

## Original Article

### Pooled sequential ejaculates for intrauterine insemination- A prospective randomized study on infertility due to oligospermia in young couples

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#### Abstract

**Background:** The use of pooled sequential ejaculates is a minimally-invasive, cost-effective and yet underutilized technique of increasing total motile sperm counts for intrauterine insemination (IUI) before opting for expensive and unnecessary infertility treatments. At present, no guidelines are available regarding the use of pooled sequential ejaculates for IUI in cases with very low seminal parameters; therefore, we speculated that the use of pooled sequential ejaculates for IUI in patients with male factor infertility may be an efficient technique for achieving a successful pregnancy. The aim of this study is to assess the efficiency of the pooled semen technique by comparing the outcomes of IUI using a single semen sample versus pooled sequential ejaculates in young patients with severe oligospermia.

**Materials and Methods:** Young couples with only male factor infertility were randomly assigned single semen or pooled sequential ejaculates technique for a single cycle of IUI and analyzed. We strictly included males aged 25-30 years with sperm concentration < 6 million/mL, total motile sperm count (TMSC) < 10 million and sperm motility < 50%. The second ejaculate was obtained within two hours of the first ejaculate.

**Results:** Pooling the sequential ejaculates significantly increased the TMSC for insemination ( $P < 0.0001$ ) compared with the single semen sample group. There were 5 successful pregnancies in the pooled sequential ejaculate group (pregnancy rate 33.3%, OR = 1.58; 95% CI 1.04, 2.41) as compared to 1 in the single semen sample group. The receiver operating characteristic analysis showed that the number of TMSC for IUI has a good predictive value for a successful pregnancy outcome in young couples with only male factor infertility (AUC = 0.86, p-value = 0.06).

**Conclusion:** Pooled sequential ejaculates for IUI is an effective and inexpensive minimally-invasive treatment for infertility due to severe oligospermia in young couples.

**Keywords:** Intrauterine Insemination, Male Factor Infertility, Sperm Count, Oligospermia, Ejaculate, Pregnancy

#### Introduction

Infertility is an increasingly major problem of the modern world, affecting nearly 15% of sexually active couples worldwide.<sup>1</sup> It is estimated that infertility due to male factors ranges from 20-70% with

30 million infertile males around the globe.<sup>2</sup> Several factors such as environmental and lifestyle changes have been related to the cause of male infertility and reduction in sperm counts over the years.<sup>3</sup>

Patients attending the infertility clinics diagnosed with male factor infertility are often referred for expensive treatments such as In Vitro Fertilization (IVF) or Intra Cytoplasmic Sperm Injection (ICSI). The overuse and the cost of these treatments contribute to the psychological and financial stress for the patients especially in developing countries where health insurance programs are either inexistent or do not provide major support for the health costs. Intrauterine Insemination (IUI) is a simple, non-invasive

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technique that does not require expensive infrastructure and provides successful results within a few cycles. It is observed that due to the wide promotion of IVF and ICSI for 'quick pregnancy', the advantages of IUI are indeed ignored.<sup>4</sup>

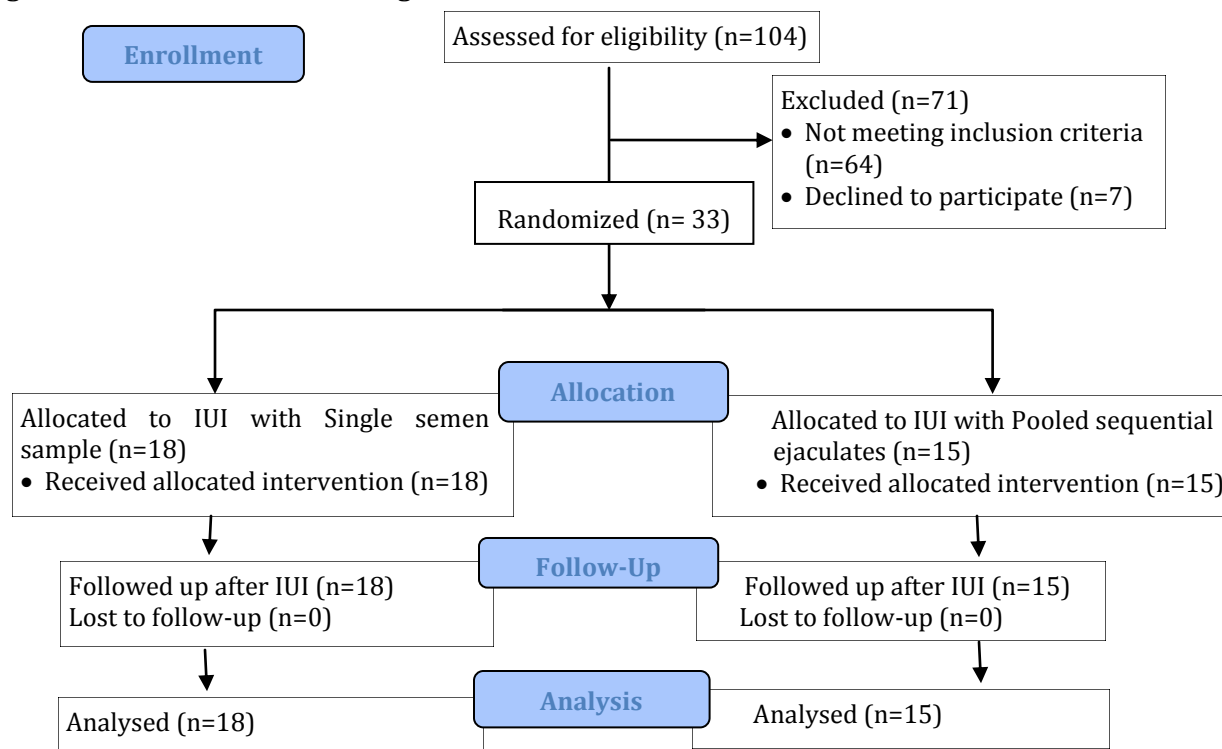
Studies have revealed that there is a linear relationship between the total motile sperm count and the success rate for IUI.<sup>5,6</sup> Therefore, IUI may not be opted as a treatment of choice for male infertility due to severe oligospermia. It has been reported that using pooled sequential ejaculates is a simple and cost-effective method to increase the total motile sperm count for IUI, IVF, or cryopreservation.<sup>7,8</sup> However, no guidelines are currently available regarding the use of pooled sequential ejaculates for IUI in cases with very low seminal parameters. We speculated that using pooled sequential ejaculate for IUI in cases of male factor infertility might be an efficient way to achieve successful pregnancies without any increase in the financial burden for the patient. This study aims to assess the efficiency of pooled semen by comparing the outcomes of IUI using a single semen sample versus pooled sequential ejaculates in young patients with low semen quality and to analyze the predictive value for a successful pregnancy using pooled semen in cases of male factor infertility due to oligospermia.

## Materials and Methods

A prospective randomized study was conducted in a tertiary care hospital in India between the years 2016-2018. Written informed consent was obtained from all the patients and ethical approval was obtained. We investigated 104 couples attending our Infertility Center and 40 patients were found eligible for this study. Those couples diagnosed with only male factor infertility according to the World Health Organization (WHO) guidelines<sup>9</sup> and, undergoing IUI for the first time were included in the study. Inclusion criteria for the males enrolled in this study was 1) sperm concentration < 6 million/mL, 2) total motile sperm count < 10 million, 3) sperm motility < 50% and 4) age < 30 years. We excluded cases with teratozoospermia, associated female factor infertility, uterine anomaly, pelvic inflammatory disease or infection, tumor, and patients with a previous history of receiving other infertility treatments.

Seven patients did not consent to this study and were excluded. The data and results from the remaining 33 patients are presented in this study Figure 1. A total of 33 patients were randomly assigned single semen or pooled sequential ejaculates technique for a single cycle of IUI using a computer program to generate a random allocation sequence.

**Figure 1: CONSORT 2010 Flow Diagram**



A detailed medical history was obtained from all the enrolled couples. The male partners were well counseled and informed to maintain the abstinence period of 5-8 days from the anticipated day of IUI. Semen samples were collected at the end of the abstinence and semen values were determined according to the WHO guidelines.<sup>9</sup> 18 patients were requested to provide a single semen sample for the IUI. The sequential ejaculate was obtained from the 15 male patients within two hours of the first ejaculate. Both the ejaculates were processed separately by using a single-layer density gradient (40%) and centrifugation at 2000 rpm for 7 minutes. Sperm pellets were washed twice with normal media by centrifugation at 2000 rpm for 3 minutes followed by resuspension of sperm pellet with normal media. Finally, two times washed sperms were resuspended in 0.5 ml glucose enriched (3%) media which showed a sperm survival of more than 24 hours. The sperms were then used for IUI by the normal conventional method. The two preparations were pooled before insemination and the final inseminated volume was maintained as 0.5 ml in glucose enriched semen preparation media. The female partner was given Clomiphene citrate 100 mg once a day for five days beginning from day 2 post menses. The follicular development was carefully monitored by ultrasound and human chorionic gonadotrophin (HCG) trigger 5000 IU was given when more than two follicles attained a size of more than 18 mm and an endometrial thickness of 10-12 mm with a triple layer differentiation. The IUI was performed post-ovulation using the glucose enriched media. Post IUI estrogen was given 2 mg thrice a day. The pregnancy test was confirmed after two weeks from the day of IUI.

The distribution of seminal parameters is not normal; therefore, the data are expressed as median (range) values. Significant differences were assessed using the Mann-Whitney U test for the continuous variables and Fisher's exact test for categorical data. The odds of achieving a successful pregnancy with pooled sequential ejaculates were analyzed using a binomial logistic regression model. Patient factors for males included age, history of smoking, and alcohol along with the number of follicles as a treatment factor for females. The receiver operating characteristic (ROC) analysis was also used to evaluate whether increasing the total motile sperm counts with pooled ejaculates could predict successful pregnancy outcomes after IUI. A P value < 0.05 was considered significant. The statistical analysis was performed with Jamovi statistical analysis software.

## Results

The patient demographics, semen characteristics, and the results of IUI between the single semen sample and pooled sequential ejaculate groups are outlined in Table 1. The patient age ranged between 25-30 years for both groups. In the pooled ejaculate group, more than 50% of the male patients smoked tobacco and consumed alcohol regularly. However, there was no significant difference in the history of smoking and/or alcohol between the two groups. The female partners in both groups did not report any past or present history of alcohol/tobacco consumption.

In the single semen sample IUI group the median of the volume of ejaculate was 4.4mL, range (3.2-5.0); sperm concentration  $4 \times 10^6$ /mL, range (2-6); progressive motility 40.5 %, range (30-50) and normal morphology 58.5%, range (51-70). In the pooled sequential ejaculates group the median of the volume of the first ejaculate was 4.1mL, range (3.1-5.0); sperm concentration  $4 \times 10^6$ /mL, range (2-6); progressive motility 44%, range (31-50) and normal morphology 67%, range (50-78).

There was no significant difference between the total motile sperm count (TMSC) of the first ejaculate between both the groups. The median of the TMSC of the second ejaculate in the pooled ejaculate group was  $4.1 \times 10^6$ , range (2-5.5). However, pooling the sequential ejaculates in this group significantly increased the TMSC for insemination ( $P < 0.0001$ ) compared with the single semen sample group.

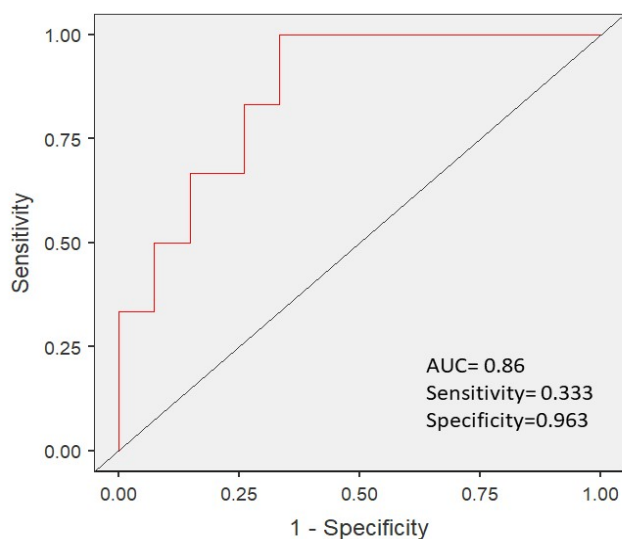
On ultrasound examination on the day of IUI it was observed that in most females, 2-3 good follicles had developed with a size of more than 18 mm following our treatment protocol. There were 5 pregnancies in the pooled sequential ejaculates group (pregnancy rate = 33.3%) whereas, in the single semen sample group, there was only 1 pregnancy (pregnancy rate = 6%) following the first cycle of IUI ( $P = 0.07$  by Fisher's exact test). Increasing the total motile sperm count for insemination was associated with an increased odds of a successful clinical pregnancy (OR = 1.58; 95% CI 1.04, 2.41). Figure 2 shows the ROC analysis indicating that the number of total motile sperm counts for insemination in a model consisting of baseline characteristics for the male patient i.e age, history of smoking and alcohol, and treatment factor for the female partner i.e number of follicles has a good predictive value for successful pregnancy following IUI (AUC = 0.86, P-value = 0.06).

**Table 1. Patient demographics, semen characteristics and results of IUIa**

	IUI (single sample) (n = 18)	IUI (pooled samples) (n = 15)	P-value
Female age (years)	25 [22-30]	26 [22-29]	0.32
Male age (years)	28.5 [25-30]	28 [25-30]	0.30
H/O smoking in female, n (%)	0 (0)	0 (0)	NA
H/O smoking in male, n (%)	7 (38.8)	8 (53.3)	0.40
H/O alcohol in female, n (%)	0 (0)	0 (0)	NA
H/O alcohol in male, n (%)	11 (61.1)	9 (60)	0.94
Sperm characteristics- Volume (mL)	4.4 [3.2-5.0]	4.1 [2.9-5.0]	0.21
Sperm concentration (million/ mL)	4.4 [3.2-6.0]	4.6 [2.8-6.0]	0.30
Progressive motility (%)	40.5 [30-50]	44 [31-50]	0.25
Normal morphology (%)	58.5 [51-70]	67 [50-78]	0.09
First sample- TMSC (million)	3.75 [1.8-6.8]	4.7 [2.2-6.2]	0.11
TMSC (million)	-	4.1 [2- 5.5]	NA
TMSC inseminated (million)	3.75 [1.8-6.8]	8.6 [4.2-15.7]	< 0.0001
No. of follicles with size > 18 mm on the day of hCG trigger (n)	2.3 [1.3-3.2]	2.5 [0.8-3]	0.55
Clinical pregnancies, n (%)	1 (6.0)	5 (33.3)	0.07

A Data are presented as medians with range in parenthesis. Abbreviations: IUI, Intra Uterine Insemination; NS, not significant; TMSC, total motile sperm count.

**Figure 2: Receiver operating characteristic analysis for total motile sperm count for intrauterine insemination as a predictor for successful pregnancy in young patients with infertility due to oligospermia. Abbreviations: AUC; area under the curve.**



## Discussion

In the present study, we tested the efficiency of using pooled sequential ejaculates for IUI in young couples with only male factor infertility and poor semen quality. We focused our study on young couples of less than 30 years of age and aimed at minimal interventions with a cost-effective treatment.

The semen characteristics in both the groups in our study were similar with no significant differences. We observed that most of our male patients had a history of smoking and/or alcohol consumption. Smoking and alcohol consumption has been reported to have a severe impact on sperm parameters causing impairment of sperm morphology, concentration, and motility.<sup>10,11</sup> Considering the deleterious effects, we had strongly advised our patients regarding the cessation of these habits to provide the best outcomes of the treatment.

Studies have shown that pooling the ejaculates increases the TMSC significantly which is an essential requirement for an IUI cycle. Tur- Kaspas et al. showed that pooled sequential ejaculates increased the median TMSC by up to 329% in oligospermic males and by 144% in normozoospermic males.<sup>7</sup> In our study also, the TMSC in the final inseminate following pooling of first and second ejaculates was significantly increased and thus compensated for the insufficient sperm counts required for the IUI cycle ( $p < 0.05$ ; paired samples t-test). Moreover, another study reported that sperm concentration and progressive motility in the second ejaculate obtained within one hour of the first ejaculate is significantly increased.<sup>12</sup> In our study, the second ejaculate was obtained within 2 hours of the first collection but compared to the first sample, it did not reveal any significant difference in sperm characteristics.

In this study, we observed a pregnancy rate of 33.3% using pooled sequential ejaculates in the first cycle of IUI. Kucuk et al. in their study reported a pregnancy rate of 15.3% using pooled ejaculates for IUI compared to the single semen sample group.<sup>8</sup> However, they showed that the TMSC in the final inseminate after pooling was significantly increased which was similar to our study. The results of the ROC analysis in our study indicate a good predictive value for a successful pregnancy by increasing the total motile sperm counts. A retrospective study on 2162 consecutive IUI procedures performed on 869 couples reported that the pregnancy rate in pooled ejaculates group was similar to the single ejaculate group; however, the study included cases with female

factor infertility which may have affected the subsequent pregnancy outcome.<sup>13</sup> In our study, we excluded the cases of female factor infertility to study the impact on pregnancy outcomes only by the improvement of male semen parameters which was reflected in our results.

A pregnancy rate of up to 22 % in young women (< 30 years of age) has been reported, with an infertility period of fewer than 4 years and fewer treatment cycles.<sup>14</sup> The pregnancy success rate in our study is high (33.3%) which may be attributed to the following factors;

- 1) We followed strict inclusion criteria and included only young couples between 25-30 years of age without any female factor for infertility,
- 2) The IUI was performed post-ovulation and
- 3) A glucose enriched media (3%) was used which had shown a sperm survival of more than 24 hours. In India, advanced treatments like IVF and ICSI are increasingly offered to most patients, neglecting the simple, less invasive techniques such as IUI which provide similar successful outcomes. IUI is an inexpensive treatment for infertility providing pregnancy rates between 8-22%.<sup>15,16</sup> Our results have shown that good pregnancy rates can be achieved in young couples with severe oligospermia by using pooled sequential ejaculates for IUI. A limitation of this study is the small sample size; which can be attributed to the strict inclusion criteria from a single institution. Our results will encourage similar studies in the future with larger patient groups. To conclude, pooled sequential ejaculates for IUI is an effective and inexpensive minimally-invasive treatment for infertility due to severe oligospermia in young couples.

## Disclosure Statement

The authors have no conflicts of interest to declare.

## Author contributions

D.J. designed the study, collected, analyzed, and interpreted the data and drafted the manuscript; A.S. collected, analyzed, and interpreted the data; A.K.J. interpreted the data, revised the manuscript, and approved the final draft.

## Statement of Ethics

All patients had provided their written informed consent for participation in the study.

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