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Role of sequential semen samples in infertile men candidates for assisted reproduction: A prospective study

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Abstract
Objective: To study the beneficial effect of repeated sequential ejaculation in infertile men who are candidates for assisted reproduction.
Materials and methods: The study included a total of 237 infertile males attending our infertility and IVF center from January 2016 till December 2017. All patients were asked to provide two semen samples (1–3 h apart) after an abstinence period of 3–7 days. The two consecutive semen samples were analyzed according to the 2010 WHO criteria for semen analysis and their parameters were compared.
Results: The mean age for our study group was 35.7 years (20–56 year). Of the 237 subjects, 157 showed oligoasthenozoospermia on their initial semen sample while the remaining 80 were azoospermic. A statistically significant difference was detected between the 2 sequential semen samples regarding all semen parameters except grade A motility. Despite the significant decrease in seminal volume by sequential sampling, there was a statistically significant increase in sperm concentration in the second ejaculate compared to the first (6.2 ± 0.61 versus 3.4 ± 0.52 million/mL, respectively, $p = 0.016$). The mean normal sperm morphology also demonstrated a significant increase (2.1 ± 1.8–$5.1 \pm 2.6\%$, $p < 0.002$). Mean progressive sperm motility increased from 1.13 ± 0.31 to $1.7 \pm 0.31\%$ ($p = 0.010$) on repeated sampling. Also, we were able to retrieve viable sperm in 15% of the azoospermic patients whom were known to be azoospermic on previous occasions.

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Conclusions: Obtaining consecutive semen samples leads to improvements in the quality of many semen parameters (sperm concentration, motility and morphology) which may be of special importance for management of infertile couples especially those attempting assisted reproductive techniques.

Introduction

Sexual abstinence has been shown to influence semen parameters. The WHO recommends an abstinence period of 3–7 days prior to semen collection [1]. However, the European Society of Human Reproduction and Embryology suggested an abstinence period of 3–4 days only [2]. Prolonged sexual abstinence may have a negative impact on sperm motility and viability [3]. There has been a growing body of evidence on the improvement of semen quality by daily ejaculation and sequential semen sampling within the same day with improvement not only on the level of regular semen parameters but also sperm DNA quality [4–6]. Sperm concentration and motility show high variability among different samples of the same subject which may explain the incidence of poor semen quality on the day of assisted reproductive procedures in patients with previously acceptable semen analyses. With the widespread utilization of intracytoplasmic sperm injection (ICSI) for male factor infertility comprising almost fifty percent of infertile couples, improving semen parameters becomes crucial with the priority for quality over count and volume which are usually decreased in the second ejaculate [6]. Providing a consecutive semen sample only hours after a prior one has been attempted to obtain better quality semen parameters [4]. Nevertheless, no consensus exists regarding whether to request a second successive sample in cases of low semen quality or not. Even cases of azoospermia are to be confirmed by repeating semen testing on 2 separate occasions 2–4 weeks apart according to the WHO abiding by the classic rule for abstinence before any semen analysis [1]. The objective of this study was to compare between the semen parameters of two successive ejaculates collected within the same day in infertile patients with poor semen quality, whether as oligozoospermia, oligo-terato-asthenospermia or non-obstructed azoospermia (NOA).

Subjects and methods

Two hundred and thirty-seven patients attending our fertility clinic for male factor infertility were prospectively enrolled in this study from January 2016 till December 2017. Informed consents were obtained from all patients after study approval by the Institutional Review Board of the Faculty of Medicine, Beni-Suef University. We included all patients with potentially nontreatable oligozoospermia and azoospermia (e.g. NOA, and hypergonadotropic hypogonadism). The subjects underwent a basic andrological evaluation, which included two consecutive semen analyses evaluated according to the WHO 2010 criteria. The 1st semen sample was provided after an abstinence period of not less than 3 days and not more than 7 days while the second sample was provided 1–3 h after the first. Patients with NOA were confirmed to be azoospermic by extended sample centrifugation. The fertility hormonal milieu for all subjects including follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone was studied in morning blood samples. All semen samples were collected at our fertility center lab via masturbation. Samples were allowed to liquefy completely for 15–30 min at 37 °C before further processing. Each sample was examined manually by a single experienced observer. Analysis of the two consecutive semen samples with regards to semen volume, sperm concentration, motility grading and morphology was performed. Mean and standard deviation (SD) were calculated for all parameters.

Table 1 Description of first sample seminal characters.

Parameter	Min.	Max.	Mean	SD
Volume (mL)	0.6	7	2.9	0.16
Conc. (mill./mL)	0.1	8	3.4	0.52
Motility (%)	0	25	4.8	0.7
Grade A	0	1	0.1	0.09
Grade B	0	20	1.03	0.3
Grade A + B	0	20	1.13	0.31
Grade C	0	25	3.7	0.5
Grade D	75	99	95.2	3.9
Morphology	1	6	2.1	1.8

Statistical analysis

Calculations were analyzed using SPSS 19.0 software (SPSS Inc., Chicago, Illinois, USA). All numeric data were presented as the mean value ± SD. The statistical analysis was performed using paired t-test to compare the two sequential sample results. Differences between the values were considered statistically significant when p value was below 0.05.

Results

Our study population ages ranged from 20 to 56 years with a mean of 35.7 years. Upon initial semen sampling, 157 patients showed oligozoospermia while 80 revealed no sperm after excluding for cryptozoospermia. The basic values for the 1st and 2nd semen sample parameters for the oligozoospermic men are displayed in Tables 1 and 2. Analyzing the difference between 1st and 2nd samples regarding semen parameters, a statistically significant difference could be detected between consecutive samples regarding all parameters ($p < 0.05$) except grade A motility (Table 3, Figs. 1 and 2). Although there was a significant decline in seminal volume on the second ejaculate (1.5 ± 0.9 mL) compared to the first (2.9 ± 0.16 mL) ($p < 0.001$) in all men, 121 out of the oligozoospermic group (77%) demonstrated a statistically significant increase

Table 2 Description of second sample seminal characters.

Parameter	Min.	Max.	Mean	SD
Volume (mL)	0.4	5	1.5	0.09
Conc. (mill./mL)	0.1	10	6.2	0.61
Motility (%)	0	35	6.8	0.8
Grade A	0	5	0.1	0.04
Grade B	0	20	1.6	0.3
Grade A + B	0	20	1.7	0.31
Grade C	0	20	5	0.6
Grade D	70	99	93.3	4.5
Morphology	1	7.2	5.1	2.6

Table 3 Comparison between the first and second samples regarding semen analysis parameters.

Parameter	Mean difference	Difference SD	p Value
Volume (mL)	1.4	0.12	<0.001*
Conc. (mill./mL)	−2.8	0.2	0.016*
Motility (%)	−1.8	0.43	<0.001*
Grade A	0.049	0.049	0.320
Grade B	−0.64	0.22	0.004*
Grade A + B	−0.6	0.22	0.010*
Grade C	−1.3	0.31	<0.001*
Grade D	−16.8	3.9	<0.001*
Morphology	−3	1.9	0.002*

* Statically significant.

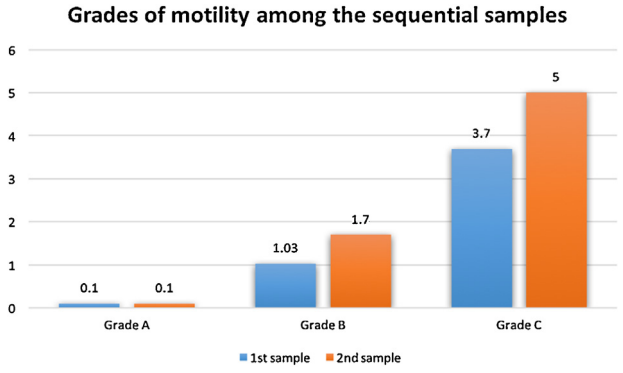

Figure 1 Comparison of grades of motility among sequential samples.

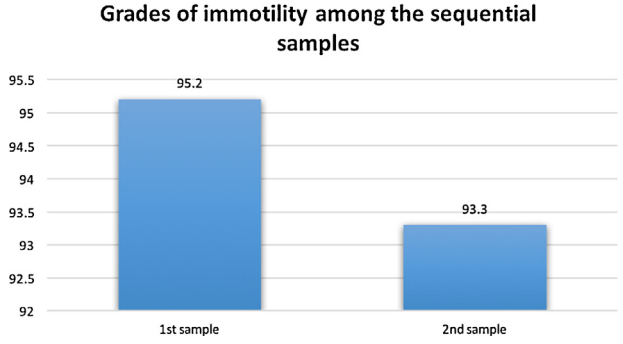

Figure 2 Comparison of grades of immotility among sequential samples.

Table 4 Change in concentration in second sample among previously azoospermic patients in 1st sample.

Parameter	1st sample	2nd sample	p Value
Volume (mL)	3.2 ± 1.2	1.7 ± 0.8	<0.001*
Concentration (sperm/20 μL)	0	3.04	0.17
Motility grade C (sperm/20 μL)	0	1.68	0.059*

* Statically significant.

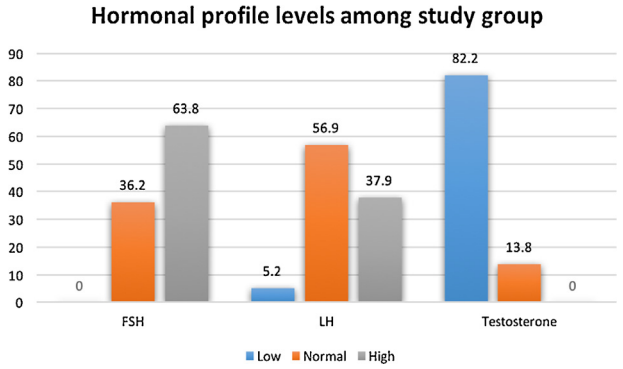

Figure 3 Frequency of hormonal profile levels among study group.

in sperm concentration in their 2nd ejaculate compared to their first, 6.2 ± 0.61 million/mL versus 3.4 ± 0.52 million/mL respectively (p = 0.016). Following the same incline in values, the mean progressive sperm motility in first samples rose from 1.13 ± 0.31% to 1.7 ± 0.31% (p = 0.010). Similarly, the mean for normal sperm morphology increased over double the value by repeating semen testing from 2.1 ± 1.8% in the 1st sample to 5.1 ± 2.6% in 2nd sample (p = 0.002). The rest 36 patients (23%) failed to show such improvement.

A remarkable result in our study was the detection of sperm in 12 men of the azoospermic group with at least one sperm per 20 μm. These men were known to be azoospermic on repeated occasions before attending our center. Hence, this represented a 15% sperm retrieval rate in azoospermic subjects without intervention (Table 4). Despite the statistical insignificance of finding sperm in these 12 azoospermic patients on sequential sampling (p = 0.17), we found it to be clinically significant as these patients were waived the need for a testicular biopsy for attempting assisted reproduction by utilizing their ejaculated sperm after collecting a considerable amount via sperm banking. The mean sperm concentration in these positive samples was 3.04 sperm/20 μL. The sperm detected in these second samples showed only nonprogressive motility (Table 4). All azoospermic patients who failed to recover sperm on their second sample consequently underwent ICSI after relying on surgical sperm retrieval.

63.8% of our subjects had above normal FSH hormone levels, 37.9% had high LH hormone levels while 86.2% had below normal levels of testosterone (Fig. 3). In an attempt to correlate between the successful recovery of sperm in initially negative sampling and the hormonal milieu of those patients, we fell short of detecting a significant correlation. Thus we could not draw a clear hormonal cut off value for requesting a second semen sample in azoospermic patients (Table 5).

Table 5 Hormonal profile correlation with positive sperm recovery in the azoospermic group.

Hormonal profile	Not-improved (n = 68)		Improved (n = 12)		p Value
	Mean	SD	Mean	SD	
FSH (IU/L)	17.6	11.5	14	6.3	0.4
LH (IU/L)	9.8	6.5	8.03	4.6	0.5
Testosterone (nmol/L)	5.01	2.4	7.3	6.9	0.2

Follicle stimulating hormone (FSH), luteinizing hormone (LH).

Discussion

Based on studies that evaluated the relation between abstinence period and semen quality in normospermic males [7,8], the recommended abstinence period for proper evaluation of a semen analysis is 3–7 days [1] while that for regular intercourse aiming to achieve natural conception is every other day sexual intercourse [9]. However, several recent studies proposed another two different recommendations which are the benefit of successive ejaculates and daily ejaculations in oligoasthenozoospermic and oligo-teratoasthenospermia patients. Daily ejaculations every 24 h for four days in combination with sperm selection using density gradient centrifugation used for ICSI resulted in an improved pregnancy rate when compared to patients undergoing abstinence for 4 days [6]. The time frame for successive ejaculates ranged from minutes [4], to one hour [10], to 2 h [11], and up to 4 h [12]. In our study we instructed the patients to have the second semen sample within a 1–3 h window from the first sample. The choice of that time range was for the patients' own comfort. Some studies reported a decrease in sperm count in the second ejaculate [5,13] which could be explained by epididymal tail depletion of sperm after the first ejaculate [14]. Contrarily, others reported the sperm count to improve. Although these studies agreed on a statistically significant increase in total motile sperm count, sperm motility [4,10,11] and decrease in sperm DNA fragmentation [5], a statistically significant decrease in semen volume was noted [4,10,11]. The improvement in sperm motility could be explained by the fact that the proximal part of the epididymal body has fresher sperm with better motility than the epididymal tail, the former being expelled in the second ejaculate [15]. Concurring with the previous studies we were able to detect a statistically significant increase in sperm concentration, normal sperm morphology, and progressive sperm motility in the second ejaculate compared to the first one despite a statistically significant decrease in semen volume. Our study group (n = 237) included 157 oligozoospermic patients which outnumbered other studies which in a study by Ortiz and his group included 32 patients undergoing intrauterine insemination [13], 73 subfertile oligozoospermic men by Bahadur et al. [4] and 34 patients by the Zhai group [12]. The majority of the patients in our study demonstrated a significant improvement in sperm concentration and motility upon providing a second sample. Hence, the collection of two sequential ejaculates from oligozoospermic males may not only be utilized to improve both the yield and quality of sperm required for assisted reproductive technologies and cryopreservation as formerly described [16], but even optimize success rates for fertilization, pregnancy and ultimately the potential for take home baby. Considering the fact that there is no consensus whether to request a second ejaculate in oligozoospermic men or not, we present our study as additional evidence in support to all previously cited studies. Moreover, we were able to retrieve viable sperm in 15% of patients (12/80) who were found to be azoospermic on initial sampling. We consider succeeding in retrieving sperm in the 2nd ejaculate of previously confirmed azoospermic men without the use of the invasive testicular biopsy a significant finding. Not only may it spare the patient psychological and financial burdens but more importantly avoid unnecessary trauma to a testis with a very low potential for yielding sperm by surgical retrieval regardless of the technique used. We believe that our data adds extra evidence to the concept of the potential advantage of consecutive semen sampling when fertility becomes the point of interest.

The limitation of our study is that despite the objective improvement of semen quality upon sequential sampling yet we lack information regarding fertilization and pregnancy rates pertaining to these samples with special emphasis on the 12 NOA cases that were able to provide sperm via their 2nd sample. We are aware of the importance of following up such data in order to confirm the added value of sequential semen collection.

In conclusion, obtaining a second successive semen sample may play a crucial role in improving the quality of poor quality semen samples used in assisted reproductive technologies. Better results may be achieved via this simple step without adding a physical or psychological cost to infertile couples attempting assisted reproduction especially those in low income countries. We hope this concept may be a future addition to current guidelines for semen sample collection.

Conflict of interests

None.

Ethical committee approval

The study has been approved by the Institutional Review Board of the Faculty of Medicine, Beni-Suef University.

Funding

No competing financial interests exist.

Authors' contributions

AM Ragheb: study design, paper writing, critical review.

RM Ibrahim: study design, paper writing.

AM Elbatanouny: critical review.

AS Moussa: critical review.

AM Abdelbary: critical review.

OM Sayed: critical review.

MS Eladawy: critical review.

HA Shaker: critical review.

SO Hamdi: study design.

Consent from the patients

Informed consents were obtained from all patients.

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