Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

🔓 OPEN ACCESS Check for updates

Sperm morphology by strict criteria does not predict clinical pregnancy rate following intra-uterine insemination

Lamia Sayegh, Rupin Shah, Mohamad Shmoury, Leyla Depret-Bixio 🄳 and Michael Fakih

Department of Infertility, Fakih IVF Fertility Center, Dubai, United Arab Emirates

ABSTRACT

Objective: To determine the impact of abnormal sperm morphology of the pre-washed semen sample on the day of intrauterine insemination (IUI) on clinical pregnancy rates (CPR)

Design: Cross sectional retrospective chart review.

Setting: Academic fertility center

Patient(s): Couples undergoing (IUI) from May 2014 to March 2022.

Intervention(s): Sperm morphology, by strict criteria, on the pre-washed IUI sample.

Main outcomes Measure(s): To determine the association of sperm morphology with CPR.

Result(s): Semen analysis reports, including Kruger strict criteria for morphology from the pre-washed IUI sample, were reviewed for 1,059 cycles, comprising 825 total treated couples.

Of the total 1,059 cycles, 15.1% resulted in clinical pregnancy. When categorized by strict morphology ≥4% (normal morphology), (3%–2%) [mild-moderate teratozoospermia (TZS)], and ≤1% (severe TZS), the CPR was 16%, 13%, and 10%, respectively (*p* value 0.30). Early spontaneous miscarriage rate was 4% and when stratified by morphology ≥4% (3%–2%), and ≤1%, was 3%, 1%, and 0%, respectively (*p* value 0.20).

In couples with isolated TZS, the pregnancy rate was 16% in the normal morphology group, 14% in the mild–moderate group, and 8% in the severe group. (*p* value 0.30).

In the multivariate logistic regression, sperm morphology, mild/moderate TZS vs normal forms (OR = 0.99, 95% CI [0.94–1.1]), severe TZS vs normal forms (OR = 0.98, 95% CI [0.0.83–1.1]), was not a predictor of CPR. The Pre-wash TMSC (OR = 1.0, 95% CI [0.996–1.00]) was also not predictive of CPR.

The only predictive factor of CPR in IUI was the PWTMSC (OR = 1.03, 95%CI [1.00–1.06).

Conclusion(s): The morphology of the pre-washed sample on the day of IUI did not find a difference in CPR, neither in miscarriage rate following IUI, in couples with normal or abnormal sperm morphology, including severe TZS.

Mild, moderate, or severe TZS in the semen sample should not exclude couples to attempt an IUI procedure.

Introduction

Infertility affects one of every six couples trying to conceive [1], and a male factor is involved in about half of these couples [2]. The diagnosis of male infertility is mainly based on abnormal semen parameters, in which morphological evaluation is an integral part. In 2010, the World Health Organization (WHO) listed strict sperm morphology of ≥4% as the lower reference value for normal morphology [3]. Numerous studies have examined the effect of sperm morphology on IUI success; however, the results are conflicting and the prognostic value of the initial semen morphological characteristics in assessing the likelihood of successful IUI is still the subject of debate [4]. The American Society for reproductive Medicine (ASRM) and American Urological Association (AUA) guidelines state that there is no consensus regarding the influence of TZS on the selection of a specific assisted reproductive method in couples attempting to conceive [5,6]. Thus, couples undergoing previously In-Vitro Fertilization (IVF) with the only indication of isolated TZS [7] could have been served equally by IUI, a procedure ten times less costly than IVF [8]. The most recent meta-analysis that evaluated the impact of TZS on clinical pregnancies via IUI, published in 2018, included 20 observational studies involving a total of 41,018 cycles and concluded that abnormal sperm morphology does not have any impact on pregnancy rate [9]. However, these studies were based on the morphology of a semen analysis done prior to the day of IUI, and not on the prewashed sample which could have affected the results.

Hence, we decided to conduct the present study to check the impact of the strict sperm morphology, performed on the pre-washed sample, on pregnancy rate.

CONTACT Leyla Depret-Bixio ✉ leyladepretbixio@gmail.com 🖂 Department of Infertility, Fakih IVF Fertility Center, villa 37, 65b st - 32b st Al wasl road, Jumeirah 1, Dubai 72960, UAE

Materials and methods

Study population

Institutional Review Board approval was obtained.

We retrospectively reviewed IUI outcomes from May 2014 to March 2022 at a private fertility center.

Inclusion criteria

Couples presenting for IUI procedure during the study period:

- Women younger than 42 years old, with patent tubes as documented by hysterosalpingogram, with infertility causes due to low ovarian reserve (AMH less than 1 ng/mL), ovulatory dysfunction, unexplained infertility.
- Women with one to three dominant follicles more than 20 mm of size on the day of trigger.
- Men with normal sperm analysis.
- Men with Isolated teratozoospermia
- Men with impaired semen parameters affecting motility and/or concentration irrespective of the morphology parameter (because this is the main variable of our study), with Pre-wash TMSC < 12 million. This value was based on the WHO fifth manual edition, which considers the normal total sperm count as 39 million and progressive motility as 32%, we multiplied 39million × 32% = 12 million, and thus considered any TMSC < 12 million as abnormal.

Exclusion criteria

- Women aged more than 42 years old, blocked tubes, or diagnosis of endometriosis.
- Women with no response (no dominant follicle) after ovulation induction or multi-follicular response defined by more than three dominant follicles on the day of IUI.
- Men with more than 5 days of abstinence. We opted for a short abstinence time of between 2 and 5 days to decrease the effect of abstinence on sperm DNA fragmentation index and because the couples were trying to conceive.
- Use of frozen sperm sample, no availability of a baseline semen analysis.
- Lack of data pertinent to the study (the indication of IUI/ovulatory drug used/undocumented pregnancy outcome).

No lower threshold for Pre-Wash TMSC was used to exclude any patient, however if the TMSC was less than 2 million couples were counseled of the less likelihood of success, and the decision to proceed was left to the patient discretion.

To estimate the pregnancy rate of patients undergoing IUI with a desired power of 80%, a type I error of 0.05, and a hypothesis of a10% pregnancy rate, a minimal difference of 3.0% was assumed. Under these assumptions, 1,000 cycles were needed to detect the minimal difference. MedCalc© statistical software was used to calculate the needed sample size (version 20.114)

In total 1,381 cycles of IUI have been done during the study period in the center, 322 cycles were excluded because they did not meet the inclusion criteria and thus 1059 were studied.

Semen analysis and IUI sample preparation

All patients were asked to do a baseline semen analysis, followed by another one on the day of IUI, on which morphology was assessed before wash. The time-lapse between the two samples was 1–3 months. All samples were collected in the clinic by masturbation in a provided sterile container. The semen analysis was performed by well-trained, experienced laboratory andrologists following the methodology of the fifth edition of the WHO manual for human semen examination.

Upon the collection of samples, patients were given a sheet with instructions for reading and completion, which also contained provisions for documenting potential sample loss. After collection, samples underwent a 30-minute incubation at 37°C to encourage liquification, maintaining a maximum time limit of 60 minutes between liquification and analysis. The analysis encompassed aspects such as sperm concentration, total motility, progressive motility, and morphology.

To assess viscosity and volume, a graduated pipette was utilized to draw up the ejaculate. Any identified abnormal debris, round cells, and viscosity were carefully documented. Sperm concentration was ascertained by averaging the sperm count from two readings (ten squares each) on the counting grid of a Makler chamber, observed through a phase-contrast microscope at 20 magnifications.

The proportion of progressively motile sperm was established by counting sperm in 10 chambers (two readings) of the Makler chamber, classifying them as progressive, motile nonprogressive, or nonmotile. For the evaluation of morphology, 15 μL of semen was spread on a glass slide, allowed to dry, and subsequently stained using the Kwik-Diff Stain Kit (Thermos Scientific, USA). Slides underwent immersion 5 to 10 times in each solution of the kit and were then left to dry.

A total of 200 sperm per slide were scrutinized according to Kruger strict criteria at 1,000 magnifications using an oil immersion objective. The percentage of normal forms was computed based on the assessment of 200 sperm, considering abnormalities in the

head, midpiece, and tail, and the findings were systematically recorded. Sperm wash was performed by double density gradient centrifugation. The Gradient used was (PureCeption™ 80%, Cooper Surgical, USA) as lower layer and (PureCeption™ 40%) as upper layer. The liquefied semen sample was added on the top of the upper Phase and centrifuged for 20 minutes at 300 g. Then the pellet was washed twice with sperm washing medium (Cooper Surgical, USA) at 300 g for 5 minutes each time. After the second wash supernatant was removed and the pellet was resuspended in 0.5 ml sperm washing medium.

IUI cycles protocols and characteristics

The IUI cycles were either natural cycles following follicular tracking or cycles using ovulation induction and superovulation. Clomid and letrozole were used daily on cycle day 3–7, gonadotrophins alone from cycle day 6 to 10, and some cycles used a combination of gonadotrophins and letrozole/clomid. A midcycle vaginal ultrasound was done between day 9 and 11 of the cycle and a trigger by human chorionic gonadotrophin (HCG) was timed when one to three follicles were more than ≥20 mm in mean diameter as measured by ultrasound. IUI was done 36 h after the trigger. A SoftPass insemination catheter (Cook Medical) was utilized for IUI.

Patients were instructed to do a blood test to detect the B-HCG hormone 2 weeks after the IUI. First ultrasound was done at 7 weeks GA. Clinical pregnancies were defined as sonographic evidence of fetal cardiac activity at 7 weeks GA, and this was the primary outcome of the study.

Spontaneous miscarriages were defined as arrested pregnancies at/or after 7 weeks GA (blighted ovum, arrested fetal heart rate secondary to bleeding/other reasons). The pregnancies were followed till 12 weeks.

Statistical analysis

Continuous variables were presented as mean and standard deviation. Median and interquartile range were presented when the continuous variables were not normally distributed. Categorical variables were presented as number of observations and percentages.

General linear model was used to calculate the mean difference of continuous variables among semen morphology categories.

For count variables, a Poisson distribution analysis was applied (genmode procedure). For continuous no normally distributed variables, a nonparametric Wilcoxon statistics test was used for the morphology analysis.

To evaluate the association between pregnancy rate and semen morphology categories, a tweedie distribution was assumed which allows for frequent zero-valued observations.

The chi-square and Fisher exact tests were used to calculate the p value among category variables and semen morphology classes. A paired t-test was performed to compare the baseline semen analysis morphology and the sperm morphology of the Pre-Wash sperm.

For the multivariate analysis, the GLMSELECT procedure was used to find the predictive factors for clinical pregnancy. The selection method used was backward with the choose option of cross validation to estimate prediction errors. Cross validation was used to account for the unbalance data of the sperm morphology categories. The selection criterion employed for variables selection was the Schwarz Bayesian Information Criterion (SBC) [10], based on average square error. The choice of SBC was based on the low number of variables included in our model, and its application against overfitting.

The multivariate analysis was modeling the probability that PREGNANCY='Yes'. Besides semen morphology categories, the model was adjusted by age, Pre-Wash TMSC and PWTMSC because of statistical difference found in the bivariate analysis.

P-values, odds ratios, and confidence interval at 95% (OR [95% CI]) are presented in the summary tables and figures, in association with the descriptive statistics.

For the multivariate analysis and all analysis, a p value of 0.05 (both sides) was considered statistically significant. All analyses were performed using SAS studio (SAS® Studio). There were no missing values for any of the collected variables that were analyzed.

Results

A total of 825 couples and 1,059 cycles were included in the analysis.

Five hundred and twenty-seven couples (64%) had ≥4% normal morphology, 279 couples (34%) had mild-to-moderate TZS (2%–3%) and 19 couples (2%) had severe TZS (≤1%) normal forms.

Table 1 shows the characteristics of the total cohort of patients included. The mean age of the women was 33.0 ± 5.0 years old. Approximately half of couples had a diagnosis of unexplained infertility, and a third of couples had a diagnosis of ovulatory dysfunction. Ovulation induction with letrozole was done in 30% of cases, with clomid in 15% of cases, and natural cycle were performed in 21% of couples.

The median Pre-wash TMSC was 44.1×10^6, and the median PWTMSC was 14.4×10^6.

Of the total 1,059 cycles included, 160 resulted in clinical pregnancy and thus the pregnancy rate per cycle was 15.1%. 7 cycles ended by a miscarriage, with an overall miscarriage rate of 4%.

Patient's characteristics were compared according to sperm morphology categories (Table 2).

Table 1. Characteristics of the total cohort.

Variable	Mean ± std or N (%)
Number of cycles	1,059
Number of couples	825
Female age	33.0 ± 5.0
Number of dominant follicles on the day of the trigger	2.1 ± 0.8
Abstinence (days)	3.5 ± 2.3
Diagnosis n (%)	
Diminish Ovarian Reserve	108 (10.20)
Impaired motility and/or concentration concentration*	110 (10.39)
Ovulatory Dysfunction	348 (33.86)
Unexplained infertility	493 (46.55)
IUI sample parameters median (IQR)*	
Pre Wash TMSC (10^6)	44.1 (56.4)
Post Wash TMSC (10^6)	14.4 (71.7)
Pre- Wash Normal Forms	4.1 ± 2.3
Normal (≥4%)	679 (64.1)
Pre-wash abnormal forms(TZS)(<4%)	380 (35.9)
Mild/Moderate TZS (3%-2%)	361 (95.0)
Severe TZSa (≤1%)	19 (5.0)
Pre Wash Volume	2.4 ± 1.1
IUI cycle protocol	
Clomid + Gonadotrophin	25 (2.4)
Clomid	147 (14.8)
Letrozole + Gonadotrophin	204 (19.3)
Letrozole	321 (30.3)
Gonadotrophin	144 (13.6)
Natural	218 (20.6)
Clinical Pregnancy rate	160 (15.1)
Miscarriage rate (%)	7 (4.0)

*TMSC: total motile sperm count IQR: Interquartile range.
**Impaired sperm motility and/or : if total motile sperm count (prewashed) is less than 12 million/mL, according to the WHO manual 5th edition : total sperm count × progressive motility= normal total motile sperm count = 39 million × 32% = 12 million.

The main infertility diagnosis for the normal morphology group was idiopathic/unexplained infertility, and included around 50% of the couples, in the mild/moderate group and the severe TZS group, the most common diagnosis was impaired sperm concentration and/or motility with TMSC < 12 million. This difference in the diagnosis was statistically significant ($p < 0.0001\%$) specifically, the diagnosis of impaired motility and/or concentration was statistically different, between normal, mild/moderate TZS when compared to the severe TZS group.

Compared to couples with ≥4% morphology, couples with mild, moderate, and severe TZS had a significantly lower pre-wash and PWTMSC ($p < 0.0001$). Regarding IUI cycle protocol, natural cycle protocol was higher in the Mild/Moderate TZS category (25.2%) compared to normal (18.4%) and severe TZS (10.5%) groups. Gonadotrophins use alone, or with Letrozole, was higher

Table 2. Characteristics of patients and cycles according to sperm morphology.

Characteristics	Normal (≥4%)	Mild/Moderate TZS (3%-2%)	Severe TZS (≤1%)	P value
Number of cycles	679	361	19	
Number of couples	527	279	19	
Female age (mean ± std)	33.0 ± 4.6	32.4 ± 5.0	33.0 ± 5.2	0.45
Number of dominant follicles on the day of the trigger	2.1 ± 0.8	2.0 ± 0.8	2.4 ± 0.9	0.29
Abstinence days	3.4 ± 2.2	3.6 ± 2.5	3.8 ± 2.3	0.51
Diagnosis n (%)				
Diminish Ovarian Reserve	73 (10.7)	32 (8.9)	3 (15.8)	0.45
Impaired motility and/or concentration	27 (4.0) [a]	74 (20.5) [a]	9 (47.4) [b]	<.0001*
Ovulatory Dysfunction	228 (33.6)	116 (32.1)	4 (21.0)	0.48
Unexplained infertility	351 (51.7) [b]	139 (38.5) [a]	3.0 (15.8) [a]	<.0001*
IUI cycle protocol n (%)				
Clomid + Gonadotrophin	15 (2.2)	10 (2.8)	0 (0.0)	0.67
Clomid	89 (13.1) [a]	52 (14.4) [a]	6 (31.6) [b]	0.06
Letrozole+ Gonadotrophin	148 (21.8) [b]	53 (14.7) [a]	3 (15.8) [a]	0.01*
Letrozole	194 (28.6)	121 (33.5)	6 (31.6)	0.25
Gonadotrophin	108 (15.9)*** [b]	34 (9.4)) [a]	2 (10.5)) [a]	0.01*
Natural	125 (18.4) [a]	91 (25.2) [b]	2 (10.5)) [a]	0.02*
IUI sample parameters median (IQR)**				
Pre-Wash TMSC (10^6)	54.7 (58.8) [a]	26.3 (37.4) [b]	28.6 (66.0) [a]	<.0001
Post Wash TMSC (10^6)	17.2 (18.7) [a]	9.5 (12.9) [b]	5.6 (20.2) [a]	<.0001
Clinical pregnancy n(%)	111 (16.0)	47 (13.0)	2 (10.0)	0.30
Miscarriage rate n(%)	5(3.0)	2 (1.0)	0 (0.0)	0.20

*p values > 0.05, **TMSC: total motile sperm count IQR: Interquartile Range: Groups with the same letter are not statistically different (a,b).

in normal morphology group compared to the mild/moderate and severe TZS group, with observed statistical significance.

We compared the 1% normal morphology of the baseline semen analysis done a priori for each patient, one to 3 months before the IUI to the morphology of the pre-wash sample given on the day of IUI. For the same patient, morphology of the pre-washed sample was 0.16 higher than the morphology of the baseline sample. Although it reached statistical significance of $p = 0.02$, we consider this difference not clinically relevant.

Of the total 1059 cycles included,160 (15.1%) resulted in clinical pregnancy. Total clinical pregnancy rate was 16.3%,13%, and 10.5% when categorized by strict morphology of ≥4%, (3%–2%), and ≤1% respectively and was not statistically different among semen morphology categories, comparing normal forms to mild/moderate TZS (16.3% vs 13%; $p = 0.15$); normal forms vs severe TZS (16% vs 10.4% $p = 0.75$) (fig1). In patients with isolated TZS (pre-wash TMSC > 12 × 106), we compared clinical pregnancy rate according to sperm morphology. The clinical pregnancy rate was 16%, 14%, 8% for the ≥4%, (3%–2%), ≤1% morphology groups, respectively (Figure 1b). There was no statistically significant difference in CPR following IUI between couples with >4% normal sperm morphology, and those with isolated TZS irrespective of the severity of the TZS, with a $p = 0.36$ and a $p = 0.19$ for normal versus mild/moderate and normal versus severe, respectively (Figure 1b).

During the comparative analysis of PWTMSC levels in the normal morphology group, those with PWTMSC ≥ 9 million had a CPR of 17.6%, whereas for those with PWTMSC < 9 million, the CPR was 12.2%, with no statistical significance observed (p value 0.10). Likewise, within the group presenting abnormal morphology (Mild/Moderate TS (3%-2%) and Severe TZS (≤1%), CPR values for PWTMSC ≥ 9 million and <9 million stands at 15.3% and 10.1%, respectively, presenting not statistically significance (p value 0.12) (Figure 2a).

Furthermore, an intergroup comparison between normal morphology with PWTMSC ≥ 9 million and abnormal morphology with PWTMSC < 9 million showed statistical difference in CPR of 17.6% and 10.1% observed, respectively, with a statistically significant (p-value of 0.01) (Figure 2b). Of the 160 patients pregnant, 7 patients (4%) had an early spontaneous miscarriage. Miscarriage rate was 3%,2%, and 0% when categorized by strict morphology of ≥4%, (3%–2%), and ≤1% respectively and was not statistically different between semen morphology groups (p value 0.20).

A multivariate analysis was applied to find the predictive factors for clinical pregnancy (Figure 2a,2b).

The model was adjusted for age, infertility diagnosis, IUI cycle protocol, and Pre-Wash TMSC PWTMSC.

Sperm morphology was not found to be a predictive factor for clinical pregnancy, severe TZS vs normal forms (OR = 0.98, 95% CI [0.0.83–1.1]), mild/moderate TZS vs normal forms (OR = 0.99, 95%
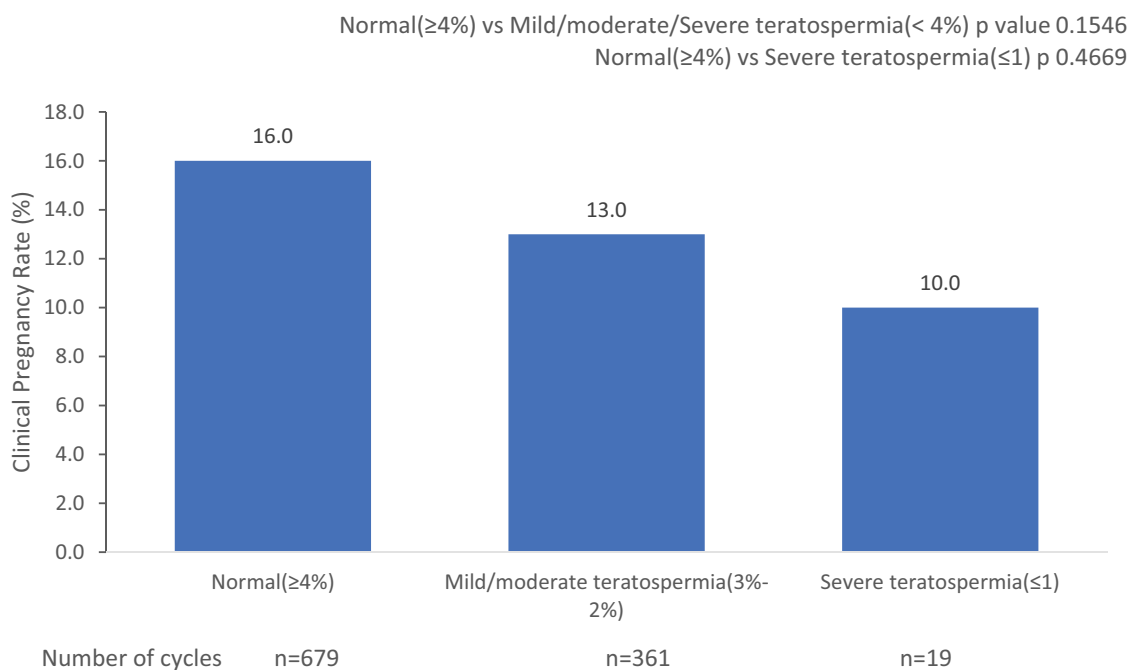

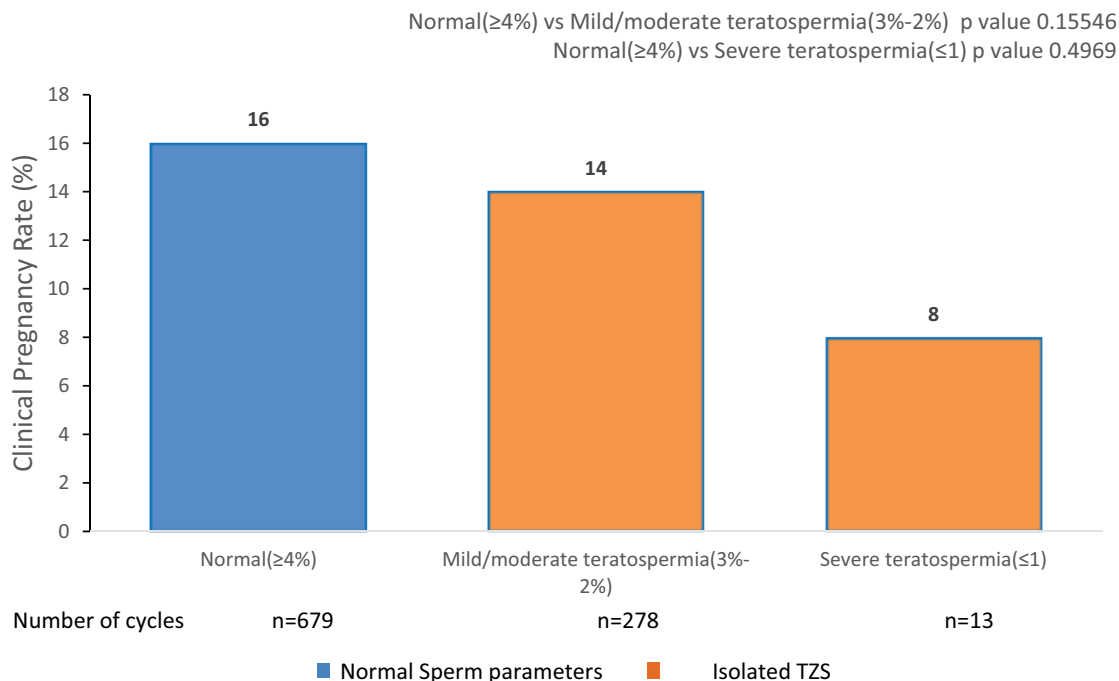**Figure 1a.** Clinical pregnancy rate by semen morphology categories.

Figure 1b. Clinical pregnancy rate by semen morphology categories and normal semen parameters versus/isolated TZSTZS).
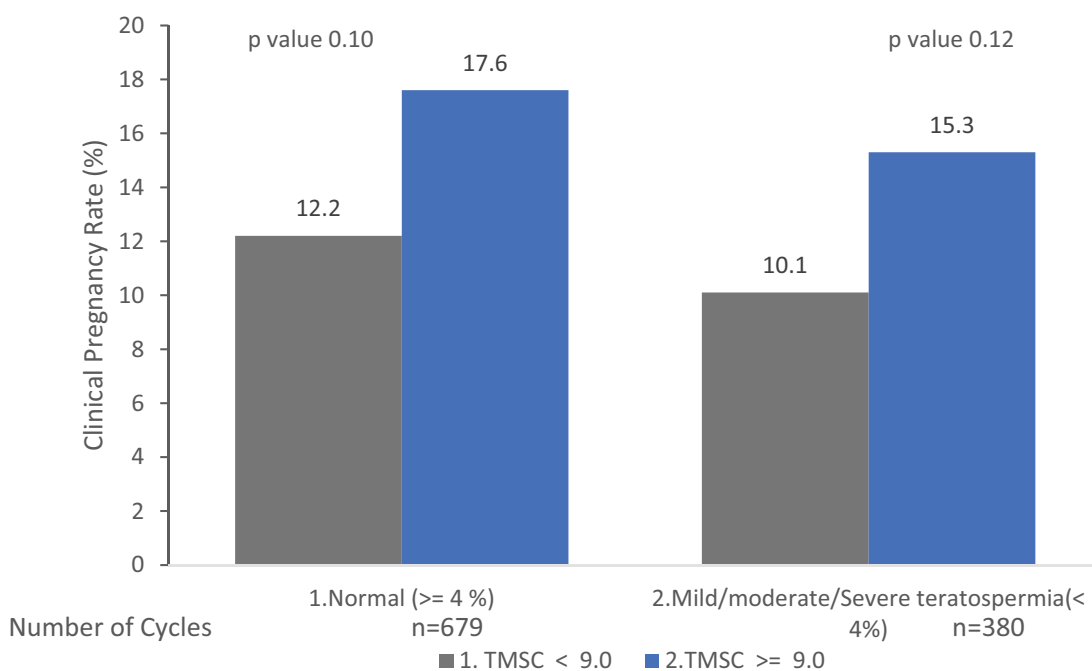

Figure 2a. Clinical pregnancy rates within the same morphology group (normal or abnormal) and PWTMSC (<9 million or ≥ 9 million).

CI [0.94–1.1]). The pre-wash TMSC (OR = 1.0, 95% CI [0.996–1.00]) was neither predictor of clinical pregnancy. The only predictive factor of CPR in IUI was the PWTMSC (OR = 1.03, 95% CI [1.00–1.06]). The other variables included in the model were not statistically significant (Figure 3).
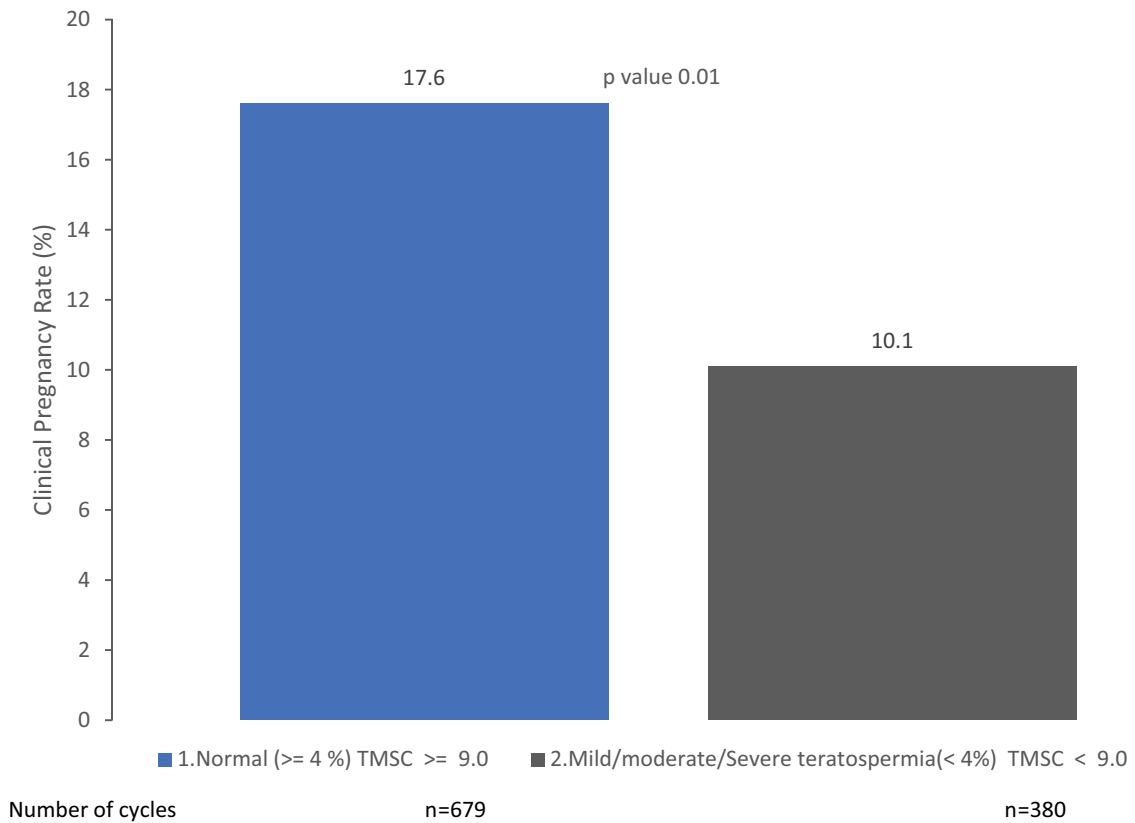
Figure 2b. Clinical pregnancy rate between normal morphology group (≥4%) with PWTMSC ≥ 9 million and abnormal morphology group (<4%) with PWTMSC < 9 million.
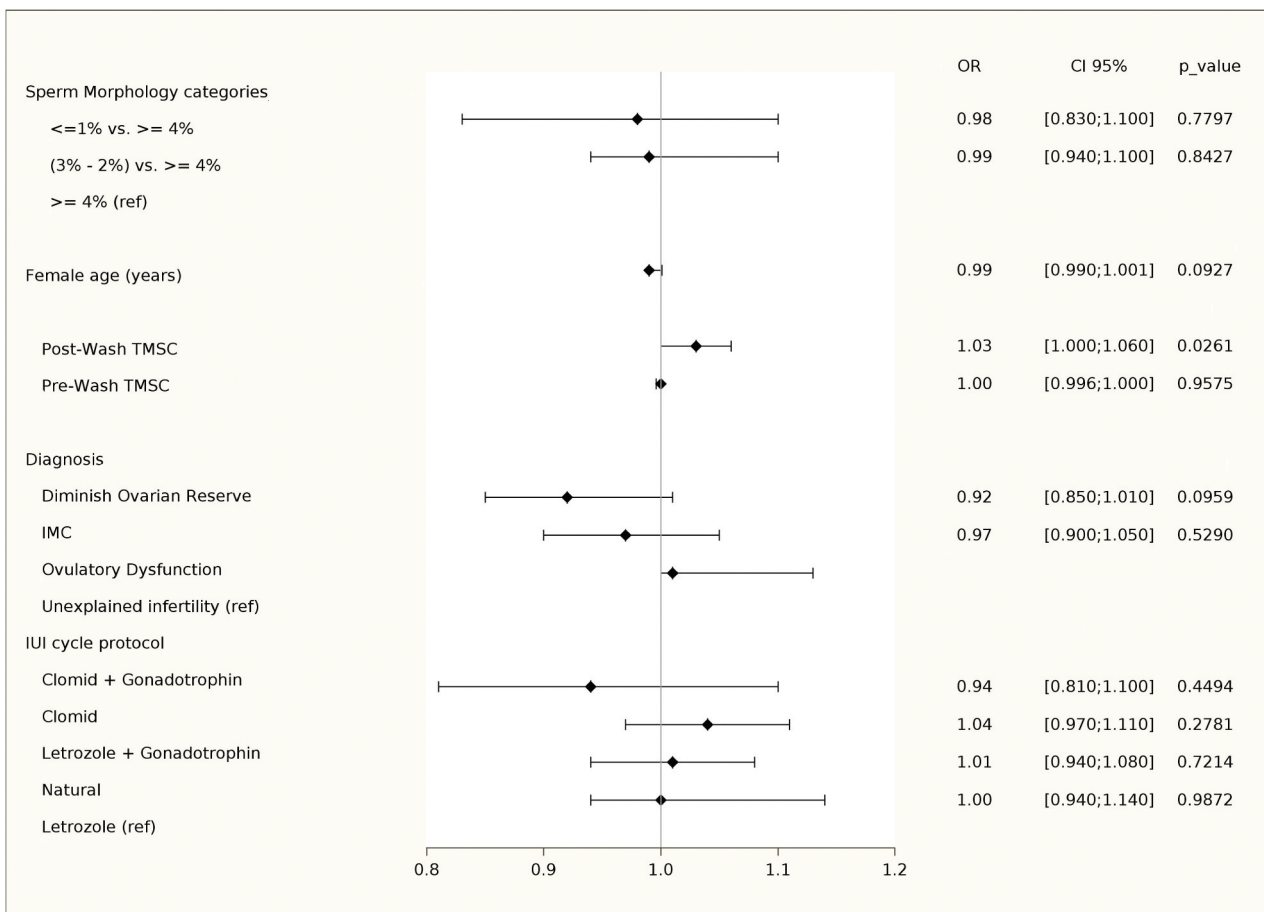

Figure 3. Multivariate logistic regression analysis - predictive factors of clinical pregnancy. IMC = Impaired sperm motility and/or concentration; OR = odds ratio; CI 95% = 95% Confidence Interval TMSC = total motile sperm count (10^6).

Discussion

In this study, 160 clinical pregnancies were achieved after 1059 IUI, for a total clinical pregnancy rate/cycle of 15.1%. This rate is higher than the one reported by Stanhiser et al. (8.6%) [11] and by Wainer et al. (12.9%) [12]. However, it is within the range of the study of Muthigi et al. [13] including 92,471 IUI and showing that pregnancy rate per cycle ranged from 11.9% to 18.5%.

When semen was categorized by strict morphology, clinical pregnancy rate was not statistically different among the various morphology categories. Furthermore, results were scrutinized, and we compared clinical pregnancy after IUI between semen with normal parameters and men with isolated teratozoospermia (normal count and motility), the CPR in the normal morphology group was two times more than the CPR in the severe TZS group without reaching statistical significance.

These results demonstrating no difference in pregnancy rate irrespective of morphology severity, are similar to those of Kohn et al. [9] Patel et al. [14] and Stanhiser et al. [11] however, they are contrary to the relatively old publications reporting that normal morphology is associated with higher pregnancy rate [7,15,16].

This study showed a higher percentage of 0.16% for normal sperm morphology of the baseline semen analysis, which was done one to 3 months before the IUI, compared to the sperm morphology of the pre-washed sperm.

This minor percentage change in the morphology does not have any clinical significance.

Our result is in concordance with the study of Ombelet et al. in 1995 [17] showing that morphology is a stable characteristic that will not vary from a sample to another.

Accordingly, we suggest reviewing the clinical pertinence of performing a morphology test on the pre-wash semen sample, on the day of IUI, if we have a baseline semen analysis done within 3 months.

In addition, the study showed that pre-wash TMSC has no prognostic value to predict the probability of becoming pregnant in couples with IUI treatment. Although TMSC is considered as a relatively good indicator for male factor infertility in general [18,19], it has been shown by a recent study by Mankus et al., as a poor predictor of live birth in insemination cycles [20].

In contrast to Pre-wash TMSC, this study revealed that PWTMSC was predictive of the ongoing pregnancy rate after IUI, and this is an agreement with the previous literature [21–23]. A recent publication by Muthigi et al. in 2021 [13], confirmed our finding and showed that PWTMSC was highly predictive of pregnancy and optimized when post-wash TMSC is ≥9 million X 106, below which the rates gradually decline. Using Muthigi et al. threshold for PWTMSC

(<9 million and ≥9 million), our study did not show statistical difference in CPR within the same morphology group. However, when comparing between normal morphology group with PWTMSC ≥ 9 million and abnormal morphology group with PWTMC < 9 million, the CPR was statistically significant. This finding suggests that oligospermia combined with teratozoospermia can influence negatively IUI outcomes/success rates. According to these data, pooling of sperm by combining two semen samples, where each pellet is resuspended in 0.25 ml sperm washing medium to get the final desired volume of 0.5 ml, could be offered to couples who have a history of TZS associated with oligospermia. This may allow to reach an optimal PWTMSC, and further studies should address the clinical utility and outcome of this suggested intervention.

The overall early spontaneous miscarriage rate was 4%, which is lower than the one mentioned recently by Qiongxiu Luo et al. [24] In this study 31,933 cycles of IUI were included and showed an early spontaneous miscarriage rate of 11.7%.

We decided to investigate the reason behind the controversy in the literature concerning sperm morphology and IUI success rates, since old studies have shown that sperm morphology has a significant impact on IUI outcomes, and recent studies are demonstrating no effect. Thus, we categorized these studies according to year of publication, edition 4th or 5th of the WHO semen manual used for strict morphology criteria, and clinical pregnancy rate (Table 3).

This classification revealed that studies that relied on the 4th edition of the WHO semen manual found a positive correlation, contrary to the studies that relied on the 5th edition.

Although we acknowledge that heterogeneity in the preparation and reading of smears [25], inter-observer variation, and the subjective nature of the morphological assessment [26] between these studies could have affected the clinical outcome, however we believe that the various renditions of the WHO manual for semen analysis testing [27] are the principal culprit that has put the clinical utility of morphology parameter under scrutiny.

This is due to increase in strictness of the morphology parameter in the 5th edition of The WHO semen manual, compared to the 4th edition, that has affected predictiveness, making the barometer 'normal' sperm progressively smaller to the extent that most sperm are classified as abnormal.

Thus, the morphology based on the current Kruger's classification system (Kruger's strict criteria) may have lost its clinical relevance, but sperm morphology itself could still be an important parameter. In addition, detection of monomorphic TZS (macrozoospermia and globozoospermia) associated with DNA genetic alteration and poorer reproductive outcome,

Table 3. Association between sperm morphology and pregnancy in IUI according to WHO manual edition adopted by the study.

study	Year of publication	WHO Manual edition	Association between Morphology and Clinical pregnancy	Comments
Stanhiser J et al	2020	5th edition	No	
Patel P et al	2019	5th edition	No	
Danis RB et al	2019	5th edition	No	Sperm Morphology non predictive of poor reproductive outcomes
Kohn TP et al	2018	5th edition	No	
Kohn TP et al.(meta-analysis)	2018	5th edition (14 studies) 4th edition(6 studies)	No	
N. Gatimel J et al	2017	5th edition	No	
Van den Hoven L et al	2015	5th edition	No	
Tomlinson M et al	2013	5th edition	No	Sperm morphology remains controversial
Jarow J et al	2010	5th edition	No	
Deveneau NE et al	2010	5th edition	No	
Spiessens C et al	2003	4th edition	Yes	
Van Waart J et al	2001	4th edition	Yes	
Hauser R et al	2001	4th edition	Yes	
Lindheim, S.R et al	1996	4th edition	Yes	
J. P. Toner et al	1995	4th edition	Yes	

compared to polymorphic teratozoospermia, is primordial for patients treatment strategies and prognosis [28]. Future research to develop an assessment of sperm morphology that is more predictive is essential to return the use of this parameter to clinical significance. The use of some indexes, discussed in WHO manual 6th edition [29], published in 2021, like the teratozoospermia index (TZI), the multiple anomalies index (MAI) and the sperm deformity index (SDI) could be pertinent and could have a clinical relevance.

The main limitations of this study are its retrospective design, and very small number of men in the group with severe TZS, which was however addressed using a cross-validation procedure in the multivariate analysis.

Nevertheless, this study has many strengths. The total sample size is adequately large with 1059 cycles of IUI included in the study. It evaluated the impact of sperm morphology from the pre-wash sample on clinical pregnancy rate and it compared the change in morphology parameter between the baseline semen analysis done one to 3 months before the IUI, and the pre-washed sample for the same individual.

Furthermore, possible confounding parameters like female age, infertility diagnosis, IUI cycle protocols, pre-wash TMSC and PWTMSC, were adjusted in the multivariate analysis. Abstinence period before the baseline semen analysis and the IUI sample was stable. Semen analysis including baseline sample and the sample produce on the day of the IUI, were performed in the same laboratory with fully trained andrologists and stable standardized laboratory techniques.

Conclusion

This study demonstrated no difference in the pregnancy rate, nor in the miscarriage rate for couples who underwent IUI with normal sperm morphology ≥4% compared to abnormal sperm morphology <4%, irrespective of its severity, using strict Kruger criteria.

Our data also revealed that PWTMSC was predictive of clinical pregnancy rate in IUI cycles.

Accordingly, future research should concentrate on the development of a nomogram including the PWTMSC with other relevant variables that could have a clinical correlation with CPR in IUI.

At this moment, we should not adopt a quantitative percentage threshold for normal sperm morphology, as a sole factor, to offer or deny an IUI procedure to infertile couples.

Abbreviations

CPR	Clinical pregnancy rates
IMC	Impaired sperm motility and/or concentration
TZS	Teratozoospermia
IVF	In-vitro Fertilization
IUI	Intrauterine Insemination
UAE	United Arab Emirates
PWTMSC	Post- wash total sperm motile count
TMSC	Total sperm motile count
pre-wash TMSC	Pre- wash total motile sperm count
WHO	World Health Organization
ASRM	American Society for reproductive Medicine
AUSA	American Urological Association
HCG	Human Chorionic gonadotrophin
BHCG	B Human Chorionic gonadotrophin

GLM	General linear model
GEE	Generalized Estimating Equations
TZI	Teratozoospermia index
MAI	Multiple anomalies index
SDI	Sperm deformity index

ORCID

Leyla Depret-Bixio http://orcid.org/0000-0003-1336-8130

Author contribution

L.S: study concept and design. L.S. and L.DB.: Drafted the manuscript. L.DB: Analyzed data. L.S., M.C., R.S and M.F: Data interpretation. L.S., R.S., and M.F: Critical revision of the manuscript for important intellectual content. All authors have read and agreed to the published version of the manuscript.

Informed consent

A waiver for informed consent was requested and obtained by Dubai Research Ethics Committee (Dubai Health Authorities)

References

[1] Chandra A, Martinez GM, Mosher WD, et al. Fertility, family planning, and reproductive health: data from the 2002 national survey of family growth. Vital Health Stat. 2005;23:25.

[2] American Society for Reproductive Medicine Diagnostic evaluation of the infertile male: a committee opinion. Fertil Steril. 2012;98 (2):294–301. doi: 10.1016/j.fertnstert.2012.05.033

[3] World Health Organization. Laboratory manual for the examination and processing of human semen. 5th ed. Geneva: WHO Press; 2010. p. 271.

[4] Deveneau NE, Sinno O, Krause M, et al. Impact of sperm morphology on the likelihood of pregnancy after intrauterine insemination. Fertil Steril. 2014 Dec;102(6):1584–90. e2. Epub 2014 Oct 25. PMID: 25439801. doi: 10.1016/j.fertnstert.2014.09.016

[5] Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: a committee opinion. Fertil Steril. 2015 Mar; 103(3):e18–25. Epub 2015 Jan 15. PMID: 25597249. Fertil Steril 2015. 10.1016/j.fertnstert.2014.12.103

[6] Jarow J, Kolettis PN, Lipshultz LR. et al. Components of a full evaluation for male infertility. In: American Urological Association, editor. The optimal evaluation of the infertile. Male: AUA Best Practice Statement; 2010. p. 15

[7] Spiessens C, Vanderschueren D, Meuleman C, et al. Isolated teratozoospermia and intrauterine insemination. Fertil Steril. 2003 Nov;80(5):1185–9. PMID: 14607572. doi: 10.1016/s0015-0282(03)01172-5

[8] Kohn TP, Kohn JR, Lamb DJ. Role of sperm morphology in deciding between various assisted reproduction technologies. Eur Urol Focus. 2018 Apr;4(3):311–313. Epub 2018 Aug 22. PMID: 30143470. doi: 10.1016/j.euf.2018.07.029

[9] Kohn TP, Kohn JR, Ramasamy R. Effect of sperm morphology on pregnancy success via intrauterine insemination: a systematic review and meta-analysis. J Urol. 2018 Mar;199(3):812–822. Epub 2017 Nov 9. PMID: 29129781. doi: 10.1016/j.juro.2017.11.045

[10] Schwarz G. Estimating the dimension of a Model. Ann Stat. 1978;6(2):461–464. doi: 10.1214/aos/1176344136

[11] Stanhiser J, Mersereau JE, Dock D, et al. Sperm morphology from the actual inseminated sample does not predict clinical pregnancy following intrauterine insemination. F S Rep. 2020 Dec 9;2(1):16–21. PMID: 34223268; PMCID: PMC8244316 doi: 10.1016/j.xfre.2020.11.007

[12] Wainer R, Albert M, Dorion A, et al. Influence of the number of motile spermatozoa inseminated and of their morphology on the success of intrauterine insemination. Hum Reprod. 2004 Sep;19 (9):2060–2065. Epub 2004 Jul 8. PMID: 15243004. doi: 10.1093/humrep/deh390

[13] Muthigi A, Jahandideh S, Bishop LA, et al. Clarifying the relationship between total motile sperm counts and intrauterine insemination pregnancy rates. Fertil Steril. 2021 Jun;115(6):1454–1460. Epub 2021 Feb 18. PMID: 33610321. doi: 10.1016/j.fertnstert.2021.01.014

[14] Patel P, Carrasquillo R, Madhusoodanan V, et al. Impact of abnormal sperm morphology on live birth rates following intrauterine insemination. J Urol. 2019 Oct;202(4):801–805.Epub 2019 Sep 6. PMID: 31009287. doi: 10.1097/JU.0000000000000288

[15] Hauser R, Yogev L, Botchan A, et al. Intrauterine insemination in male factor subfertility: significance of sperm motility and morphology assessed by strict criteria. Andrologia. 2001 Jan;33(1):13–7. x. PMID: 11167514. doi: 10.1046/j.1439-0272.2001.00404.x

[16] Van Waart J, Kruger TF, Lombard CJ, et al. Predictive value of normal sperm morphology in intrauterine insemination (IUI): a structured literature review. Hum Reprod Update. 2001 Sep-Oct;7(5):495–500. PMID: 11556497. doi: 10.1093/humupd/7.5.495

[17] Ombelet W, Menkveld R, Kruger TF, et al. Sperm morphology assessment: historical review in relation to fertility. Hum Reprod Update. 1995 Nov;1(6):543–557. PMID: 9079395. doi: 10.1093/humupd/1.6.543

[18] Hamilton JA, Cissen M, Brandes M, et al. Total motile sperm count: a better indicator for the severity of male factor infertility than the WHO sperm classification system. Hum Reprod. 2015 May;30(5):1110–1121. Epub 2015 Mar 18. PMID: 25788568. doi: 10.1093/humrep/dev058

[19] Van Voorhis BJ, Barnett M, Sparks AE, et al. Effect of the total motile sperm count on the efficacy and cost-effectiveness of intrauterine insemination and in vitro fertilization. Fertil Steril. 2001 Apr;75 (4):661–668. PMID: 11287015. doi: 10.1016/s0015-0282(00)01783-0

[20] Mankus EB, Holden AE, Seeker PM, et al. Prewash total motile count is a poor predictor of live birth in intrauterine insemination cycles. Fertil Steril. 2019 Apr;111 (4):708–713. PMID: 30929730; PMCID: PMC6446576. doi: 10.1016/j.fertnstert.2018.12.025

[21] Van Weert JM, Repping S, Van Voorhis BJ, et al. 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 Sep;82(3):612–620. PMID: 15374704. doi: 10.1016/j.fertnstert.2004.01.042

[22] Nikbakht R, Saharkhiz N. The influence of sperm morphology, total motile sperm count of semen and the number of motile sperm inseminated in sperm samples on the success of intrauterine insemination. Int J Fertil Steril. 2011 Oct;5 (3):168–73. Epub 2011 Dec 22. PMID: 25101161; PMCID: PMC4122832.

[23] Tomlinson M, Lewis S, Morroll D. British fertility society. Sperm quality and its relationship to natural and assisted conception: British fertility society guidelines for practice. Hum Fertil (Camb). 2013 Sep;16 (3):175–193. Epub 2013 Jul 17. PMID: 23862664. doi: 10.3109/14647273.2013.807522

[24] Luo Q, Qin J, Wang H, et al. Factors associated with spontaneous miscarriage risk in IUI treatment: a retrospectively cohort of 31,933 cycles. Eur J Obstet Gynecol Reprod Biol. 2023;286:61–68. ISSN 0301-2115. doi: 10.1016/j.ejogrb.2023.05.005

[25] Van den Hoven L, Hendriks JC, Verbeet JG, et al. Status of sperm morphology assessment: an evaluation of methodology and clinical value. Fertil Steril. 2015 Jan;103(1):53–58. Epub 2014 Oct 24. PMID: 25450299. doi: 10.1016/j.fertnstert.2014.09.036

[26] Danis RB, Samplaski MK. Sperm morphology: history, challenges, and impact on natural and assisted fertility. Curr Urol Rep. 2019 Jun 15;20(8):43. PMID: 31203470. doi: 10.1007/s11934-019-0911-7

[27] Menkveld R. Clinical significance of the low normal sperm morphology value as proposed in the fifth edition of the WHO Laboratory Manual for the examination and processing of human semen. Asian J Androl. 2010 Jan;12(1):47–58. PMID: 20111081; PMCID: PMC3739680. doi: 10.1038/aja.2009.14

[28] De Braekeleer M, Nguyen MH, Morel F, et al. Genetic aspects of monomorphic teratozoospermia: a review. J Assist Reprod Genet. 2015 Apr;32(4):615–23. Epub 2015 Feb 25. PMID: 25711835; PMCID: PMC4380889. doi: 10.1007/s10815-015-0433-2

[29] Boitrelle F, Shah R, Saleh R, et al. The sixth edition of the WHO manual for human semen analysis: a critical review and SWOT analysis. Life (Basel). 2021 Dec 9;11 (12):1368. PMID: 34947899; PMCID: PMC8706130. doi: 10.3390/life11121368