicolaou stain, following an assessment of at least 200 sperms. Male factor was diagnosed if any of the semen parameters were out of the reference range as per WHO 1999 criteria, hence not included in this study.

Only couples undergoing their first conventional IVF cycle, having normal semen parameters, and at least two mature cumulus-oocyte complexes (COC) retrieved were included. A total of 68 cycles met this criterion with the male partner's prewash and post-wash semen parameters on the day of oocyte pick up (OPU), evaluated along with the respective female partner's baseline characteristics of age, number of follicles, level of estradiol on day of trigger, and number of mature COC's retrieved.

Semen Assessment & Preparation

On the day of OPU, the semen sample was produced, usually before oocyte collection. Following liquefaction, the volume, sperm concentration, and motility were assessed in order to calculate TMSC in the neat sample. Semen was subjected to density gradient centrifugation using 90% spermgrad (Vitrolife, Sweden). Following centrifugation for 10 minutes at 450g, the pellet was resuspended in 02-03 ml of pre-equilibrated GIVF+ media

for washing (Vitrolife, Sweden). It was further centrifuged for 10 minutes at 350g. Lastly, the pellet was suspended in a final volume of 1 ml GIVF+. The post-wash TMSC was evaluated in the final suspension. The post-wash TMSC was categorized into three groups of 4 to <10 Million, ≥10 to 19.99 Million, and ≥20 Million or Higher.

IVF Protocol

Female partners were subjected to a standard long stimulation regime using GnRH agonist Buserelin acetate (Suprefact, Aventis Pharma) at a standard dose of 0.5 mg starting in the mid-luteal phase of the preceding cycle or subjected to a short antagonist IVF protocol using 0.25 mg Cetrorelix (Cetrotide® Merck Serono SA, Aubunne, Switzerland) commencing on day 6 of stimulation. Ovarian Stimulation was started using human menopausal gonadotrophin (Menogon Ferring SA, Sainet Prex, Switzerland) or recombinant follicle-stimulating hormone (rFSH), (Gonal F Merck Serono SA, Aubunne, Switzerland). OPU was performed 36-38 hours after a 10,000 IU hCG trigger (Pregnyl Organon, Oss, the Netherlands). Mature COC's were group cultured (2-6 COC's per well), in pre-equilibrated four-well dishes containing 0.5 ml GIVF+ and 0.5 ml oil overlay. Mature COC's were inseminated using 60,000 motile sperms per egg. Fertilization was assessed 16-20 hours post insemination, with two or more evident pronuclei considered as fertilized. Fertilization rates were categorized into three groups of <30%, ≥30 to 69.9 %, and ≥70% observed fertilization.

Ethical Approval

The research protocol was approved by Salma Kafeel Medical Services No.010-2016. Informed oral consent was obtained from each participant included in this study.

Statistical Analysis

Categorical data were analyzed using Fisher's exact test with pairwise post hoc analysis using Bonferroni adjustment. Continuous data variables with normality and homogeneous variance were tested using ANOVA with post hoc analysis using the Scheffé test. For non-parametric data or data with non-homogenous variance, Kruskal Wallis was used. To assess the capacity of prewash and post-wash TMSC to predict fertilization receiver operating characteristic (ROC) analysis was conducted. P value <0.05 was considered statistically significant unless stated otherwise. All tests were performed using SPSS 22.0 (Chicago, IL, USA) statistical package. Due to the limited number of subjects in this study post hoc power and effect size analyses were performed, with results presented for significant differences. Power analysis was conducted using G*Power.

Results

There was no significant difference between any parameters of the female clinical characteristic among the three different post-wash TMSC groups. These characteristics included female age, number of follicles, level of estradiol on trigger day, mature COC's collected The calculated prewash TMSC on the day of OPU, the prewash motile sperm count/mL and prewash TMSC varied significantly across all the three groups of 04 to <10 Million, ≥10 to 19.99 Million and ≥20 Million or higher post-wash TMSC (P<0.001), as presented in Table 1.

The incidence of fertilization classified into groups of <30%, 30 to 69.99%, and 70% or higher, in relation to the three post-wash TMSC groups of 04 to <10 Million, ≥10 to 19.99 Million and ≥20 Million. The incidence of lower than 30% fertilization was significantly higher in the post-wash TMSC group of 04 to <10 Million (N=38 P-value <0.005 statistically significant with Bonferroni adjustment.) compared with ≥20 Million group. Moreover, a significantly lower trend of 70% or greater fertilization was observed in this respective group in comparison with ≥20 Million, post-wash TMSC group (Figure 1) [P=0.004, effect size=0.99 (large), observed power=0.99].

While a higher trend of 30% to 69.99% fertilization and ≥70% fertilization was evident in the ≥20 Million post-wash TMSC groups in comparison with ≥10 to 19.99 Million post-wash TMSC group, however, this finding was not statistically significant. (N=61, P-value=0.31). Moreover, ROC analysis of all cycles was conducted in the prediction of total failed fertilization (TFF). The analysis revealed post-wash TMSC as a statistically significant predictor of this outcome (P=0.046) with Area under curve (AUC)=0.79, a cut off value of 10.89 Million post- wash TMSC, having 96.9% sensitivity and 75% specificity (Figure 2B). On the contrary, prewash TMSC (Figure 2A) was not a significant predictor of TFF (P=0.34). Furthermore, ROC analysis in order to predict

30% or higher fertilization revealed post-wash TMSC to be a significant predictor (P=0.026) for this outcome with AUC=0.77 however, not the parameter of prewash TMSC (P=0.27). A cut-off value of 10.89 Million TMSC demarked the prediction of this outcome with 98.4% sensitivity and 67% specificity (Figure 3).

Table 01: Clinical characteristics of female and male partner according to post-wash TMSC groups.

Variables	4 to <10 Million Post-Wash TMSC	≥10 to 19.99 Million Post-Wash TMSC	≥20 Million Post-Wash TMSC	p-value
	(n=4)	(n=14)	(n=50)	
Female Age	32.0 ± 2.42	30.6 ± 1.18	31.71 ± 0.81	0.79
Female Infertility Etiology	0.00 %	2.6 %	5.3 %	0.06
PCOS	2.6 %	26.4 %	23.7 %	
Tubal Factor	0.0 %	2.6 %	7.9 %	
Endometriosis				
Ovulatory Dysfunction	2.6 %	0.0 %	26.3 %	
Number of Follicles	7.3 ± 4.84	10.64 ± 1.58	9.34 ± 0.64	0.50
E2 Level on Trigger Day	257.5 ± 24.50	809.57 ± 224.68	1026.08 ± 126.58	0.43
Mature COC's Collected	8.25 ± 2.86	5.50 ± 1.63	7.54 ± 0.69	0.13
Male Age	40.3 ± 2.73	41.2 ± 4.27	36.4 ± 1.09	0.28
Semen Volume	1.86 ± 0.16	1.84 ± 0.08	1.9 ± 0.04	0.38
Pre-wash Motile Sperm Count/mL	22.01 ± 7.45	28.72 ± 3.7	51.54 ± 3.17	<0.001
Pre-wash TMSC	39.65 ± 12.25	54.02 ± 8.10	100.62 ± 6.53	<0.001

Values are represented as Mean ± standard error of mean (SEM). ANOVA test performed between groups for parameters of female age, number of follicles, pre-wash motile sperm count/ml and pre-wash TMSC. Fisher's exact test performed between groups to assess distribution of female infertility etiology. Kruskal Wallis test performed between groups for all remaining parameters. p<0.05 was considered as statistically significant.

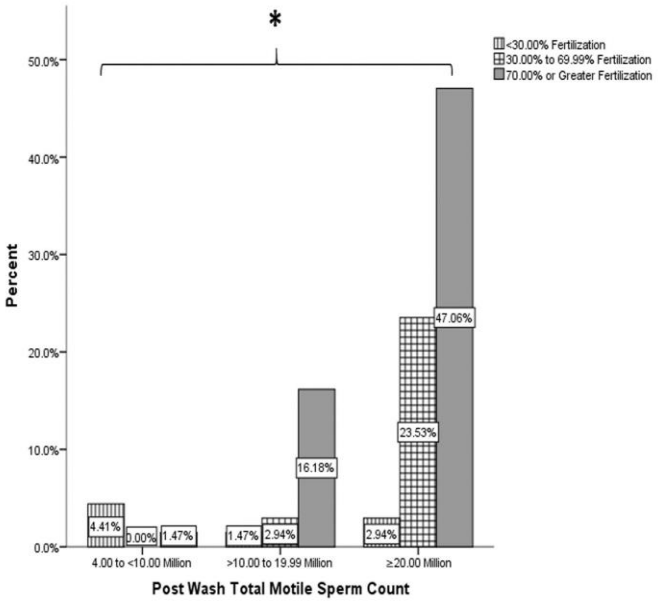

Figure 1. The incidence of fertilization classified into lower than 30%, 30% to 69.99% and 70% or higher, in relation with post-wash TMSC groups of 4.0 to <10 Million, ≥10 to 19.99 Million and ≥20 Million post-wash TMSC, respectively: presented as % of the total across all respective post- wash TMSC groups. Fisher's exact test performed with Bonferroni adjusted P-value between pairwise group comparisons. P<0.005 was considered statistically significant.

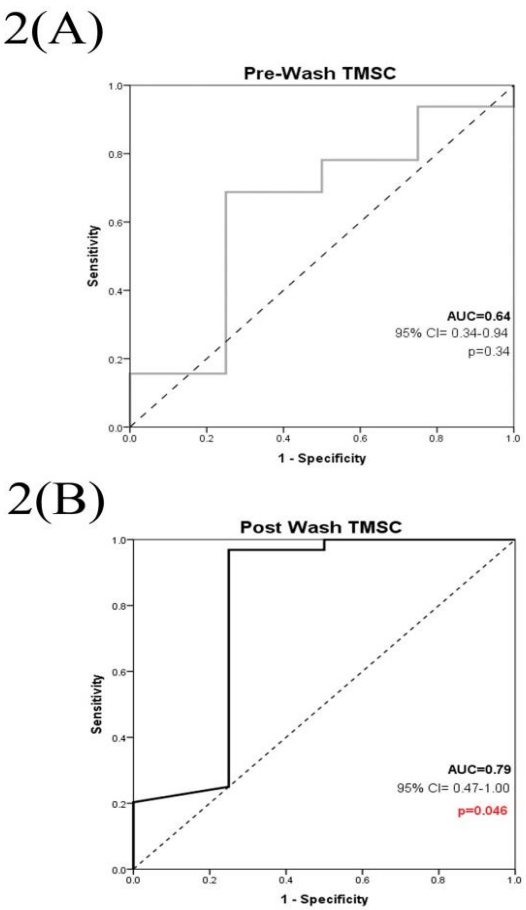

Figure 2. Receiver operating characteristic analysis for all cycles in the prediction of total failed fertilization by (A) Pre-wash TMSC and (B) Post wash TMSC. AUC= Area under curve, CI= Confidence Interval, dotted line represents reference line, bold p values indicate statistical significance.

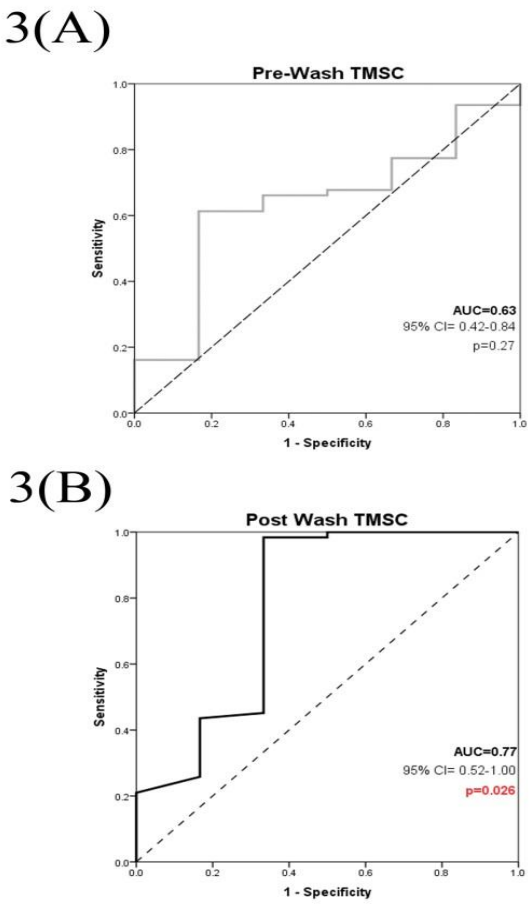

Figure 3. Receiver operating characteristic analysis for all cycles in the prediction of 30% or higher fertilization by (A) Pre-wash TMSC and (B) Post wash TMSC. AUC= Area under curve, CI= Confidence Interval, dotted line represents reference line, bold p values indicate statistical significance.

Discussion

Finding the most appropriate assisted reproductive treatment for a couple can prove to be challenging, essentially in terms of determining a modality that is least invasive, most cost-effective and offers the highest chance of achieving a healthy offspring.[17] For cases of non-male factor infertility, the use of ICSI as the default option is questionable.[3] Moreover, in such cases, the plausible negative effects associated with the use of ICSI include compromised embryonic development.[19] lower implantation and pregnancy rates per cycle.[7] increased risk of births defects.[4] None of these negative effects can be overlooked when compared with conventional IVF. Moreover, the selection of sperms in conventional IVF is extremely complex, dynamic, and rigorous in determining

spermatozoa possessing all the essential mechanisms of oocyte recognition, fusion and crucial intracellular factors.[19] Therefore, impacting fertilization and subsequent embryo development. All these intricate processes are bypassed in ICSI, and sperm selection is based on an embryologist's subjective assessment of sperm morphology.[20]

Thus, the aim of this study was to identify a practically useful parameter to predict fertilization in couples with non-male factor infertility that are undergoing IVF cycles. Furthermore, to enable a more robust selection of treatment modality, ensuring that unnecessary ICSI is avoided while still maximizing fertilization rates. This study considered only a single treatment cycle of each couple as multiple cycles of the same couple would be a source of bias. The study focused on post-wash TMSC as sperm preparation method of density gradient centrifugation was utilized, which essentially allows isolation of morphologically normal spermatozoa possessing a density of at least 1.10 g/ml.[21] Post-wash TMSC has been identified as a useful tool in the decision to perform conventional IVF or ICSI in cases of isolated teratozoospermia,[10] in the prediction of total fertilization failure including patients with male subfertility or unexplained infertility.[14] However, for couples with non-male factor infertility exclusively a criterion is not well established.

The results of this study showed that the incidence of <30% fertilization including total failed fertilization is significantly higher in 04 to the <10 Million post-wash TMSC group compared with the ≥20 Million post-wash TMSC group. Consequently, a significantly lower incidence of ≥70% fertilization was evident in the 04 to <10 Million group compared with the ≥20 Million post-wash TMSC group. This finding is consistent with that of an earlier study highlighting that 7.6 Million post-wash TMSC demarked IVF cycles showcasing no observed fertilization.[14] Furthermore, in order to identify a threshold of prewash TMSC and post-wash TMSC in predicting total failed fertilization, a ROC analysis was conducted in this study. The main finding of the ROC analysis in this study, revealed that post-wash TMSC but not the prewash TMSC is a significant predictor of successful fertilization. The rationale for this finding is also supported by previous studies. Supporting that while pre and post-wash TMSC fundamentally are highly associated. Nonetheless, post-wash TMSC obtained by density gradient sperm preparation method allows for isolation of motile, morphologically normal sperm and recovery of sperms with good DNA integrity in comparison with pre-wash specimens.[10, 22] Therefore, post-wash TMSC parameter reflects overall sperm quality, thus offers a higher predictive utility than pre-wash TMSC.[10] Additionally, another study investigated sperm anomalies/deformity indices assessed between pre and post-wash sperms. The grouped sperm anomalies indices of post-wash sperms were predictive of pregnancy outcomes in intrauterine insemination cycles, however not indices of pre-wash sperms.[23] Further, providing evidence for post-wash TMSC reflecting greater predictive utility in clinical practice and in demonstrating overall sperm quality.

In terms of identifying a cut-off point for the prediction of total failed fertilization, post-wash TMSC (AUC=0.79) demarked 10.89 Million bearing a sensitivity of 96.9% and specificity of 75% in the prediction of TFF. Below this threshold, ICSI would be beneficial in view of the potentially high risk of complete fertilization failure. While other studies propose a cut-off point of lower than 1.5[10] and 02 Million[14] post-wash TMSC as an indication for ICSI, which appears quite lower than the findings of this study. However, this is due to the selected patient population, as the findings presented here are specifically addressing non-male factor infertility couples and does not include patients with isolated teratozoospermia or with male factor subfertility.[14] Additionally, a threshold of 10.89 Million post-wash TMSC demarked a slightly reduced predictive capability of 98.4% sensitivity and 67% specificity in order to predict 30% or higher fertilization outcome (AUC=0.77). While the prognostic value for identifying a post-wash TMSC to predict 70% fertilization, would be clinically useful as it matches the average fertilization rates achieved by the ICSI technique. However, the small sample size in this study restricts the predictive utility for this outcome. Nonetheless, a higher trend of 30 to 69.99% fertilization and ≥70% fertilization was observed in ≥20 Million TMSC group as compared with 10.00 to 19.99 Million post-wash TMSC group. This finding did not prove to be statistically significant (P=0.31). Although, a recent study has showcased that a threshold of 25 Million post-wash TMSC can predict 70%

fertilization in conventional IVF cycles of couples with no apparent male factor infertility.[4] Therefore, providing evidence to support that post-wash TMSC threshold offers a robust treatment selection criterion for optimal fertilization rates.

Although sperm morphology has not been re-evaluated on the day of OPU and is a weakness of the study, yet all subjects had prior confirmed normal semen parameters including sperm concentration, motility, and normal sperm morphology as per WHO 1999.[18] A previous study has shown that normal and subnormal sperm morphology does not influence fertilization rates in conventional IVF cycles given that, the other semen parameters are normal.[24] Additionally, worth considering while additional assays such as sperm chromatin evaluation may offer value in deciding upon performing IVF or ICSI as the treatment modality. However, the variety of sperm chromatin/DNA assays lack standardized consensus, offers limited prognostic value, is expensive and essentially not part of the routine assessment in most ART laboratories.[25] Furthermore, it is known that sperm DNA/chromatin integrity is significantly improved following density gradient centrifugation however, its association with fertilization rates in IVF and ICSI still lacks significant correlation. Thus, post-wash TMSC offers a robust, simple and quick assessment to aid in the decision between IVF/ICSI.[10, 14, 17] Moreover, specifically for non-male factor infertility cases as highlighted in this study.

While the findings presented are of clinical significance, it is important to highlight a few limitations of this study. The retrospective nature, small sample size, and potential for selection bias due to lack of proper randomization are weaknesses of this study. However, corroboration of these observations with future studies could lead to definitive recommendations being proposed. In conclusion, our findings offer evidence-based strategies in the decision to perform IVF or ICSI for non-male factor infertility couples and suggests that post-wash TMSC is a good predictor for fertilization in such couples which should be measured routinely prior to conventional IVF inseminations. An identified threshold of 10.89 Million post-wash TMSC or lower is suggestive of increased risk of low fertilization and TFF in such couples and thus should warrant the use of ICSI procedure.

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