

# Retrospective Analysis of the First Collection versus the Second Collection in Severe Oligo-asthenoteratozoospermia Cases in Self-Intracytoplasmic Sperm Injection Patients

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ABSTRACT

**Background:** It is well established that high-quality semen can lead to an improved fertilisation rate. Ejaculatory abstinence (EA) certainly can influence sperm quality such as volume, count, motility and morphology. However, very few studies have addressed the influence of EA on intracytoplasmic sperm injection (ICSI) outcome and especially in males with severe oligo-asthenoteratozoospermia (OAT) syndrome. **Aim:** This study was undertaken with the purpose of evaluating the advantage of shorter abstinence period (1-h sequential ejaculation) in males with severe OAT syndrome on total usable embryo rate and thereby emphasising the potential application of consecutive ejaculate. **Study Setting:** This retrospective cohort study consisted of all the infertile couples undergoing ICSI cycle with the indicated seminal characteristics who had consulted the tertiary care hospital between January 2021 and July 2021. **Materials & Methods:** All couples in the study had idiopathic male infertility. Retrospectively, two groups were analysed, i.e., Group A with 56 subjects in which first semen sample was used for ICSI cycle and another Group B with 41 subjects in which second semen sample collected within a shorter abstinence period of 1 h was used. **Statistical Analysis:** The data were descriptively analysed using GraphPad Prism (vs. 9.2). Unpaired *t*-test and analysis of variance test were used to determine the significance.  $P < 0.05$  was considered statistically significant. **Results:** The age of female subjects in Group A was  $29.9 \pm 3.5$  years while it was  $29.4 \pm 3.4$  years in Group B. Similarly, the age of male subjects was  $32.2 \pm 3.6$  years and  $31.9 \pm 4.1$  years in Group A and Group B, respectively, with no statistical differences in any gender between the groups ( $P > 0.05$ ). Apart from initial progressive motility ( $P = 0.004$ ), none of the parameters such as total volume, total sperm count and morphology were significantly different ( $P > 0.05$ ) between Group A and samples of Group B. Similarly, parameters such as volume ( $P = 0.006$ ) and post-wash motility ( $P < 0.001$ ) were significantly different between Group A and samples of Group B. However, there was no significant difference in sperm count and morphology ( $P > 0.05$ ). Grade 1 embryos on day 3 were 345 (62.8%) in Group A and 170 (54.3%) in Group B. Overall, the total usable embryos in Group A and Group B were 222 (40.4%) and 148 (47.3%), respectively ( $P > 0.05$ ).

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**Conclusion:** With regard to compromised sperm parameters, our findings do suggest that the second ejaculate is quite relevant to 'in vitro' reproductive treatments and a simple request for a second consecutive ejaculate (shorter abstinence period of 1 h) could provide the same results in terms of fertilisation. We observed the increased chances of usable embryos in the second ejaculate group.

**KEYWORDS:** *Ejaculatory abstinence, infertility, intracytoplasmic sperm injection, severe oligo-asthenoteratozoospermia*

## INTRODUCTION

Infertility, which itself is a broad term, can be due to underlying disorders in either of the sexes, but approximately 50%<sup>[1]</sup> of infertility cases can be traced to male factors, i.e., 'impaired spermatogenesis'. However, male reproductive health is rarely discussed and is still considered taboo. In 2004, the World Health Organization (WHO) estimated the overall prevalence of primary infertility in India between 3.9% and 16.8%.<sup>[2]</sup> On global terms, the prevalence rate of female infertility increased by 14.96% from 1990 to 2017 marking a shift of 0.37% per year, while the prevalence rate of male infertility over the same period increased by 8.22% with an increasing rate of 0.29% per year.<sup>[3]</sup> In this regard, most of the couples with male infertility are not absolutely infertile but are subfertile and hence a logical approach to improve the total motile sperm count is the need of the hour. Development of intracytoplasmic sperm injection (ICSI) came at rescue for infertile patients with severe oligo-asthenoteratozoospermia (OAT). Ejaculatory abstinence (EA) is certainly one of the available factors that can influence sperm quality such as volume, count, motility and morphology. In the recently published guidelines, the WHO (2010) recommends the sexual abstinence of 2–7 days while the American Urology Association recommends 2–3 days of EA and ironically the European Society of Human Reproduction and Embryology has recommended the abstinence window of 3–4 days. Differences in the demographics of the subjects enrolled in such kind of studies make it more difficult to come to a conclusion due to various confounding factors. Moreover, there is a lack of evidence regarding the recommendations for abstinence in cases of subfertile men.

The issue of association of EA with seminal parameters and sperm quality is still debatable because of the controversial reports emerged from several studies. For instance, reports from three different studies by Magnus *et al.*<sup>[4]</sup> in 1991, Pellestor *et al.*<sup>[5]</sup> in 1994 and De Jonge *et al.*<sup>[6]</sup> in 2004 have concluded that lengthening EA can increase the sperm concentration, but at the same time, it decreases sperm motility while sperm morphology is believed to be independent of length of EA. In a study by Jurema *et al.*<sup>[7]</sup> in 2005, Marshburn *et al.*<sup>[8]</sup> in 2010

and Sánchez-Martín *et al.*<sup>[9]</sup> in 2013, higher pregnancy rates were observed in couples with shorter abstinence length. This complexity and controversy in these findings are due to the fact that some parameters improve with longer abstinence while others improve with shorter abstinence. As we scrutinise more literature, controversies surrounding the idea of EA will increase proportionally. From their past experiences, the authors of the present study certainly believe that fertility clinic can optimise the sperm quantity and quality for prospectively planned fertility treatments by selecting the right EA window period. To our knowledge, very few studies have addressed the influence of EA on ICSI outcome.

In order to compensate the reduction in total sperm count that primarily occurs during semen processing, the authors of the study hypothesised that a second semen sample can be collected within a shorter abstinence period of 1 h. In this way, fertilisation rate and embryo quality can be enhanced following ICSI procedure.

## METHODS

The study group consisted of all the infertile couples undergoing ICSI cycle with the indicated seminal characteristics who had consulted the tertiary care hospital between January 2021 and July 2021. All female partners had undergone a diagnostic workup including hysterosalpingography, transvaginal ultrasonography and measurement of baseline levels of follicle-stimulating hormone, luteinising hormone, oestradiol, thyroid-stimulating hormone, triiodothyronine (T3), thyroxine (T4), prolactin and midfollicular progesterone. Male infertility was diagnosed when sperm abnormalities according to the WHO criteria (2010) were seen in at least two semen samples. All couples in the study had idiopathic male infertility. Retrospectively, two groups were analysed, i.e., Group A in which first semen sample was used and another Group B in which second semen sample collected within a shorter abstinence period of 1 h was used. All of them had an abstinence of 2–7 days, as suggested by the WHO 2010 criteria that were followed for sperm analysis. Female patients with more than 35 years of age or for genetic analysis were excluded from the study. All semen samples were collected by

masturbation within the fertility centre to minimise conditions that might alter sperm parameters. The second sample was analysed and compared to the first for the following parameters: concentration (mill/mL), total and progressive motility (%) and morphology (%). All laboratory procedures were performed by the andrology and embryology personnel, who were blinded regarding the study's experiments. None of the patients underwent any intervention prior to providing the semen sample that was examined and none of our subjects failed to provide the second sample.

All care and caution were exercised while utilising the patient data for current research as outlined in the hospital guidelines pertaining to the usage of patient's data for this study and confidentiality was maintained throughout. Written informed consent was obtained from the patient to publish the article along with relevant clinical information that needs to be reported in the journal. Patient identifiable data (name, contact, address, etc.) will not be presented in journal or any public forum. The study was also approved by the Institutional Ethics Committee (Reg. No. ECR/1435/Inst/GJ/2020).

### Statistical analysis

The baseline patient characteristics are presented as frequencies and percentage for the categorical variables and as the means and standard deviations for continuous variables. After appropriate data filtration, the data sheet was transferred and analysed using GraphPad Prism (vs. 9.2). Unpaired *t*-test and analysis of variance test were used to determine the significance.  $P < 0.05$  was considered statistically significant.

## RESULTS

This retrospective study evaluated 56 patients in Group A (first semen sample used) and 41 patients in Group B (second semen sample collected within a shorter abstinence period of 1 h used for ICSI procedure). The age of female subjects in Group A was  $29.9 \pm 3.5$  years while it was  $29.4 \pm 3.4$  years in Group B. There were no statistical differences in female age between the two groups [Table 1]. Similarly, the age of male subjects was  $32.2 \pm 3.6$  years and  $31.9 \pm 4.1$  years in Group A and Group B, respectively, with no statistical differences ( $P > 0.05$ ). Volume, total sperm count, morphology and total and progressive motility were compared between samples of Group A versus samples of Group B [Table 2]:

- Apart from initial progressive motility ( $P = 0.004$ ), none of the parameters such as volume, total sperm count and morphology were significantly different ( $P > 0.05$ ) between Group A and samples of Group B

**Table 1: Comparison of female age and male age between patients of Group A and Group B**

Variable	Group A (n=56)	Group B (n=41)
Female age (years)	29.9±3.5	29.4±3.4
Male age (years)	32.2±3.6	31.9±4.1

**Table 2: Comparison of sperm parameters between patients Group A and Group B**

Parameters	Group A (n=56)	Group B (n=41)	
		First ejaculate	Second ejaculate
Volume (ml)	1.80±0.87	1.87±0.99	1.39±0.57
Initial count (mill/ml)	12.84±10.57	11.65±10.91	11.54±10.12
Initial motility (%)	25.8±8.6	18.64±13.38	21.29±13.18
Normal morphology (%)	1.31±0.6	1.55±1.06	1.55±1.06
Post-wash count (mill/ml)	6.16±4.96	-	7.78±6.59
Post-wash motility (%)	76.3±25.3	-	51.77±38.3

- Similarly, parameters such as volume ( $P = 0.006$ ) and post-wash motility ( $P < 0.001$ ) were significantly different between Group A and samples of Group B. However, there was no significant difference in sperm count and morphology ( $P > 0.05$ )
- The same comparisons were made between samples of Group B. Significant difference was observed only in volume ( $P = 0.009$ ) but not in total sperm count, morphology and motility ( $P > 0.05$ ).

Out of 965 expected follicles in Group A and 598 expected follicles in Group B, 883 (91.5%) and 606 (101.3%) were retrieved, respectively. From retrieved follicles, 665 (75.3%) metaphase II oocytes in Group A and 408 (67.3%) in Group B were retrieved in the fresh cycles. In the ICSI cycle, 665 and 408 oocytes in Group A and Group B, respectively, were injected. No statistical differences were observed between the two groups in terms of retrieved follicles and injected oocytes, which shows the good randomisation of the subjects between the groups. The fertilised oocytes in Group A and Group B were 549 (82.6%) and 313 (76.7%), respectively. The mean fertilised oocytes in each patient of Group A was  $9.8 \pm 5.8$  while it was  $7.6 \pm 5.1$  in Group B with borderline significant difference ( $P = 0.051$ ) between the two groups. Grade 1 embryos on day 3 were 345 (62.8%) in Group A and 170 (54.3%) in Group B. One hundred and nine (31.6%) embryos in Group A and 63 (37.1%) embryos in Group B were cryopreserved on the same day. Later on day 5 and day 6, 113 (32.8%) and 85 (50%) embryos were cryopreserved for further utilisation. Overall, the total usable embryos in Group A and Group B were 222 (40.4%) and 148 (47.3%), respectively [Table 3].

**Table 3: Outcome comparison between control group (Group A) and study group (Group B)**

Variable	Group A (n=56)		Group B (n=41)		Significance
	n (%)	Mean±SD	n (%)	Mean±SD	
Expected follicles	965	17.2±8.3	598	14.6±6.5	0.087
Retrieved follicles	883/965 (91.5)	15.8±8.7	606/598 (101.3)	14.8±7.2	0.538
Metaphase II	665/883 (75.3)	11.7±6.4	408/606 (67.3)	9.9±5.6	0.145
Injected oocytes	665/665 (100)	11.9±6.4	408/408 (100)	9.9±5.5	0.102
Fertilised oocytes	549/665 (82.6)	9.8±5.8	313/408 (76.7)	7.6±5.1	0.051
Cleaved embryos	538/549 (98.0)	9.6±5.7	306/313 (97.8)	7.5±5.1	0.060
Grade I embryo (D3)	345/538 (62.8)	6.2±4.6	170/306 (54.3)	4.4±3.5	0.031
Total usable embryos	222/549 (40.4)	3.9±1.9	148/313 (47.3)	3.7±2.3	0.651

SD=Standard deviation

**Table 4: Statistical analysis of sperm parameters between patients Group A and Group B**

Parameters	Group A versus first ejaculate of Group B	Group A versus second ejaculate of Group B	First ejaculate versus second ejaculate of Group B
Volume	0.718	0.006	0.009
Initial count	0.592	0.541	0.962
Initial motility	0.004	0.060	0.369
Normal morphology	0.197	0.21	0.98
Post-wash count	-	0.19	-
Post-wash motility	-	<0.001	-

## DISCUSSION

It is well established that high-quality semen can lead to an improved fertilisation rate. Apart from testicular sperm production and accessory gland secretion, the ejaculate output depends on a significant confounder called 'the duration of EA'. However, conflicting results exist in terms of correlation between sperm quality and EA. Hence, the present retrospective study was undertaken with a purpose of evaluating the advantage of shorter abstinence period (1-h sequential ejaculate) in males with severe OAT syndrome on fertilisation rate and thereby emphasising the potential application of consecutive ejaculate. The mean volume in the initial and consecutive ejaculates was  $1.87 \pm 0.99$  ml and  $1.39 \pm 0.57$  ml, respectively, while the Total sperm count (TSC) was  $11.65 \pm 10.91$  and  $11.54 \pm 10.12$  million/ml in the initial and consecutive ejaculates, respectively. Although, the volume in the consecutive ejaculate was significantly lower ( $P = 0.009$ ; Table 4); sperm count, progressive motility and morphology did not show any significant differences between the initial and consecutive ejaculates of Group B subjects. Second ejaculation had higher concentration and motility compared to the first ejaculation of Group B subjects.

Good quality embryos at day 3 (embryos preferably about 6 to 10 cells and with less than 10% fragmentation at day 3) in group A were 62.8% while it was 54.3% in group B ( $P=0.031$ ). While total useable embryos on day 5/day 6 were 32.8% and 50.0% in Group A and Group B, respectively. Although the difference of

good quality day 3 and useable embryo formation was non-significant ( $P < 0.05$ ) between the two study groups, Group B showed a higher percentage of embryos that can be cryopreserved for further utilisation as compared to Group A (37.1% vs. 31.6%, respectively). Similarly, on day 5/day 6, the percentage of embryos that were cryopreserved for further utilisation in Group B was higher as compared to Group A (50% vs. 32.8%, respectively). A second semen sample collected after 1 h of EA allowed the same results in terms of fertilisation. With regard to compromised sperm parameters, our findings do suggest that the second ejaculate is quite relevant to 'in vitro' reproductive treatments and a simple request for a second consecutive ejaculate could provide the same results in terms of fertilisation. Several studies have concluded that shorter duration of EA may protect the sperm from oxidative stress damage caused by reactive oxygen species. As compared to 4–7 days of abstinence period, Alipour *et al.*<sup>[10]</sup> in 2017 observed improved sperm kinematic parameters and percentage of progressively motile sperm in the males with 2 h of abstinence while a study by Bahadur *et al.*<sup>[11]</sup> in 2015 reported improved sperm concentration and morphology in the males with a 40-min abstinence. Sugiyam *et al.*<sup>[12]</sup> in 2008 too reported the increased chances of fertilisation rate (53.3% vs. 28.9%) when repeated spermatozoa were collected after 30–60 min from the first ejaculation. In the present study, we observed a statistically non-significant increased chance of usable embryos in the second ejaculate group as the total usable

embryos in Group A and Group B were 222 (40.4%) and 148 (47.3%), respectively.

Without any consensus on optimal abstinence in cases of subfertile men and keeping in mind the complexity of the reproductive system fuelled by lifestyle and local environmental factors, it is high time that semen parameters should be individualised depending on the country and/or specific geography. Our study of consecutive ejaculates produced within 60 min of the initial ejaculate holds ground and is quite realistic with backing from other similar studies. The strength of the study is indeed the control group, i.e., the Group A subjects with the same inclusion criteria as that of Group B, that allowed us to compare the total usable embryos that can be cryopreserved for future usage. However, a prospective sibling oocyte study or randomised controlled trials are needed in order to validate whether this approach can improve pregnancy and live birth rates. Because of the retrospective nature of the study, we could not access the sperm nuclear maturity and DNA fragmentation status with EA.

As the infertility treatment procedures are not subsidised by the majority of the government and are dominated largely by the private sector, our results (the increased chances of usable embryos in the second ejaculate group) can benefit infertile couples in developing countries with inadequate health infrastructure. Since sperm quality becomes more clinically important than total volume or count, a proper diagnostic and therapeutic workup could allow the embryologist to increase the number of motile spermatozoa available to freeze in cases of fertility preservation for worsening OAT patients.

#### Data availability statement

Data shall be available from the corresponding author upon specific request.

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Nil.

#### Conflicts of interest

There are no conflicts of interest.

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