Background: Male factor is a predominant cause of infertility. Success rates of intrauterine insemination (IUI) for male factor depend upon minimum semen parameters such as progressive motility > 30%, strict morphology > 4%, total motile count in the native sample (TMSC) > 5 million and inseminating motile count (IMC) > 5 million. Couples with parameters lesser than these are usually advised IVF-ICSI. In developing countries, affordability for IVF is an important deterrent to its widespread utilisation.

Aims: To evaluate pregnancy rates after the use of a pooled consecutive ejaculate in infertile men with semen parameters of < 5 million TMSC, and to compare with pregnancy rates achieved among couples with semen parameters > 5 million TMSC after processing of a single ejaculate.

Settings and Design: Private infertility practice. Retrospective study from Oct 2012 to June 2019.

Methods and Materials: All consecutive patients (n=1979) who underwent IUI in the given study period were included. Patients undergoing donor sperm insemination and low IMC (<1 mill) were excluded from the study. The study group (n=128) included patients in whom the total motile sperm count (TMSC) of the native sample was < 5 million. The control group (n=1851) included patients with normal semen parameters. All participants of the study group with TMSC < 5 million were asked to give a second ejaculate within an hour or two of producing the first. The pooled consecutive ejaculates were used for sample processing and insemination. The primary outcome measure was clinical pregnancy rate. The secondary outcome measures were semen quality of the second ejaculate, TMSC and IMC of both groups.

Statistical Analysis: Quantitative parameters were compared using Independent sample t-test and Mann Whitney u test. Multivariate binary logistic regression analysis was performed to test the association between the explanatory variables and outcome variable. P value < 0.05 was considered statistically significant.

Results: 6.47% (128/1979) of men were required to give a consecutive ejaculate. The initial ejaculate had significantly higher volume (2ml vs 1 ml; P < 0.001); but lower concentration (8 million/ml vs 19 million/ml; P <0.001) and lower progressive motility (25% vs 35%; P <0.001) in comparison with the second. The final IMC of the pooled ejaculate was 9.01 million vs 21.6 million in the control group (P<0.001). The clinical pregnancy rate was comparable between the control group and the consecutive ejaculate group (15.4% vs 15.63%; P =0.94).

Conclusion: Consecutive semen samples produced immediately after the
first have significantly lower volume but higher sperm count and progressive motility. In couples with moderate male factor infertility with semen parameters inadequate for IUI, pooled consecutive ejaculates yield higher inseminating motile counts which enhance IUI success rates.

**KEYWORDS:** Consecutive ejaculate, male factor infertility, intrauterine insemination

**INTRODUCTION**

Male factor infertility accounts for nearly 40%–45% of the global burden of infertility.[1] The options for the management of male factor include medical or surgical therapy, intrauterine insemination (IUI), and *in vitro* fertilization (IVF) – intracytoplasmic sperm injection (ICSI). IUI is a simple, cost-effective, and less invasive modality of treatment requiring minimal infrastructure compared to IVF-ICSI. Recent evidence from well-designed randomized trialsstate that IUI offers acceptable cumulative live birth rates in three to four cycles compared to IVF and can be a preferred cost-effective first-line treatment in mild male factor or unexplained infertility, especially so in low-resource settings.[2-5] Large observational studies have strengthened and established the fact that IUI is more cost effective than IVF in delivering one live birth and is associated with lower risk of multiple pregnancy, maternal, and neonatal complications.[6] In the light of emerging evidence, it must be reiterated that, in view of its efficacy, safety, and cost-effectiveness, IUI should be offered before IVF where permissible and indicated.

The success rates of IUI depend on several factors such as female age, ovarian reserve, duration of infertility, factor causing infertility, stimulation protocol, number of follicles, semen processing method, and quality of the processed semen sample.[7-9] Several studies have evaluated the accuracy of semen parameters in the prediction of IUI success. A recent systematic review suggested that, based on Level 2 and 3 evidence, parameters such as inseminating motile count (IMC) >1 million/ml, sperm morphology >4%, total motile sperm count (TMSC) in native sample >5–10 million, and progressive motility (PM) >30% are important and significant discriminatory parameters in any IUI program.[10] The success rates of IUI appear to be significantly improved above these threshold values. Infertile men with IMC <1 mill/ml or TMSC <5 million are generally advised IVF.[10]

The WHO guidelines recommend an abstinence period of 2–7 days to ensure the best quality of the semen sample.[11] As a convention, this rule has been followed for all infertile couples undergoing evaluation or treatment. However, in 1990, it was surprisingly discovered that, when men were asked to produce a second sample within 1 or 2 h of ejaculation, reasonably good or in fact better samples were obtained; questioning the time honored correlation between abstinence and semen quality.[12] Improvements in either sperm count[13-15] or PM[16-20] in consecutive ejaculates in oligospermic men have been reported in many subsequent publications. This combined and clear defining evidence questions the fundamental concepts in andrology and may pave the way for modified practice in future.

In men with moderate male factor who are unable to meet the minimum requirements for IUI, taking a second consecutive ejaculate may help to enhance the numbers above the threshold and enhance their IUI success. It has been observed that no pregnancy resulted when the prewash TMSC was <5 million[21] or <9 million[22] and that pregnancy rates increased when TMSC increased over 10 million.[23] Many studies have reported lower pregnancy rates when the postwash IMC was <5 million.[24-26] Hence, it was hypothesized that men with moderate male factor infertility with a TMSC <5 million or IMC <5 million would have lower or no successful pregnancy with IUI. In such men, a consecutive ejaculate immediately after the first would enhance the inseminating sperm count and consequently IUI success rates.

There have been five published studies till date evaluating the benefit of a consecutive ejaculate in oligozoospermic men to improve IUI outcome.[12,27-30] Most of these studies have small sample sizes and varying inclusion criteria and just three mentioning pregnancy rate as an outcome. Inclusion criteria are inconsistent and include IMC <1 million, IMC 1–5 million, TMSC <10 million, and TMSC <4 million. Evidence from these studies is conflicting with two studies showing benefit with this approach and one showing no increase in success rates. This large retrospective study was hence planned in view of inconclusive observations in prior studies. The main objective of the study was to evaluate pregnancy rates after the use of pooled consecutive ejaculate in infertile men with semen parameters of <5 million TMSC and to compare with pregnancy rates achieved among couples with semen parameters >5 million TMSC after processing of a single ejaculate.

**MATERIALS AND METHODS**

This was a retrospective study conducted in a private
infertility setting from October 2012 to June 2019. The study was approved by the Institutional Review Board and the Ethics committee of the institute. Informed consent was obtained from all couples. All consecutive patients (n = 1979) who underwent IUI in this study period were included. Patients who underwent donor sperm insemination and those in whom the IMC was <1 million were excluded from the study. The study group (n = 128) included patients who had a TMSC in the native sample of <5 million and had at least one patent fallopian tube. The control group (n = 1851) included patients with normal semen parameters. The indications for IUI in this group were unexplained infertility, anovulation, mild endometriosis, or nonconsummation.

All women underwent a baseline scan on day 2 or 3 of the menstrual cycle to rule out follicular cysts and to assess antral follicle count. They underwent mild ovarian stimulation with letrozole, clomiphene, combination of letrozole, and gonadotropins or only gonadotropins. They underwent follicular monitoring till the dominant follicle reached a diameter of 17–18 mm. A maximum of 2–3 dominant follicles were allowed, and IUI was cancelled if follicular number was >3 to avoid multiple pregnancy. They received urinary human chorionic gonadotropin trigger 10,000 i.u. (Fertigyn, Sun Pharma) and a single IUI was performed 36–38 h posttrigger. A single clinician performed all the IUIs using a soft catheter under ultrasound guidance.

A single ejaculate was assessed for sperm count, PM, and morphology. TMSC (volume × concentration × PM) was calculated. If TMSC was <5 million, they were asked to produce a second ejaculate within an hour or two of the first. The first sample was placed at 37°C in an incubator till the second was obtained. The semen parameters of the first, second, and pooled ejaculate were determined. The pooled consecutive (first and second) ejaculates were used for sample processing. All the samples in the study period were processed using density gradient method. The final IMC of the processed sample was calculated. The primary outcome measure was clinical pregnancy rate. Clinical pregnancy rate was defined by the presence of fetal heart beat at 6–7-week gestation. The secondary outcome measures were comparison of semen parameters of the first and second ejaculate and TMSC and IMC of both groups.

Statistical analysis
Descriptive analysis was carried out by mean and standard deviation for quantitative variables; frequency, and proportion for categorical variables. Nonnormally distributed quantitative variables were summarized by median and interquartile range (IQR). Data were also represented using appropriate diagrams such as bar diagram, pie diagram, and box plots.

All quantitative variables were checked for normal distribution within each category of explanatory variable using visual inspection of histograms and normality Q-Q plots. Shapiro–Wilk test was also conducted to assess normal distribution. Shapiro–Wilk test P > 0.05 was considered as normal distribution.

For normally distributed quantitative parameters, the mean values were compared between study groups using independent sample t-test (2 groups)/ANOVA (>2 groups). For nonnormally distributed quantitative parameters, medians and IQR were compared between study groups using Mann–Whitney U-test (2 groups)/Kruskal–Wallis test (>2 groups).

Categorical outcomes were compared between study groups using Chi-square test/Fisher’s Exact test (If the overall sample size was <20 or if the expected number in any one of the cells is <5, Fisher’s exact test was used).

Multivariate binary logistic regression analysis was performed to test the association between the explanatory variables and outcome variable (pregnancy). Unadjusted odds ratio (OR) along with 95% confidence interval (CI) is presented.

P < 0.05 was considered statistically significant. IBM SPSS version 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY) was used for statistical analysis.

Results
A total of 1979 couples were included in the final analysis. Thousand eight hundred and fifty-one (93.53%) couples were in the control group. One hundred and twenty-eight men (6.47%) had an initial TMSC <5 million and were required to produce a second ejaculate and this comprised the consecutive ejaculate group.

Both the groups were comparable with respect to their demographic characteristics. A list of confounding variables was created from published literature and these variables were compared between both the control group and consecutive ejaculate group. The mean age in control group was 30.11 ± 3.94 years versus 29.51 ± 4.09 years in consecutive ejaculate group (P = 0.102). The mean body mass index (BMI) in control group was 25.97 ± 4.56 and 25.77 ± 4.67 in consecutive ejaculate group (P = 0.69). The mean follicles in control group were 1.84 ± 1.01 and 1.91 ± 1.05 in consecutive ejaculate group (P = 0.468). The mean endometrial thickness (mm) in control
Gurunath, et al.: Consecutive ejaculates improve IUI success

The semen characteristics of the first and consecutive ejaculates were compared. It was observed that volume of the consecutive ejaculate was significantly lower than the first (2 ml vs. 1 ml; \( P < 0.001 \)). Surprisingly, the consecutive ejaculate showed a statistically significant increase in concentration, total motility, and PM compared to the first ejaculate (\( P < 0.005 \) [Table 2].

Comparing the semen characteristics between the control group and the first ejaculate, it was observed that semen volume, sperm concentration, total motility, PM, and TMSC were significantly higher in the control group (\( P < 0.005 \) [Table 3]. The TMSC of the native sample in the control group was significantly higher 51.39 ± 37.46 million as against 4.63 ± 3.81 million in the first ejaculate (\( P < 0.001 \)).

With the combination of the first and the consecutive ejaculates, the final processed sample was derived, and its IMC was calculated. Comparing the final IMC of the control group and the consecutive ejaculate group, the mean IMC in control group was 21.68 ± 10.19 million and 9.01 ± 6.42 million in the consecutive ejaculate group. The difference in IMC between two groups was statistically significant (\( P < 0.001 \)) [Figure 1].

The primary outcome measure was clinical pregnancy rate. Two hundred and eighty-five out of 1851 (15.4%) women achieved a clinical pregnancy in the control group and 20/128 (15.63%) in the consecutive ejaculate group. The difference in clinical pregnancy rate between the two groups was not statistically significant (\( P = 0.945 \)) despite the fact that the consecutive ejaculate group had a lower IMC of 9.01 million [Figure 2].

Multivariate logistic regression analysis was done to evaluate the impact of potential confounders. After controlling for the effect of all the potential confounders, the odds of pregnancy were 0.827 times in consecutive ejaculate group as compared to control group (adjusted OR: 0.827, 95% CI: 0.409–1.672, \( P = 0.827 \)), which was statistically not significant.

### DISCUSSION

The results of our current study indicate that a consecutive semen sample produced within an hour or two of the first has lower volume but significantly higher sperm count and PM compared to the first sample. Combination of the first and consecutive ejaculates in oligoasthenoteratozoospermic men yields notably higher IMC in the processed samples. Utilization of such pooled consecutive ejaculates in men who do not meet the minimum requirements of sperm adequacy for IUI is found to improve IUI success rates similar to normozoospermic men. Our findings reaffirm earlier studies that pooled consecutive ejaculates in IUI are a simple and noninvasive intervention to overcome moderate male factor infertility and enhance IUI success rates which is a safer and cost-effective treatment option in comparison with IVF. It may be reasserted that IUI must be offered to any infertile couple before IVF wherever allowable and indicated. This practice would benefit infertile couples in resource-poor countries with inadequate access to fertility treatment.

It has been a time-honored practice to recommend an abstinence period of 2–7 days for optimal semen quality

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**Table 1: Comparison of different baseline parameters between study groups (\( n=1979 \))**

| Parameter                              | Control group (\( n=1851 \)) | Consecutive ejaculate (\( n=128 \)) | \( P \) |
|----------------------------------------|------------------------------|-------------------------------------|--------|
| Age (\( n=1906 \))                     | 30.11±3.94                   | 29.51±4.09                          | 0.102  |
| BMI (\( n=1026 \))                     | 25.97±4.56                   | 25.77±4.67                          | 0.690  |
| Follicles (\( n=1669 \))              | 1.84±1.01                    | 1.91±1.05                           | 0.468  |
| Endometrial thickness (mm) (\( n=1659 \)) | 8.54±1.76                   | 8.69±1.88                           | 0.383  |

SD=Standard deviation, BMI=Body mass index

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**Figure 1:** Comparison of inseminating motile count between study groups (\( n=1979 \))
and this has been endorsed by the latest WHO guideline published in 2010. It has been presumed that an abstinence of <24 h produces lower sperm counts. Consequently, men who produce suboptimal samples on the day of planned fertility treatments such as IUI are denied the same and advised more complicated and expensive treatments such as IVF-ICSI. However, in contrast to the existing dogma, some researchers did observe that a second consecutive ejaculate resulted in increased sperm count in oligospermic men compared to the first ejaculate. Increase in PM without increase in sperm count was also noted by some. Reported evidence is rather conflicting, and a similar improvement in parameters was not seen in all populations with some publications reporting reduction in total sperm count and volume and no improvement in concentration, PM, morphology, and DNA fragmentation index with daily ejaculation. In our study, we observed a significantly lower volume but much higher sperm concentration and PM in the second consecutive ejaculates.

IUI success rates have been found to be lower in male factor infertility when the TMSC is <5–10 million. There have been five publications attempting to address whether a consecutive ejaculate would improve IUI outcome. There were two retrospective studies, two prospective nonrandomized studies and one randomized trial. The first published study was by Tur-Kaspa et al. in 1990. They included 16 normospermic and 18 oligospermic men and observed improved TMSC in the pooled samples. They however did not demonstrate an improved count or motility in the second ejaculate in oligospermic men, and pregnancy outcome was not mentioned. Ortiz et al. included 32 patients with sperm count <5 mill/ml, PM <30%, and TMSC <4 mill and pooled two ejaculates for IUI. They found a significantly higher count, PM, and TMSC in the second ejaculate and obtained a pregnancy rate of 9.3%. This study lacked adequate sample size and had no control group to compare its observations. Bahadur published a prospective study, in which men with an initial TMSC of <10 million were asked to produce a consecutive ejaculate and they observed a similar pregnancy rate in both groups. A retrospective study by Moreau J including 2066 controls and 96 oligospermic men was published in 2018. The study group included 96 men in whom the IMC was <1 million, and they were asked to produce a second ejaculate. The target IMC of >1 mill/ml could be achieved in 82% of the study group using the second ejaculate. Although the IMC of the pooled ejaculate was significantly lower, similar pregnancy rates were achieved in both groups. The only randomized controlled trial was published by Küçük et al. in 2008. They included 89 patients who had an IMC between 1 and 5 million in postwash samples. Thirty-nine were

| Parameter       | Median (IQR) | Wilcoxon test (P) |
|-----------------|--------------|-------------------|
| Volume (ml)     | 2 (1.2-2.57) | 1 (0.60-1.95)     | <0.001 |
| Concentration   | 8 (5-12)     | 19 (10-34.75)     | <0.001 |
| Motility        | 40 (28-50)   | 49 (37.2-54.7)    | <0.001 |
| Progressive     | 25 (18-36.75)| 35 (25-43)        | <0.001 |

IQR=Interquartile range

| Parameter       | Study group (mean±SD) | P     |
|-----------------|-----------------------|-------|
| Abstinence      | 2.58±1.47             | 2.86±2.9 | 0.076 |
| Volume (ml)     | 2.36±1.22             | 2.1±1.29 | 0.018 |
| Concentration   | 49.69±23.71           | 11.22±12.38 | <0.001 |
| Motility        | 55.45±8.54            | 38.58±15.58 | <0.001 |
| Progressive     | 44.31±9.14            | 26.52±13.12 | <0.001 |
| Concentration   | 44.31±26.47           | 18.54±12.98 | <0.001 |
| Motility (%)    | 98.4±3.47             | 96.92±6.29 | <0.001 |
| Total motile sperm count | 51.39±37.46 | 4.63±3.81 | <0.001 |

SD=Standard deviation
randomized to the study group where they obtained a second ejaculate. Fifty men in the control group gave a single sample. They found the pooled ejaculate to have a significantly higher TMSC. The study group had a pregnancy rate of 15.3% versus 10% in the control group which was not statistically different, and the study may have been underpowered.[27] The inclusion criterion in most groups is varied, and sample sizes are small. Using IMC as an inclusion criteria delays the time from sample collection to insemination in view of the time spent in collection and processing the second ejaculate after the first sample is processed. Although most studies demonstrate a higher IMC with the pooled ejaculate, few have not mentioned impact of the intervention on pregnancy rate nor do they have a control group.

The strength of our study is that the sample size is fairly large and inclusion of a control group with normospermia in comparison. We used an inclusion criterion of TMSC <5 million as this is a simple calculation that combines multiple parameters such as volume, count, and PM and reduces the time delay from collection to insemination. The limitations are the retrospective design and the absence of comparison with an oligospermic group with same inclusion criteria using just one ejaculate for insemination. A larger sample size may have improved the quality of the evidence. A formal cost-effectiveness analysis was not performed. However, there is ample evidence from large observational and randomized studies that IUI is more cost effective than IVF in achieving one live birth. In the UK, at baseline tariffs and success rates, IUI was £42,558 cheaper than IVF to deliver one live birth with enhanced benefits.[6]

This practice can be extended to other areas in reproductive medicine as in IVF. Enhanced sperm counts or progressively motile sperm in second ejaculate could assist the embryologist to obtain more motile sperm for ICSI in severe oligoasthenoteratozoospermia, perform conventional insemination in IVF rather than to resort to invasive micromanipulation such as ICSI, and avoid surgical sperm aspiration. There is some evidence that shorter abstinence times may yield samples with lower DNA fragmentation.

**Implications for future research**

The results of the current study justify two research objectives. The first is a randomized trial comparing single versus pooled ejaculates to assess live birth outcomes in IUI. The second research question is to compare live birth rates and cost-effectiveness of IUI with consecutive ejaculates and IVF-ICSI.

**Conclusions**

Oligospermic men who do not meet the minimum requirements for sperm adequacy for IUI can benefit by being asked to produce a second consecutive sample within 1 h of the first. The consecutive ejaculate usually has a lower volume but significantly higher count and PM. The use of pooled consecutive ejaculates enhances the IMC of the processed sample for IUI. This improves the IUI success rates similar to men with normozoospermic samples. This simple intervention can be attempted, especially in resource-poor settings before an early referral for IVF. These findings prompt us to question the association between abstinence time and semen quality. Our findings reaffirm earlier studies that pooled consecutive ejaculates in IUI are a simple and noninvasive intervention to overcome moderate male factor infertility and enhance IUI success rates which is a safer and cost-effective treatment option in comparison with IVF.

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**Conflicts of interest**

Sumana Gurunath is currently associate editor of JHRS.

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**Table 4: Factors associated with pregnancy in study population multivariate logistic regression analysis (n=1979)**

| Factor                      | Adjusted odds ratio | 95% CI of OR  | P     |
|-----------------------------|---------------------|---------------|-------|
| Study group (baseline=control) |                     |               |       |
| Consecutive ejaculate       | 0.827               | 0.409-1.672   | 0.827 |
| Age                         | 0.983               | 0.940-1.028   | 0.442 |
| BMI                         | 1.055               | 1.017-1.094   | 0.004 |
| Follicles                   | 1.140               | 0.964-1.348   | 0.124 |
| Endometrial thickness (mm)  | 1.057               | 0.954-1.172   | 0.288 |
| IMC                         | 1.012               | 0.995-1.029   | 0.174 |

CI=Confidence interval, OR=Odds ratio, IMC=Inseminating motile count.
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