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The effect of consecutive ejaculation on the sperm parameters in the oligo-astheno-teratozoospermia (OAT) men

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Research Article

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Summary

Recently, the World Health Organization recommendation for abstinence time for semen analysis has been challenged in some studies and many of them have supported the advantages of a second short abstinence ejaculation. More evidence is needed to approve this for clinical use. This study aimed to compare the average routine abstinence time (2–7 days) with the short time (1–2 h) on sperm quality based on functional parameters in a population of oligo-astheno-teratozoospermia (OAT) men. The semen samples were retrieved from 50 men with OAT two times: one standard 2–7 days (long ejaculation) and short duration trimming (1–2 hours later the first ejaculation). All semen parameters as well as sperm DNA integrity were compared between groups. Results showed that mean sperm concentration (10.40 vs. 8.76), total sperm count (28.53 vs. 12.24) and mean semen volume (2.69 vs. 1.40) were higher in the first ejaculation (2–7 days of abstinence), while progressive motility (20.52 vs. 13.32), non-progressive motility (53.46 vs. 48.86), morphology (2.46 vs. 1.46) and viability (83.90 vs. 77.96) were significantly higher in the second ejaculation ($P < 0.05$). The second sample also showed lower immotile (26.82 vs. 38.02) and DNA fragmentation (19.5 vs. 26.96) ($P < 0.05$). Taking all data into account, an additional short abstinence period (AP) may be a simple and helpful strategy to obtain better sperm quality in couples with male infertility causes, especially in OAT patients. The recommended current guidelines regarding the AP may need to be revisited in severe male factors.

Introduction

The time between ejaculatory events, or abstinence time, is one of the leading modifiable intrinsic factors that affect semen quality. However, the World Health Organization's (WHO) Manual for the Examination and Processing of Semen has undergone many updates since 1980; until the last edition in 2021, the duration of sexual abstinence remained unchanged (Barbagallo *et al.*, 2023). There is no distinct document that supports the best ejaculatory abstinence (EA) time recommended for routine semen analysis is 2–7 days (De Jonge *et al.*, 2004). The European Society of Human Reproduction and Embryology and the Nordic Association for Andrology suggest a shorter abstinence time of 3–4 days (Ayad *et al.*, 2018a). However, scientific documents do not sufficiently support the basis for these recommendations and require more evidence. Optimizing the best time for abstinence is important to ensure both the quantity and quality of sperm, which are necessary for successful, natural and assisted conception (Agarwal *et al.*, 2016b).

Some studies challenged the recommended time of 2–7 days by showing semen parameters improvement after different abstinence intervals (Levitas *et al.*, 2005). Also, it was found that shorter abstinence is accompanied by better results in the aim of assisted reproductive technology (ART).

Abstinence duration has been shown to impact various sperm parameters in different ways. For example, short duration improves sperm motility while decreasing total sperm count (Sokol *et al.*, 2021). There is a controversy in the study's results regarding the best time for abstinence. It was found that in normozoospermic samples, semen parameters will be improved by the increase in EA. Still in oligozoospermic samples, motility and morphology will be decreased (Levitas *et al.*, 2005). In another study, it was shown that short abstinence time has no detrimental effect on sperm quality in normozoospermic samples and is also recommended for

reducing sperm DNA Fragmentation Index (DFI) (Agarwal *et al.*, 2016b). Furthermore, in an analysis of 9,489 oligospermia samples, the results showed that just one day of sexual abstinence is accompanied by better sperm quality. Sperm collection with the aim of standard analysis or cryopreservation for donor samples should not exceed ten days of abstinence (Levitas *et al.*, 2005).

On the other hand, in contrast to prolonged abstinence, one theory is that lengthy abstinence could increase reactive oxygen species (ROS) exposure, which is generated mainly by abnormal spermatozoa and granulocytes. High levels of ROS have damaging effects on sperm DNA (Agarwal *et al.*, 2016b). In this regard, it was shown that higher abstinence time decreased intrauterine insemination outcomes and increased oxidative stress (OS) (Degirmenci *et al.*, 2020, Jurema *et al.*, 2005). It may be because of senescence and functional damage of sperm, which are not identified by standard semen analysis (Jurema *et al.*, 2005).

Sperm parameters have a high impact on assisted reproductive outcomes, and growing concerns have been raised regarding the effect of different EA intervals on various semen parameters (Ayad *et al.*, 2018a). Due to the conflicting nature of the current evidence, no clear recommendations can be made regarding the ideal abstinence time (Hanson *et al.*, 2018a). Interestingly, many results favoured extremely shorter periods, ranging from 1 to 4 hours (Ayad *et al.*, 2018a).

While there is much evidence that supports the shortening of the abstinence period (AP) compared to the conventional recommendation (standard), few studies have examined the efficacy of a very short abstinence time (1–2 hours) in the sperm parameters of oligo-astheno-teratozoospermia (OAT) patients. There is a growing interest in a very short time for abstinence (second ejaculation after the first) in infertile men, to improve sperm quality as well as ART outcomes, especially in the OAT. Besides, there is some controversy in the reported data on this population. This challenge may be a simple way to improve sperm quality, especially in cases of very poor semen analysis. So in this study, the aim was to evaluate the effect of short abstinence of 1–2 hours (h) on sperm quality based on functional parameters compared to the standard recommendation, in the OAT males.

Material and methods

Ethical approval and consent to participate

This study was approved by the Ethics Committee of Esfahan Medical University, Esfahan, Iran, which follows the Helsinki Declaration of 1975 (RI.MUI.REC.1403.002).

This study was designed as a prospective study in the Maryam Infertility Center, Isfahan, Iran, for four months, from April to July 2024. The enrolled population was OAT patients who were referred to our fertility centre after signing informed consent. According to the WHO 2010, OAT is defined as when the concentration is < 15 million/ml, progressive motility < 32% and morphology < 4% (Organization, 2021).

Inclusion criteria were as follows: infertile men aged 20 to 45, at least one previous semen analysis result with a sperm concentration of less than 15×106/ml with an AP of 2–7 days, no history of previous testicular/penile surgery or vasectomy, and no history of cancer, radiation therapy, or chemotherapy. Candidates should have been ungratified for 2–7 previous days to collect study samples. The exclusion criteria consisted of severe azoospermia and oligospermia, a frozen sample, and a history of treatment with particular drugs.

According to the time of semen collection (AP), samples were classified into two groups: (1) after 2–7 days of abstinence (standard recommendation), and (2) semen samples collected 1–2 h later than the first sample. Each case was a control for himself.

Semen sample collection and sperm parameters

Samples were collected in a sterile plastic container from 50 volunteers by masturbation. The usual information was recorded about the exact duration of abstinence (the time between the current and previous ejaculations) for each participant. Delivered samples were incubated in a water bath at 37°C until analysis. Sperm parameters were analyzed according to WHO guidelines immediately after liquefaction (Organization, 2021).

For this purpose, the semen sample was well mixed, and 10 µl of non-diluted liquefied sample was loaded in the middle of a Neubauer counting chamber, and subsequently covered with a cover glass and observed under 400× magnification. Information regarding sperm volume, total sperm concentration, motility, morphology, viscosity and sperm DNA damage were recorded.

Semen volume and sperm count

After liquefaction, semen volume was measured using a conical tube, and concentration was determined by counting the spermatozoa using a validated Makler chamber (mill/ml). Total sperm count was calculated by multiplying the semen volume with the sperm concentration (mill/ejaculation).

Sperm motility

Motility was evaluated using Computer Aided Sperm Analysis (SCA2000, Microptic, Barcelona, Spain) software based on the criteria of the World Health Organization. Accordingly, motility is classified as A, rapid progressive motility; B, slowly progressive; C, non-progressive motility; and category D, immotile sperm. According to our centre, which modifies this classification, classes A and B were merged into one group, so in the present study, three motility categories were as follows: progressive (A+B), non-progressive (C), and immotile sperm (D).

Sperm morphology

Morphology was checked using a Diff Quick staining kit, based on Kruger's strict criteria, which is summarized and performed as follows: About 20 microliters of the diluted sample were placed on the slide, and the prepared smear was dried at room temperature. Then, it is placed in three solutions: methanol (for the slide fixation), orange eosin solution (for cytoplasm staining) and dark blue thiazine solution (for DNA staining). After drying at room temperature, 200 spermatozoa in each slide were assessed using a light microscope (×100 magnification).

Sperm viability, sperm membrane integrity

Viable motile sperm was identified with the laser-assisted immotile sperm selection method, a fast, simple, repeatable and safe method with no need for any chemical compounds. In this system, one single laser shot of 129 µJ of approximately 1.2 Ms is emitted (OCTAX Laser Shot®, MTG, Germany). If the sperm tail is coiled in response to the laser shot, it is considered viable. Besides (Nordhoff, 2015).

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Semen viscosity measurement

According to a previous study, the semen viscosity was measured using a disposable eight chamber 20-µm depth slide (SC 20-01-08-B; Leja® Products B. V., Nieuw-Vennep, the Netherlands). The mechanism is based on the time for filling one chamber, and the results are presented in the unit centipoise (Pc) (Ayad *et al.*, 2018b).

Quality control

To ensure quality control and minimize probable variations in the assessment, the two technicians, who were well-trained in this field (andrology), analyzed all the procedures. All the instruments were calibrated because high accuracy is important in assisted reproductive techniques. The lab supervisor performed the laboratory's daily quality control (daily, weekly, or monthly, as recommended).

Sperm DNA fragmentation assay

Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling was used to detect sperm DNA damage. After labelling, DFI was analyzed by flow cytometry, according to Chatzimeletiou *et al.* (Chatzimeletiou *et al.*, 2022). For this purpose, 100 µl of each semen sample was washed in PBS (Sigma-Aldrich, Germany) and centrifuged (5 min at 300 g). The obtained precipitant was added to a mixture of TNE buffer (NaCl (0.15M), Tris HCL (0.01M), Ethylenediaminetetraacetic acid (0.0011M), pH 7.4, and detergent solution (NaCl 0,15M, TRITON X-100) (Bioline Scientific, Athens, Greece). After 5 min, acridine orange (Bioline Scientific Athens Greece) was added, and incubation was continued for a further 5 min in the dark.

Finally, the prepared stained samples were analyzed via a flow cytometer (BD FACSCalibur U.S.A.). The intact sperm is detected in the green detection region (wavelength of 546 nm), and fragmented ones (Acridine orange inserted into the fragmented portion of the sperm) are detected in the red detection region (wavelength of 670 nm). DFI is calculated by dividing the total red and green fluorescence (Chatzimeletiou *et al.*, 2023).

Statistical analysis

For each sperm parameter, comparisons were made within each time frame of abstinence (short vs. conventional recommendation) using paired *T*-tests. To describe the relationship between each semen parameter value and abstinence time, the linear mixed-effects model was used, and a linear slope was estimated (with a 95% confidence interval (CI). The significance was established at $P < .05$.

Results

A total of 100 semen specimens were obtained from 50 infertile men with oligo-asthenic-teratozoospermia. After two periods of abstinence, 10 primary and advanced semen characterizations were evaluated. Figure 1 shows the variation in semen volume, sperm concentration, total sperm count, progressive, non-progressive, immotile sperm, normal morphology, viability, viscosity and DNA fragmentation.

Semen volume (2.69 vs. 1.40, in first and second ejaculation, respectively), sperm concentration (10.4 vs. 8.76) and total sperm count (28.53 vs. 12.24) remained below the 2010 WHO 5th percentile in the short abstinence of 1 –2 h ($P < .05$). It's in while that progressive (13.32 vs. 20.52), non-progressive motility (48.86 vs. 53.46), normal morphology (1.46 vs. 2.46) and sperm viability (77.96 vs. 83.90) increased during second ejaculation ($P < .05$). Short abstinence was accompanied by lower immotile ($P < .05$), and lower DNA fragmentation index ($P < .05$), and semen viscosity was relatively unchanged (Table 1).

In Table 2, the linear mixed model showed the relationships of the changes observed in the semen parameters during the different AP. As expected, progressive, non-progressive motility, normal morphology and viability showed a positive slope ($P < .001$). These parameters increased with shorter AP and decreased with lengthier AP. Although, volume concentration and total sperm count decreased with the shorter AP, immotile sperm, sperm DNA fragmentation also decreased ($P = .001$). Viscosity showed no relationship with AP.

Discussion

Duration of EA is one of the factors that may affect routine semen analysis. This study showed that, except for viscosity, other conventional and advanced semen parameters (semen volume, sperm concentration, total sperm count, sperm motility, morphology, vitality and DFI) were influenced significantly by abstinence time. Also, the previous findings suggested that shorter APs may be beneficial for men with OAT in improving sperm parameters. One possible explanation for this phenomenon is that longer APs can lead to sperm DNA damage and decreased sperm quality in men with OAT.

The results showed that the magnitude of changes was greater for total sperm count than sperm concentration (2.33 vs. 1.18 fold respectively) over the same period. It's because that total sperm count is more related to semen production consisting of secretions from multiple organs (the testis and epididymis (~10%), prostate (~25%), periurethral glands (~1%) and seminal vesicles (~65%)) (Drabovich *et al.*, 2014), so this parameter is affected by the frequency of ejaculation (the reserve decreased by the increase in the ejaculatory frequency) (Taaffe *et al.*, 2022). On the contrary, sperm viability (slope = 5.94), motility (slope = 7.2) and morphology (slope = 1) were positively affected by AP. According to the mixed linear statistical model, immotile sperm (slope = –11.2) and DNA fragmentation (slope = –7.46) have indicated inverse trends with shorter AP. It may be explained by the fact that spermatozoa are more exposed to higher levels of OS when they stay longer in the epididymis (OS arising from dead spermatozoa and leukocytes) (Borges *et al.*, 2019). The same OS may be more harmful to semen with abnormal characteristics compared to a normozoospermic sample (Barbagallo *et al.*, 2022). Yet, the viscosity remained unchanged for both long and short ejaculatory APs.

Some studies have assessed sperm parameters in consecutive ejaculates. In line with our research, Sokol *et al.* (2021) showed that progressive motility, sperm morphology and vitality increased during short abstinence. Still, semen volume, sperm concentration and total sperm count decreased. They also reported DNA fragmentation improved, and embryo euploidy rates and pregnancy outcomes increased in short abstinence time (Sokol *et al.*, 2023).

We found that semen volume and sperm count decreased in the short abstinence time. It's in line with the other studies, which showed a decrease in sperm count with a reduction in abstinence time (Hanson *et al.*, 2018b), and showed that longer abstinence is associated with increases in semen volume and sperm count (De Jonge *et al.*, 2004), and higher numbers of sperm and higher semen

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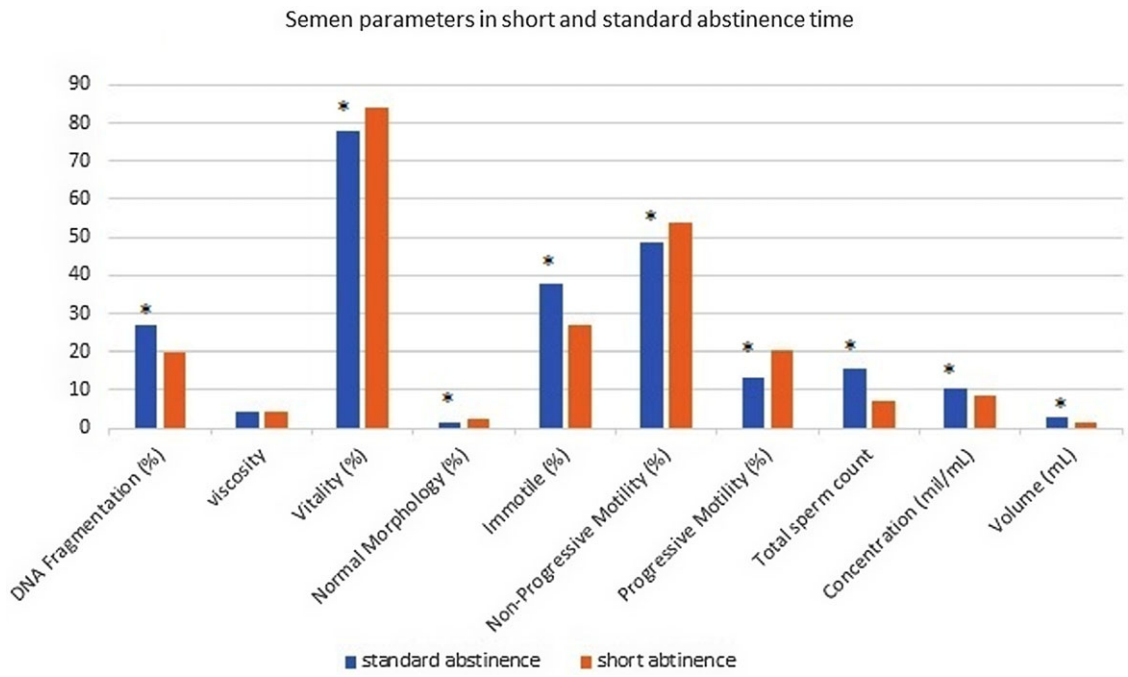

Figure 1. Semen parameters in short and standard abstinence time: semen volume (decrease, $P < .05$), sperm concentration (decrease, $P < .05$), total sperm count (decrease, $P < .05$), progressive motility (increase, $P < .05$), non-progressive (increase, $P < .05$), immotile (decrease, $P < .05$), normal morphology (increase, $P < .05$), vitality (increase, $P < .05$), viscosity (unchanged), DNA fragmentation (decrease, $P < .05$).

volume were achieved with an increase in abstinence duration (De Jonge *et al.*, 2004). But there is also some controversy result. For example, one systematic review and meta-analysis study found an increase in sperm count in OAT patients in the second ejaculation (within 4 h) (Barbagallo *et al.*, 2022). One reason for this may be the different population studies and the time for categorizing short and long abstinence. For example, in one retrospective study, the authors suggested that in the aim of fertility treatments, in oligozoospermia patients, semen samples should be collected after only one day of sexual abstinence to reach the highest percentage of sperm motility and morphology, but in normozoospermic patients, semen parameters could further improve through 7 days of abstinence (De Jonge *et al.*, 2004).

Similar to strong evidence in the literature that suggests that sperm motility, especially progressive motility, and velocity will be increased with the decline in semen volume and sperm concentration in shorter APs (Ayad *et al.*, 2018a), our results also showed better sperm motility in the shorter AP.

In our study, the other semen parameter that improved by the short abstinence was sperm morphology. It is in line with the previous research, which showed that in oligozoospermic samples, motility and morphology are inversely related to AP (Levitas *et al.*, 2005). The author believed that among oligozoospermic samples, worsened sperm motility and morphology by prolonged abstinence time is related to higher levels of ROS in infertile semen compared with fertile samples.

In line with our study, it was shown that sperm viability decreased by prolonged abstinence (Comar *et al.*, 2017, Agarwal *et al.*, 2016a). They also believed that in long abstinence, sperm are more exposed to the harmful effects of ROS while they are stored in the epididymis (Agarwal *et al.*, 2016a).

The study's findings on sperm DNA fragmentation are in line with recent studies that showed lower DFI in the second ejaculation in consecutive ejaculates (Kulkarni *et al.*, 2022, Karavani *et al.*, 2023, Barbagallo *et al.*, 2022). There is emerging evidence that has suggested a possible link between reactive oxygen species (ROS), DNA fragmentation, Mitochondrial Membrane Potential (MMP) and reproductive outcomes (Bonanno *et al.*, 2016, Treulen *et al.*, 2015). The parameters that, when combined, may be more robust in the prediction of natural conception than the standard semen parameters (Vončina *et al.*, 2016). In this regard, Shen *et al.* found a significant improvement in DNA integrity, higher total antioxidant capacity, and higher MMP in the short abstinence (second ejaculation after 1–3 h) compared to the longer time (3–7 days) (Shen *et al.*, 2019). From these findings, it can be speculated that in lower abstinence time, the sperm is less exposed to male reproductive tract ROS than in higher abstinence time. Sperm DNA damage induced by ROS is associated with male infertility (Hosen *et al.*, 2015), poor embryo development (Wdowiak *et al.*, 2015), lower pregnancy rates after both IVF and intracytoplasmic sperm injection (ICSI) (Simon *et al.*, 2017), and recurrent pregnancy loss (Shamsi *et al.*, 2011).

Also, in one retrospective study including 479 ICSI cycles, two APs were compared for autologous and donor oocytes as short (≤ 2 days) or long (≥ 3 days), respectively. The results showed that short abstinence time is accompanied by improved fertilization and implantation rates, lower abnormal oocyte rates and increased ongoing pregnancy rates in oocyte donation cycles. One of the mechanisms for this was related to decreased sperm DNA fragmentation. (Pujol *et al.*, 2023).

In one study in 2023, it was assessed if clinical outcomes in oocyte autologous and donation cycles were related to male sexual abstinence or not. The results showed that long abstinence time was linked to higher sperm concentration, short abstinence improved fertilization and implantation rates, and lower abnormal zygote rates (1PN, 3PN, >3PN and degenerated oocytes) in oocyte donation cycles. As well, ongoing pregnancy rates were increased compared to prolonged abstinence in both oocyte donation and

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Table 1. Semen parameters after World Health Organization recommended (2–7 days), and short abstinence (1–2 h)

	First ejaculation (2–7 days)	Second ejaculation (1–2 h)	Absolute change (Short to recommended)	
Sperm parameters	Mean ± SD	Mean ± SD	Mean ± SD (CI)	*P*-value*
Volume (ml)	2.69 ± 1.25	1.40 ± 0.67	1.28 ± 0.80 (1.05–1.51)	<0.001
Concentration (mil/ml)	10.40 ± 2.64	8.76 ± 2.46	1.64 ± 3.37 (0.67–2.60)	0.001
Total sperm count	28.53 ± 5.62	12.24 ± 7.21	16.28 ± 11.38 (13.05–19.52)	<0.001
Progressive Motility (%)	13.32 ± 5.29	20.52 ± 8.19	−7.20 ± 8.21(−9.5–4.86)	<0.001
Non-Progressive Motility (%)	48.86 ± 9.47	53.46 ± 9.35	−4.60 ± 12.48 (−8.14–1.05)	0.012
Immotile (%)	38.02 ± 6.80	26.82 ± 5.32	11.20 ± 8.87 (8.67 ± 13.72)	<0.001
Normal Morphology (%)	1.46 ± 0.93	2.46 ± 0.83	−1.00 ± 0.78 (−1.22–0.77)	<0.001
Viability (%)	77.96 ± 8.06	83.90 ± 6.82	−5.94 ± 3.98 (−7.07–4.8)	<0.001
viscosity	77.96 ± 8.06	83.90 ± 6.82	0.18 ± 1.43 (0.22–0.59)	0.37
DFI	26.96 ± 6.16	19.5 ± 3.67	7.46 ± 4.10 (6.29–8.62)	<0.001

*Paired sample *T*-test showed semen volume, sperm concentration and total sperm count were below the WHO percentile in the short abstinence of 1–2 h. It's in while that progressive, non-progressive motility, normal morphology and sperm viability increased during the second ejaculation. Lower DNA fragmentation was found in the short abstinence time. The *P*-value $P < .05$ was considered significant.

Table 2. The association between sperm parameters and ejaculatory abstinence length

	Coef*	CI (95% conf.interval)	*P*-value**
Volume (ml)	−1.28	−1.67, −0.89	<0.001
Concentration (mil/ml)	−1.64	−2.63, −0.64	0.001
Total sperm count	−16.28	−21.00, −11.56	<0.001
Progressive Motility (%)	7.2	4.52, 9.87	<0.001
Non-Progressive Motility (%)	4.6	0.94, 8.25	0.01
Immotile (%)	−11.2	−13.57, −8.82	<0.001
Normal Morphology (%)	1	0.65, 1.34	<0.001
Viability (%)	5.94	3.04, 8.83	0.0001
viscosity	−0.18	−0.53, 0.16	0.309
DFI	−7.46	−9.42, −5.49	<0.001

*Coefficient regression
**Linear mixed model analysis showed progressive, non-progressive motility, normal morphology and viability increased with shorter AP and decreased with lengthier. Volume, concentration, total sperm count, immotile sperm and sperm DNA fragmentation also decreased with the shorter AP ($P < .05$). Viscosity showed no relationship with AP. The *P*-value $P < .05$ was considered significant.

autologous cycles (Pujol *et al.*, 2023). Longer periods of abstinence might have an impact on the degree of sperm DNA fragmentation, a characteristic that cannot be found in the routine semen analysis.

In practice, these results have several implications. First of all, it showed that, however, short AP in men with OAT sperm reduced limited parameters such as sperm count and volume and is not detrimental to many others. Remaining conventional semen parameters within the established WHO reference value, the sperm DNA integrity is minimal. The results indicate that long AP is detrimental to males diagnosed with OAT, and daily intercourse may improve sperm quality in these cases. Historically, it's recommended that couples who attempt to conceive naturally have intercourse every other day.

The other implication is that reduced AP may be a good strategy for lowering sperm DNA fragmentation and increasing the chances of successful assisted reproduction in OAT patients. Many studies showed that sperm DNA integrity is critical to the successful outcome of pregnancy, whether natural or assisted reproduction (Rilcheva *et al.*, 2016, Ribas-Maynou *et al.*, 2022, Alahmar *et al.*, 2022).

In this study, we investigated if a short time of 1–2 h vs. The routine recommended time (2–7 days) for abstinence could improve the sperm parameters in the OAT or not. One of our limitations was the small sample size, as well as having no data regarding pregnancy rates or ART cycles. From these data, it's hard to support the conclusions regarding the influence of short APs on OAT patients' sperm parameters. These findings, along with other future data (E.g., embryo transfer outcomes), will provide rigorous evidence for supporting shorter abstinence times, to improve IVF outcomes in these patients. Future similar studies with a larger sample size are recommended to investigate the best time for abstinence in OAT patients, which also focus more on sperm DNA health as well as ART outcomes.

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Conclusions

Our findings provide further evidence behind short APs in OAT samples, which found an increase in semen volume, sperm concentration, sperm motility, morphology and viability. Also, there's a substantial improvement in DNA integrity. Taking all data into account, an additional short AP may be a simple and helpful strategy to obtain better sperm quality in couples with male infertility causes, especially in OAT patients. The recommended current guidelines regarding the AP may need to be revisited in severe male factors.

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Competing interests. There are no conflicts of interest to be declared.

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Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

References

Agarwal, A., Gupta, S., Du Plessis, S., Sharma, R., Esteves, S.C., Cirenza, C., Eliwa, J., Al-Najjar, W., Kumaresan, D. and Haroun, N. (2016a) Abstinence time and its impact on basic and advanced semen parameters. *Urology* **94**, 102–110.

Agarwal, A., Gupta, S., Du Plessis, S., Sharma, R., Esteves, S.C., Cirenza, C., Eliwa, J., Al-Najjar, W., Kumaresan, D., Haroun, N., Philby, S. and Sabanegh, E. (2016b) Abstinence time and its impact on basic and advanced semen parameters. *Urology* **94**, 102–110.

Alahmar, A.T., Singh, R. and Palani, A. (2022) Sperm DNA fragmentation in reproductive medicine: a review. *Journal Of Human Reproductive Sciences* **15**, 206–218.

Ayad, B.M., Horst, G.V. and Plessis, S.S.D. (2018a) Revisiting the relationship between the ejaculatory abstinence period and semen characteristics. *Int J Fertil Steril* **11**, 238–246.

Ayad, B.M., Van Der Horst, G. and Du Plessis, S.S. (2018b) Short abstinence: a potential strategy for the improvement of sperm quality. *Middle East Fertility Society Journal* **23**, 37–43.

Barbagallo, F., Cannarella, R., Crafa, A., La Vignera, S., Condorelli, R.A., Manna, C. and Calogero, A.E. (2023) The impact of a very short abstinence period on assisted reproductive technique outcomes: a systematic review and meta-analysis. *Antioxidants* **12**, 752.

Barbagallo, F., Cannarella, R., Crafa, A., Manna, C., La Vignera, S., Condorelli, R.A. and Calogero, A.E. (2022) The impact of a very short abstinence period on conventional sperm parameters and sperm DNA fragmentation: a systematic review and meta-analysis. *Journal Of Clinical Medicine* **11**, 7303.

Bonanno, O., Romeo, G., Asero, P., Pezzino, F.M., Castiglione, R., Burrello, N., Sidoti, G., Frajese, G.V., Vicari, E. and D'agata, R. (2016) Sperm of patients with severe asthenozoospermia show biochemical, molecular and genomic alterations. *Reproduction* **152**, 695–704.

Borges, E., Zanetti, B.F., Setti, A.S., Braga, D.P.D.A.F., Provenza, R.R. and Iaconelli, A. (2019) Sperm DNA fragmentation is correlated with poor embryo development, lower implantation rate, and higher miscarriage rate in reproductive cycles of non–male factor infertility. *Fertility And Sterility* **112**, 483–490.

Chatzimeletiou, K., Fleva, A., Nikolopoulos, T.-T., Markopoulou, M., Zervakakou, G., Papanikolaou, K., Anifandis, G., Gianakou, A. and Grimbizis, G. (2023) Evaluation of sperm DNA fragmentation using two different methods: tunel via fluorescence microscopy, and flow cytometry. *Medicina* **59**, 1313.

Chatzimeletiou, K., Fleva, A., Sioga, A., Georgiou, I., Nikolopoulos, T.-T., Markopoulou, M., Petrogiannis, N., Anifandis, G., Patrikiou, A., Kolibianakis, E., Giannakou, A. and Grimbizis, G. (2022) Effects of different drug therapies and COVID-19 mRNA vaccination on semen quality in a man with ankylosing spondylitis: a case report. *Medicina* **58**, 173.

Comar, V.A., Petersen, C.G., Mauri, A.L., Mattila, M., Vagnini, L.D., Renzi, A., Petersen, B., Nicoletti, A., Dieamant, F., Oliveira, J.B.A., Baruffi, R.L.R. and Franco, J.G. Jr. (2017) Influence of the abstinence period on human sperm quality: analysis of 2,458 semen samples. *Jbra Assist Reprod* **21**, 306–312.

De Jonge, C., Lafromboise, M., Bosmans, E., Ombelet, W., Cox, A. and Nijs, M. (2004) Influence of the abstinence period on human sperm quality. *Fertility And Sterility* **82**, 57–65.

Degirmenci, Y., Demirdag, E., Guler, I., Yildiz, S., Erdem, M. and Erdem, A. (2020) Impact of the sexual abstinence period on the production of seminal reactive oxygen species in patients undergoing intrauterine insemination: a randomized trial. *Journal Of Obstetrics And Gynaecology Research* **46**, 1133–1139.

Drabovich, A.P., Saraon, P., Jarvi, K. and Diamandis, E.P. (2014) Seminal plasma as a diagnostic fluid for male reproductive system disorders. *Nature Reviews Urology* **11**, 278–288.

Hanson, B.M., Aston, K.I., Jenkins, T.G., Carrell, D.T. and Hotaling, J.M. (2018a) The impact of ejaculatory abstinence on semen analysis parameters: a systematic review. *Journal Of Assisted Reproduction And Genetics* **35**, 213–220.

Hanson, B.M., Aston, K.I., Jenkins, T.G., Carrell, D.T. and Hotaling, J.M. (2018b) The impact of ejaculatory abstinence on semen analysis parameters: a systematic review. *Journal Of Assisted Reproduction And Genetics* **35**, 213–220.

Hosen, M.B., Islam, M.R., Begum, F., Kabir, Y. and Howlader, M.Z. (2015) Oxidative stress induced sperm DNA damage, a possible reason for male infertility. *Iran J Reprod Med* **13**, 525–32.

Jurema, M.W., Vieira, A.D., Bankowski, B., Petrella, C., Zhao, Y., Wallach, E. and Zacur, H. (2005) Effect of ejaculatory abstinence period on the pregnancy rate after intrauterine insemination. *Fertility And Sterility* **84**, 678–681.

Karavani, G., Juvet, T.S.J., Lau, S., Lajkosz, K., Mullen, B., Lo, K.C., Grober, E.D., Akroof, B., Kattan, M.S. and Jarvi, K. (2023) Improved sperm DNA fragmentation levels in infertile men following very short abstinence of 3–4 hours. *Transl Androl Urol* **12**, 1487–1496.

Kulkarni, V., Kaingade, P., Kulkarni, N., Bhalerao, T. and Nikam, A. (2022) Assessment of semen parameters in consecutive ejaculates with short abstinence period in oligospermic males. *Jbra Assist Reprod* **26**, 310–314.

Levitas, E., Lunenfeld, E., Weiss, N., Friger, M., Har-Vardi, I., Koifman, A. and Potashnik, G. (2005) Relationship between the duration of sexual abstinence and semen quality: analysis of 9,489 semen samples. *Fertility And Sterility* **83**, 1680–1686.

Nordhoff, V. (2015) How to select immotile but viable spermatozoa on the day of intracytoplasmic sperm injection? An embryologist's view. *Andrology* **3**, 156–162.

Organization, W.H. (2021) *Who Laboratory Manual for the Examination and Processing of Human Semen*. Geneva: World Health Organization.

Pujol, A., Romera, L., Lafuente, R., Popovic, M. and Mataró, D. (2023) P-036 relationship between days of male sexual abstinence on the day of oocyte retrieval and clinical outcomes. *Human Reproduction* **38**, dead093.403.

Ribas-Maynou, J., Novo, S., Torres, M., Salas-Huetos, A., Rovira, S., Antich, M. and Yeste, M. (2022) Sperm DNA integrity does play a crucial role for embryo development after ICSI, notably when good-quality oocytes from young donors are used. *Biological Research* **55**, 41.

Rilcheva, V.S., Ayvazova, N.P., Ilieva, L.O., Ivanova, S.P. and Konova, E.I. (2016) Sperm DNA integrity test and assisted reproductive technology (art) outcome. *Journal Of Biomedical And Clinical Research*, **9**, 21–29.

Shamsi, M.B., Venkatesh, S., Pathak, D., Deka, D. and Dada, R. (2011) Sperm DNA damage & oxidative stress in recurrent spontaneous abortion (RSA). *Indian Journal Of Medical Research* **133**, 550–551.

Shen, Z.-Q., Shi, B., Wang, T.-R., Jiao, J., Shang, X.-J., Wu, Q.-J., Zhou, Y.-M., Cao, T.-F., Du, Q. and Wang, X.-X. (2019) Characterization of the sperm proteome and reproductive outcomes with in vitro, fertilization after a

reduction in male ejaculatory abstinence period. *Molecular & Cellular Proteomics* **18**, S109–S117.

Simon, L., Zini, A., Dyachenko, A., Ciampi, A. and Carrell, D.T. (2017) A systematic review and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome. *Asian Journal Of Andrology* **19**, 80–90.

Sokol, P., Drakopoulos, P. and Polyzos, N.P. (2021) The effect of ejaculatory abstinence interval on sperm parameters and clinical outcome of art. A systematic review of the literature. *Journal Of Clinical Medicine* **10**, 3213.

Sokol, P., Racca, A., Ballester, M., Rodríguez, I. and Polyzos, N.P. (2023) P-062 the influence of the ejaculatory abstinence period on semen parameters from the first diagnostic sample – results from analysis of 14000 samples. *Human Reproduction* **38**, dead093.427.

Taaffe, P., O'meara, C.M., Stiavnicka, M., Byrne, C.J., Eivers, B., Lonergan, P. and Fair, S. (2022) Increasing the frequency of ejaculate collection in young dairy bulls increases semen production and field fertility. *Theriogenology* **182**, 45–52.

Treulen, F., Uribe, P., Boguen, R. and Villegas, J.V. (2015) Mitochondrial permeability transition increases reactive oxygen species production and induces DNA fragmentation in human spermatozoa. *Human Reproduction* **30**, 767–776.

Vončina, S.M., Golob, B., Ihan, A., Kopitar, A.N., Kolbezen, M. and Zorn, B. (2016) Sperm DNA fragmentation and mitochondrial membrane potential combined are better for predicting natural conception than standard sperm parameters. *Fertility And Sterility* **105**, 637–644. E1.

Wdowiak, A., Bakalczuk, S. and Bakalczuk, G. (2015) The effect of sperm DNA fragmentation on the dynamics of the embryonic development in intracytoplasmatic sperm injection. *Reproductive Biology* **15**, 94–100.

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