Review Article

Impact of semen parameter on IUI

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Abstract Intrauterine insemination (IUI) is a commonly performed procedure in the treatment of infertility. Its outcome depends on multiple factors. Semen is an important predictor for the success of IUI. Various semen parameters like sperm concentration, motility, morphology, and number of motile sperms inseminated determine the outcome of IUI. Advanced sperm function tests are required in addition to the standard semen analysis in few infertile patients.

Keywords: Infertility, intrauterine insemination, semen

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INTRODUCTION

Intrauterine insemination (IUI) is the most common procedure performed in infertility practice. In spite of the 2013 NICE guidelines[1] stating the limited value of IUI in infertility, it still remains the first line treatment for moderate male factor subfertility, cervical factors, and unexplained infertility.

It is a simple, non-invasive and a cost-effective procedure with a reasonable cumulative live birth rate within three or four cycles. [2] The rationale behind IUI is to increase the density of sperm at the site of fertilization. The semen washing procedures help to remove prostaglandins, infective microorganisms, antigenic proteins, immotile sperms, leukocytes, and immature germ cells. This decreases the formation of free oxygen radicals and enhances the quality of sperm, thereby improving its fertilization capacity.

The success of IUI depends on various factors. These include the ovulation induction regimens used, methods used for semen preparation, timing and number of inseminations per treatment cycle, etc.

Among these the most important determinants predicting the outcomes of IUI are the ovarian stimulation protocols used and the quality of sperms inseminated. In a meta-analysis done by van Rumste et al.[3] they observed that multifollicular growth in IUI cycles was associated with increased pregnancy rates, but at the expense of an increased risk of multiple pregnancies. The authors also stated that the presence of three or four follicles was associated with an increased multiple pregnancy rate without a substantial increase in the overall pregnancy rate. They concluded that in IUI cycles, ovarian stimulation protocols should aim for not more than two follicles.

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Table 1: WHO semen analysis parameters

Parameters	Lower reference limit
Liquefaction	Complete in 60 min
Volume	1.5 ml
Color	Opalescent white
pH	>7.1
Concentration (ml)	15 million
Progressive motility	32%
Vitality	58%
Morphology	4%
Leukocytes (ml)	<1 million
Mar test	<50% sperm with bound particles

Semen parameter is an important predictor of the success of IUI. Semen analysis is a simple investigation that is used to assess male infertility. To improve the standardization between laboratories in terms of semen sample diagnosis and assessment criteria, the World Health Organization (WHO) [4] has published a manual that guides the andrology laboratories to determine sperm quality. The concept of "Lower Reference Limit" (LRL) is stated in the manual of the WHO [Table 1] The values over the lower reference limit do not guarantee a successful pregnancy, but it does increase the probability of getting pregnant. The LRL has progressively been reduced due to the changing social behaviors and lifestyle. The establishment of threshold levels for sperm parameters above which IUI pregnancy outcome is significantly improved aids in the clinical practice.

SPERM CONCENTRATION AND MOTILITY

Sperm concentration is an important parameter that influences live pregnancy rate. Sperm count is evaluated both in the native and washed semen samples. Although the WHO's reference values for semen analysis are commonly used to assess the sperm quality, threshold values of semen parameter for successful IUI are still controversial.

Studies have been done to evaluate the effects of total motile sperm count (TMSC) on pregnancy rate after IUI treatment. A significant increase in clinical pregnancy rate was observed when TMSC was >5 million.[5,6,7] In a systematic review done by Ombelet et al.[8] they observed that TMSC was an important predictor for successful pregnancy with a cut-off value of 5–10 million.

Sperm motility is an independent factor influencing IUI-related pregnancy. [9] A forward progression score of 3 to 4 in a processed semen sample is necessary for IUI success. Total motility desired in semen sample is around 30%.

Significance of sperm count obtained after semen preparation has also been studied. In the meta-analysis of 16 studies by Van Weert et al.,[10] receiver operating characteristics (ROC) curves indicated a reasonable predictive value of inseminating motile count (IMC) for outcome of IUI. At cut-off levels between 0.8 and 5 million, the specificity of the IMC to predict the failure to become pregnant, was as high as 100% but the sensitivity of it to predict successful pregnancy was limited. Ombelet et al.[11] stated that an IMC of 1 million can be used as a threshold level above which IUI can be performed.

A retrospective study was done by Cao et al. [14] to assess the relationship between number of motile sperms inseminated and the pregnancy rate after IUI. They observed a pregnancy rate of 4.05%, if less than 2×10^6 motile sperms were used. This increased to 14.55% when more than 2×10^6 motile sperms were inseminated. They concluded that IUI can be performed when the number of motile sperms inseminated exceeds 2×10^6 .

A study[13] was carried to determine whether post washed total progressively motile sperm count (TPMSC) obtained by computer-assisted semen analysis (CASA) could predict positive pregnancy test rate in IUI cycles. The pregnancy rate per cycle (PR/cycle) when post-washed TPMSC was between 0 and 0.5 million, 0.51 and 1 million, 1.01 and 5 million, 5.01 and 10 million, and greater than 10 million were 8.1% (42/520), 14.4% (41/285), 16.1% (237/1,469), 18.4% (193/1,046), and 18.8% (668/3,551) respectively. The predicted odd of positive pregnancy result was statistically significantly when TPMSC was >0.51 million compared to the TPMSC of <0.51 million (OR = 1.68, 95% CI: 1.04–2.71). The predicted odd of positive pregnancy result was greatest when TPMSC was at least 5 million (OR = 2, 95% CI: 1.38–2.9).

Koyun et al.[14] studied the impact of post-wash TPMSC and semen volume on pregnancy outcomes in IUI cycles. They observed that there was no significant relationship between the inseminated semen volume and pregnancy rate ($P > 0.05$). However, a significant linear association was observed between the TPMSC and pregnancy rate ($P = 0.042$). They suggested that the post-wash inseminated semen volume should be between 0.3 and 0.5 mL and the post-wash TPMC of above 10×10^6 may be a useful threshold value for IUI success. Miller et al. [15] in their study reported a significantly lower pregnancy rate for couples with less than 10 million processed total motile sperm.

Recommended minimum number of motile spermatozoa inseminated thresholds for IUI vary widely across the

literature, with reports of 1 million,[11,16] 2 million, [12] 5 million, [13,17] and 10 million[14,15] when calculated for female patients of all ages.

This difference may be due to various sperm preparation techniques used. The gap between pre- and post-processing, as measured by recovery, could be a major factor in patients with a borderline value for total motile sperm count.

Female age[18] is an independent predictor of success following IUI. Few studies have been done to evaluate the relationship between the number of motile spermatozoa inseminated and female age in IUI cycles.

Demir et al. [19] found that pregnancy rates differed significantly in the women age < 25 years when number of motile spermatozoa inseminated was $> 10 \times 10^6$, compared to age groups 25–30 and >30, and when number of motile spermatozoa inseminated was < 5 and 5–10. Similarly, Badawy et al.[6] observed that for patients <25 years old and number of motile sperms $>5 \times 10^6$, the pregnancy rate per cycle was significantly higher (28.2%) than that of other age groups. They also observed that in women above the age of 35 years with TMSC $>5 \times 10^6$ the pregnancy rate was very low (0.84%) and no pregnancies were reported with TMSC $<5 \times 10^6$. In another study Gubert et al.[20] observed that pregnancy rates were only significantly different in the group < 35 years when number of motile spermatozoa inseminated was < 5 million. They suggested that number of motile spermatozoa inseminated was not a good predictor of IUI outcome in patients over 35 years.

The number of motile spermatozoa inseminated is a good prognostic tool which reflects both, the sperm concentration and motility. It however should not be used for counselling during the initial infertility workup, but only during the IUI procedure.

MORPHOLOGY

Kruger et al. [21] in 1986 described a method of evaluating sperm morphology, termed Kruger/Tygerberg strict sperm morphology (SSM) which helped to predict the chances for successful fertilization in IVF. They said that an SSM >14% was normal. An SSM of 5–14% had an intermediate pregnancy chance and SSM of 0–4% had poor chance of pregnancy. In 1998, Coetzee et al. [22] reviewed the literature and stated that strict sperm morphology values ≤4% was the best predictor of decreased fertilization and pregnancy rates after IVF. Intracytoplasmic sperm injection (ICSI) was recommended with SSM ≤4%. In 2010, the WHO stated the strict sperm morphology of ≥4% as the lower reference value for normal morphology.

The association between SSM criteria of ≤4% and decreased likelihood of pregnancy with IUI has been investigated; however, study results are conflicting.

Morphology is the best predictor of clinical pregnancy. [23] Van Waart et al. [24] reviewed the literature published on the use of normal sperm morphology, as an indicator of male fertility potential in intrauterine insemination. Their meta-analysis showed a significant improvement in pregnancy rate above 4% threshold for Tygerberg strict criteria for evaluation of normal sperm morphology. Similar observation was made by Hauser R. [25]

Lockwood et al.[26] studied whether isolated abnormal strict morphology (<5% normal forms) and very low strict morphology (0–1% normal forms) affects pregnancy rates in intrauterine insemination. They observed that clinical pregnancy rate did not significantly differ between the group with abnormal strict morphology (15.7%) and the normal morphology group (13.9%). Furthermore, there was no significant difference between pregnancy rate in the very low morphology group (21.4%) compared to those with normal morphology. Patients with isolated abnormal strict morphology have clinical pregnancy rates similar to those with normal morphology for IUI. Even in those with very low normal forms, consideration of IUI for assisted reproduction should not be excluded. Similar observation has been made by few other studies also. [27,28,29]

Deveneau et al. [30] compared pregnancy rates between patients undergoing IUI cycles with SSM values ≤ 4% and >4%. They found no clinically significant difference in pregnancy rates after IUI between these two groups suggesting that morphology was not a strict predictor of IUI success. They however found that sperm morphology in men with varicocele was an important parameter. In couples with varicocele, those with SSM ≤4% have about one-fourth odds of becoming pregnant compared with SSM >4%. When morphology is normal, those without varicocele have about four times the odds of becoming pregnant compared with couples with varicocele. This may be due to the accumulation of reactive oxygen species (ROS) caused by the venous stasis close to the sperm production site, though may not have a visible effect on morphology, but may still negatively alter sperm function. Furthermore, patients with varicoceles and an abnormal strict morphology have an overall poorer semen quality.

In a systematic review Castilla et al. [31] investigated the clinical value of the sperm chromatin structure assay (SCSA) and classical semen parameters. They observed that in couples treated with IUI the clinical validity was higher for SCSA compared with sperm morphology, with a positive likelihood ratio (LR+) of 6.1 (95% CI 2.6–14.6) and 1.9 (95% CI 1.1–3.0) for SCSA and sperm morphology, respectively. They also concluded that, despite this finding, the clinical value of SCSA was not enough to introduce this parameter as a routine test in male infertility work up.

Since studies reported in literature have conflicting results there is ongoing debate regarding the advisability of using the partner's sperm for IUI when strict sperm morphology is ≤4%.

SPERM DNA

Around 15% of infertile men have normal sperm analysis according to the WHO 2010. [32] Sperm DNA damage may be the causative factor for infertility in these men. Since there is a high incidence of sperm DNA fragmentation (SDF) seen in the men with unexplained infertility, [33] recently evaluation of SDF in male infertility as an advanced sperm function test is being done along with the routine tests. [34] The importance of the SDF assay has also been stated in the latest American Urological Association[35] and European Association of Urology guidelines on male infertility.

Various factors are responsible for SDF in spermatozoa.

Sperm DNA is wrapped around the histone proteins which are gradually replaced by protamines for the effective condensation of sperm DNA. At times this torsional stress incurred by double-stranded DNA (dsDNA) leads to breaks and nicks in the DNA. Failure to repair the nicks and maintain the proper rearrangement of chromatin results in DNA damage. [36]

Another important cause of sperm DNA damage is ROS generated by immature sperms. ROS damage the sperms DNA as they transit the epididymis by activating the endonuclease or sperm caspases. [37] Sperms with poor chromatin arrangement or with high protamination are also susceptible to ROS attack. In addition, SDF is also seen in epididymal sperms with lower levels of disulphide cross-linking. [38]

Studies have shown a decline in semen parameters and an increase in the SDF after the ages of 35 and 40years, respectively. [39] Other factors like genetic abnormalities, [40] varicocele, [41] and exposure to environmental toxins and pollutants, drugs, chemo-radiation, cigarette smoking can cause increase in SDF.

Indications for SDF assay

SDF assay may be indicated in prolonged idiopathic infertility, implantation failure following IVF, repeated abortions, prolonged exposure to toxic environmental conditions affecting fertility, semen parameters below the reference ranges, advanced male partner age, varicocele patients, and cancer patients.

Tests for SDF

A number of methods have been developed for the analysis of sperm chromatin and DNA integrity.

There are two types of assays that have been developed to measure SDF:

(1) These tests directly measure the extent of DNA fragmentation through the use of probes and dyes aniline/toluidine blue staining and protamine examination by chromomycine A3.
(2) These tests measure the susceptibility of DNA to denaturation, which is often seen in fragmented DNA.

The most commonly used tests are terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), the sperm chromatin dispersion test (SCD), the sperm chromatin structure assay (SCSA), and COMET (single-cell gel electrophoresis) test.

SDF and natural pregnancy

Increased SDF index decreases the chances of natural pregnancy. Chances of pregnancy are bleak when the SDF index is higher than 30%. A meta-analysis involving three studies found that a high SDF assay was associated with failure to conceive with an odds ratio (OR) of 7.01 (95% CI, 3.68–13.36). [42]

Ford et al. [43] have shown in their study that SDF is associated with recurrent miscarriage. Khadem et al. [44] compared 30 couples with recurrent spontaneous abortion to 30 control couples. They observed a high SDF in couples with recurrent spontaneous abortion group than in the control group (43.3% vs. 16.7%, $P = 0.024$). Similar results were obtained in a study done by Absalan[45] ($P \leq 0.05$).

SDF in unexplained infertility

Role of SDF in unexplained infertility and its correlation with conventional sperm parameters has been also studied

recently. [46] Oleszczuk et al. [47] studied DNA fragmentation index (DFI) in 122 men with unexplained infertility. Of them 17.7% of the men (95% CI 10.8–24.5) presented with 20 ≤ DFI <30 and 8.4% (95% CI 3.40–13.4) had DFI ≥ 30%. A significant number of men with unexplained infertility with conventional diagnostic methods had a high degree of fragmented sperm DNA. Men with unexplained infertility may have normal semen parameters with a high SDF index

SDF and IUI success rates

High levels of SDF may be associated with lower pregnancy rates in IUI cycles. Duran et al. [48] evaluated semen samples from 154 IUI cycles. SDF was measured using TUNEL or AO testing. The SDF level was significantly higher among the failed cycles, where no woman inseminated with a sample having >12% of sperm with fragmented DNA, by TUNEL, achieved a pregnancy. In another study Bungum et al. [49] measured SDF using SCSA in 387 IUI cycles. They reported significantly lower biochemical pregnancy (3% vs. 24%), clinical pregnancy (3% vs. 23.7%) and delivery rates (1% vs. 19%) in patients with an SDF index >30% vs. ≤30%, respectively. Yang et al. [50] in their study observed that the pregnancy rate of IUI was significantly lower in couples with DFI >25% than in those with DFI < or = 25%. They said that sperm DFI obtained from SCSA partly correlated with sperm concentration and motility, and it is a robust predictor of the IUI outcome.

Correlation between DNA fragmentation rates and sperm viability was studied by Samplaski et al. [51] They observed that reduced sperm viability was associated with high sperm DNA fragmentation, and vice versa. If viability was ≥ 75% ($n = 1736$), then the DNA fragmentation was ≤ 30% for 95% of the patients. They postulated that since sperm viability correlates strongly with DNA fragmentation rates DFI testing may be not be routinely necessary, given that DNA fragmentation testing is substantially more expensive than vitality testing.

Infertile men with SDF due to modifiable lifestyle risk factor may benefit by lifestyle modification (e.g., cessation of cigarette smoking) or antioxidant therapy. Treatment with oral antioxidant vitamins can decrease formation of ROS and improve fertility. In a study, [52] the DNA fragmentation index and the degree of sperm decondensation were measured using the sperm chromatin structure assay before and after 90 days treatment with antioxidant vitamins associated with zinc and selenium. Antioxidant treatment led to a decrease in sperm DNA fragmentation (–19.1%, $P < 0.0004$), suggesting that at least part of the decay was linked to ROS.

SPERM VITALITY

The two tests performed to assess sperm vitality are the Hypo-osmotic swelling test and the sperm vitality staining.

Hypo-osmotic swelling test

Hypo-osmotic swelling test (HOST) is based on the ability of live spermatozoa to withstand moderate hypo-osmotic stress. HOS-reacted sperms can be semi-quantitatively and subjectively graded as grade A to G based on the amount of swelling and curling of the tails. Percentage of each grade can be scored and reported as a percentage after counting 200 sperms. More than 60% HOS-reacted sperms are considered as normal and abnormal if <50% show tail curling. Scores between 50% and 60% are considered intermediate. When correlated with DNA fragmentation status using HOS and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL), sperms grades D, E, and F showed significantly less DNA fragmentation compared to grades A, B, and C within each of the total sperm population. [53] Correlation is evident in both types of samples those showing normal HOS and abnormal HOS scores. A study reported that low HOS values of neat semen samples were notably ($P < 0.001$) correlated with increased DNA damage identified by the DNA fragmentation index. DFI was assessed by sperm chromatin structure assay and TUNEL assay. The HOST value was highly predictive of an abnormal DFI value by receiver operating characteristic curve analysis ($P < 0.001$). [54] HOS can be generally used as an additional indicator of sperm vitality and can be used to diagnose spermatozoa with immotile cilia syndrome. Presently with the correlation of HOS grading and DNA fragmentation status, HOS is a reliable indicator for selection of spermatozoon for selection in ICSI.

Sperm vitality staining

The sperm vitality staining test measures the proportion of spermatozoa that are "alive." It is based on the ability of sperm plasma membrane to exclude extra-cellular substances like dyes and is done in semen samples with <50% motile spermatozoa. Plain eosin stain is used. Live spermatozoa do not take up stain whereas dead spermatozoa show degree of pink or red stain. Vitality assessment also provides check on the accuracy of motility assessments; as the percentage of live spermatozoa should

slightly exceed the total percentage of motile spermatozoa. Spermatozoa stained with this kit cannot be used for any further procedures.

TIME INSEMINATION

An observational study[55] was carried to determine whether semen parameters (concentration, motility) were affected by the interval between the onset of postwash sperm incubation and IUI time. They observed that there were significant differences in values of mean sperm count, percent progressive sperm motility, and total motile sperm count between 30 minutes and 120 minutes ($P = 0.000$, $P = 0.000$, and $P = 0.000$) and between 60 minutes and 120 minutes ($P = 0.000$, $P = 0.000$, and $P = 0.001$), but there was no significant difference between 30 minutes and 60 minutes ($P = 1$, $P = 0.173$, and $P = 1$). A maximum 60-minute limit of the interval between the onset of post-wash sperm incubation and IUI time may increase pregnancy rates

ABSTINENCE

Traditionally it was said that semen samples must be given within 2–7 days of abstinence. In a study it was observed that an abstinence interval of 3 days or less was associated with higher pregnancy rates following IUI. [56] Prolonged abstinence was associated with a decrease in pregnancy rates. This may be due to sperm senescence and functional damage which is not easily identified by routine semen analysis. A study was conducted to assess the effect of ejaculatory abstinence (EA) periods on routine and advanced sperm tests in men with normozoospermia. [57] A standard semen analysis and advanced sperm test for assessing the levels of ROS and sperm DNA fragmentation was performed. Comparison was made by grouping EA periods into short (1 day), recommended by World Health Organization (WHO) (2–7 days), and long (9–11 days). It was observed that the semen volume ($P < 0.001$), sperm concentration ($P < 0.001$), and total sperm count ($P < 0.001$) increased significantly with abstinence length. However an increase in sperm DNA fragmentation was also seen with increase in the length of EA ($P < 0.001$). Both 1 and 2 days of EA had the least amount of DNA fragmentation ($P < 0.001$). Significant increase was seen in volume, pH, viscosity, total count, total motile sperm, and DNA fragmentation between short and recommended EA ($P < 0.05$), and between recommended and long EA ($P < 0.05$). Short EA had no detrimental impact on semen characteristics according to the 2010 WHO thresholds and is proposed as a method for reducing sperm DNA fragmentation. Bahadur et al. [58] did a study to evaluate semen characteristics of 73 subfertile oligozoospermic men with short abstinence periods up to 40 min. Semen characteristics compared between initial and consecutive ejaculate showed improved sperm sperms concentration (10 million/ml and 17 million/ml), higher median progressive motility (25% versus 43%, $P < 0.001$) and a higher median normal morphology (6% versus 7%, $P < 0.001$) respectively. They concluded that semen analyses of consecutive semen samples collected 30 min (mean) apart can be done in oligozoospemic men.

PREPARATION

Semen preparation procedures have an influence on the quality of inseminate. In a study[59] it was seen that mean sperm motility after semen preparation improved significantly with two-layer density-gradient and swim-up compared with whole semen ($65.6\% \pm 4.0\%$ and $73.0\% \pm 3.0\%$ versus $52.0\% \pm 3.6\%$, respectively, $P < 0.005$). There was no significant difference in motility between Percoll-treated and swim-up-treated spermatozoa however the percentage of sperms with denatured DNA was reduced significantly in swim-up-treated but not in Percoll-treated spermatozoa compared with whole semen ($4.8\% \pm 1.2\%$ and $13.6\% \pm 3.6\%$ versus $10.1\% \pm 2.3\%$, respectively, $P < 0.0001$). They concluded that although density-gradient centrifugation was comparable to swim-up technique in recovering spermatozoa with enhanced motility, spermatozoa recovered after swim-up possess higher DNA integrity. This increase in SDF was also found to be directly related to higher force and longer duration of centrifugation and to the type of Percoll gradients used. In an another study[60] the authors observed that both swim-up and DGC yielded a significantly lower sperm deformity rate and DFI in comparison to unprocessed whole semen, with DGC having more favorable results.

Zirbi et al. [61] did a study to evaluate the effect of cryopreservation on sperm motility and viability and to assess sperm DNA fragmentation and oxidation in men undergoing infertility investigation before and after cryopreservation in liquid nitrogen. They observed a significant decrease in sperm motility and viability and an increase in sperm DNA fragmentation and DNA oxidative damage after cryostorage.

Current standards in sperm preparation have proposed several techniques to reduce this impact on SDF such as short incubation time, [62] room temperature storage, [63] and addition of antioxidants to culture media.[64]

CONCLUSION

Semen parameter is an important predictor of the outcome IUI. Sperm concentration, motility, morphology, and the number of motile spermatozoa inseminated determine the successful pregnancy rate. Sperm DNA fragmentation in an emerging important parameter, especially in unexplained infertility and recurrent abortions. Advanced sperm function tests may be required in addition to the standard semen analysis at times. Research is also ongoing for the ideal test for DFI and ROS in semen samples. Period of abstinence and technique used to process semen also are of utmost importance.

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