

The Effect of Prolonged Incubation of Sperm at Testis Temperature versus Room Temperature on Semen Parameters: An Experimental Study

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ABSTRACT

Background & Objective: The effect of storage time and temperature on the prepared semen sample was evaluated, but the optimal condition is unclear. The aim of this study was to assess the effect of long-term incubation of prepared sperm at testicular temperature versus room temperature on semen parameters and DNA fragmentation index (DFI).

Materials & Methods: Sperm samples were collected from 40 patients between 2019 and 2020. Each sample was separated into two parts and underwent a non-direct swim-up method. One group was placed in a 35°C incubator, and the other group was kept at room temperature (26°C) in the dark. Both groups were evaluated at intervals of 45 minutes, 24 hours and 48 hours after sampling in terms of sperm concentration, motility, morphology, and DFI. Student t-test and repeated measures analysis of variance were used.

Results: Sperm count ($P=0.007$) and motility ($P<0.001$) at 26°C in three-time intervals of 45 minutes, 24 hours and 48 hours were significantly higher than 35°C. The proportion of normal morphology spermatozoa at 26 and 35°C at 45 min, 24 h, and 48 h did not show a significant difference ($P=0.08$). DFI at 26°C in three-time intervals was significantly lower than 35°C ($P=0.008$).

Conclusion: The results of this study indicated that when the prepared sperm samples are incubated for 24 h at 26°C compared to 35°C, they show significantly better quality and good quality of sperm can be retained for several hours if stored at room temperature.

Keywords: Assisted reproductive techniques, DNA fragmentation, Insemination, Spermatozoa, Temperature



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Introduction

Infertility is defined as the failure of achieving pregnancy after ≥ 12 months of unprotected attempts, and of all infertility cases, a male component is responsible for almost half of couple infertility (1).

Semen quality is a vital factor contributing to all infertility treatment options' success rate, including assisted reproductive techniques (ART). Most widely ART used for male factor infertility includes intrauterine insemination (IUI), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI). Furthermore, as an essential technical part of ART, sperm preparation must be performed with minimal stress to spermatozoa, reducing its functionality and DNA damage. Semen quality is affected by some laboratory factors, including different methods of sperm preparation (2), the interval between sperm preparation and clinical use (3), and the incubation temperature of the

sperm at different stages of preparation (4, 5). During the preparation of sperm, two-time intervals are very important: the time between the collection of semen and giving it to a laboratory for the preparation and the time between the processed sperm and clinical use. Prolonged in-vitro incubation of spermatozoa can have deleterious effects on its fertilizing ability and DNA integrity by oxygen radicals produced from white blood cells, bacteria and dead sperm (6, 7).

Also, incubation temperature is another important and effective factor in the quality of ejaculated spermatozoa. Testicular temperature is about 2 to 3 degrees lower than the body temperature, which is necessary for the maintenance of spermatogenesis. Acrosome reactions and capacitation can be achieved by placing sperm in conditions such as incubation at body temperature (8).

The effect of storage time and temperature on prepared semen samples was evaluated in some studies, but there is no consensus; while some studies indicated unfavorable results, the others were in accordance (9-12)

It is still under consideration in most IVF laboratories to supply prepared sperm samples at this temperature prior to use in ART. The aim of this study was to investigate the efficacy of long-term incubation of prepared sperm at testicular temperature (35°C) versus room temperature (26°C) on semen parameters and DNA fragmentation index (DFI).

Methods

Experimental Design

This study was conducted in the Department of Andrology, Mehr Medical Institute, Rasht, Iran. In this experimental study, sperm samples were collected from 40 patients between 2019 and 2020.

Inclusion criteria were sperm concentration ≥ 15 million/mL, motility $\geq 32\%$, and normal sperm morphology $\geq 4\%$ according to WHO criteria (13) for a normal semen sample. Samples with volume < 2 mL and leukocyte more than 2×10^6 mL⁻¹ were excluded.

Each sample was divided into two parts and subjected to a non-direct swim-up method. The filtered serum culture medium was added to each portion in a ratio of 1:4 and centrifuged at 3000 rpm for 3 minutes. Following the supernatant collection, 0.5 mL of serum culture medium (5% albumin + 9.5% HAMS F10) was added to the sediment in both groups. One group was incubated at 35°C, and the other was kept at room temperature (26°C) in the dark. The room temperature was constant and was kept constant by the air conditioner. Laboratory temperature was recorded continuously.

Both groups were evaluated at intervals of 45 min, 24 hours, and 48 hours after sampling in terms of sperm number, motility, morphology, and DFI.

Sperm Analysis:

Semen samples were obtained following 2 days of abstinence. To liquefy the semen sample, it was first placed at 35°C for 30 minutes, and semen analysis (number, mobility) was performed according to WHO guidelines.

All samples were assessed twice for concentration and the percentage of motility of spermatozoa both in raw semen and in each experimental group (26°C and 35°C) during time intervals under a magnification of $\times 40$ and took into account the mean value of the two evaluations.

Morphology assessment was performed by Diff Quick assay in raw semen and experimental groups in time intervals. To calculate the percentage of sperm-

atozoa with normal morphology using strict criteria, 200 spermatozoa in each slide were evaluated under a magnification of $\times 100$ after Diff-Quik staining.

Sperm DNA Fragmentation

A sperm chromatin dispersion kit (Avicenna, Tehran, Iran) was used to assess sperm DNA fragmentation. To fuse the agarose, tubes with aliquots of low-melting-point agarose were put in a water bath for 5 min at 94°C. After 5 min, fused agarose was mixed with 20 μ L of the semen samples. To the pre-coated slides, the semen/agarose mix was pipetted. To permit the agarose to make a microgel embedded with the spermatozoa, the slides were then refrigerated (4°C) for 5 min. Subsequently, the slides were immersed horizontally into solution A for 7 min and solution B for 15 min. Then the slides were washed by placing in distilled water for 5 min and were dehydrated in ascending ethanol (70%, 90%, and 100%) for 2 min each and ultimately air-dried. Following the staining procedure, 300 spermatozoa were counted, and the following formula was used to calculate the percentage of sperm DNA fragmentation: ((fragmented cells + degraded cells)/total cells).

Statistical Analyzes

Statistical analyses were performed using SPSS software version 21. Continuous variables with normal distribution were presented as mean \pm standard deviation (SD). The significance level was considered 0.05. Student t-test was used to evaluate the significant difference between the two groups. Repeated measures analysis of variance was used to assess statistically significant differences over time.

Ethical Consideration

The study has been approved by the ethics committee of Guilan University of Medical Sciences (ethical code: IR.GUMS.REC.1398.204).

Results

Forty men with a mean sperm count of 42.9 ± 15.3 and motility of 66.3 ± 10.2 were included in the present study. A schematic overview of the design of the study is presented in [Figure 1](#).

Sperm count ($P=0.007$) and motility ($P<0.001$) at 26°C in three-time intervals of 45 minutes, 24 hours, and 48 hours were significantly higher than 35°C. The differences in the percentage of spermatozoa with normal morphology at 26 and 35°C at 45 minutes, 24 hours, and 48 hours were not statistically significant ($P=0.08$). Our results showed that there was no significant difference in normal morphology sperm at 26 and 35°C ($P=0.103$); however, it showed statistically significant differences at different time intervals. DFI at 26°C in three-time intervals was significantly lower than 35°C ($P=0.008$) ([Table 1](#)).

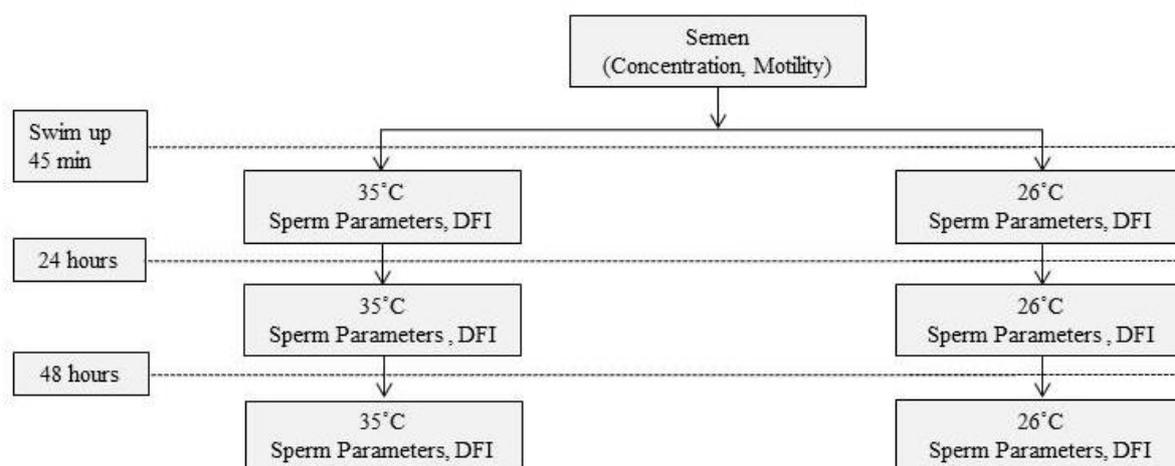


Figure 1. Schematic overview of the design of the study DFI: DNA fragmentation index

Table 1. Sperm concentration, motility, morphology, and DFI (mean \pm SD), obtained at 45min, 24 hours, and 48 hours after the start of the experiment.

	45min	24 hours	48 hours	P-value Time- Temperature	P- value Time	P-value Temperature
Sperm concentration ($\times 10^6$)						
26°C	22.43 \pm 1.6	5.96 \pm 1.9	2.4 \pm 2.1	0.007	<0.001	0.045
35°C	28.46 \pm 1.7	2.87 \pm 1.8	0.95 \pm 1.5			
Sperm motility (%)						
26°C	97.98 \pm 1.37	87.4 \pm 17.78	63.6 \pm 38.5	<0.001	<0.001	<0.001
35°C	98.6 \pm 0.75	40.25 \pm 30.7	5.5 \pm 15.7			
Sperm morphology (%)						
26°C	11.8 \pm 1.5	9.9 \pm 1.5	6.9 \pm 1.6	0.08	<0.001	0.103
35°C	9.04 \pm 1.8	8.4 \pm 2	3.7 \pm 1.8			
DFI						
26°C	16.08 \pm 2.4	19.7 \pm 2.4	21.2 \pm 2.2	0.008	<0.001	0.04
35°C	16.08 \pm 2.7	20.9 \pm 2.4	22.98 \pm 2.2			

Repeated measures analysis of variance, DFI: DNA fragmentation index

Discussion

In the present study, we aimed to evaluate the effect of long-term incubation of prepared sperm at testicular temperature (35°C) versus room temperature (26°C) on semen parameters and DFI. Sperm concentration and motility at 26°C were significantly higher than 35°C. Normal morphology of spermatozoa at 26 and 35°C did not significantly differ over time. There was a significant increase in DFI at 26 and 35°C during time intervals but, DFI at 26°C in three-time intervals was significantly lower than 35°C.

Preparation of semen samples during diagnostic or therapeutic steps usually involves exposing the sperm to room temperature for various periods. Some reports indicate the effect of temperature on sperm motility and spontaneous acrosomal reaction (8, 14). In-vitro studies have shown several transmembranes and intracellular signaling pathways, which reveal that

protein tyrosine phosphorylation may be affected by the incubation temperature of spermatozoa. It affects the permeability and peroxidation of membrane lipids as well as the distribution of antigen in the sperm membrane (15, 16).

Following 24 and 48 hours of incubation at 35 and 26°C, sperm quality (concentration, motility, and morphology) were decreased, and an increase in the number of dead spermatozoa was observed. This decline was significantly lower at 26°C than 35°C. It seems that increased temperature increases the metabolic activity of sperm, the number of sperm with vacuolated nuclei, and decreases its viability (17).

Aboulmaouhib *et al.* (18) conducted a study in 2016 to evaluate the effects of various sperm incubation conditions including temperature (23 vs. 35°C) and

CO₂ on sperm quality during 24 hours. They concluded that sperm quality could be retained for several hours at room temperature, while density-gradient centrifugation technique/80P without CO₂ is used to process sperm preparation.

The results of the study by Petrella *et al.* (19) showed that prepared sperm could live at room temperature for weeks. According to Petrella *et al.* study, the samples were divided into 4 groups: the first group - raw sample at 23°C, the second group - prepared sample + 0.5 mL of human tubal fluid at 37°C, the third group - prepared sample + 0.5 ml of modified human tubal fluid at 4°C, group 4 - Prepared sample + 0.5 ml of modified human tubal fluid at 23°C. Mobility in the first group decreased rapidly over 3 days, although viable sperm were available two weeks later. In modified human tubal fluid, storage at room temperature was superior to other conditions.

In another study by Lachaud *et al.* (20), it was shown that after incubation at 37°C for 4 hours, there was no change in sperm parameters. Still, after 24 hours of incubation, a significant reduction in sperm motility and viability was observed. However, these changes were not associated with an increase in the incidence of markers of active apoptotic processes.

The present study results indicated that long-time incubation of spermatozoa has a significantly negative impact on sperm parameters quality.

A study by Yavas *et al.* (21) showed more pregnancy rate if the time between processing of sample and insemination was under 60 minutes. In another study, it was shown that the best rate of clinical pregnancy is 40-80 minutes after sperm preparation (22). In a study conducted by Jansen *et al.* (23), two groups of patients underwent IUI. The first group - performing insemination within 24 hours after semen collection and preparation (delayed insemination); the second group - performing insemination immediately after collection and preparation (immediate insemination). The two groups did not show any significant difference in ongoing pregnancy rate.

In the present study, DFI was evaluated at time intervals of 45 min and 24 hours and 48 hours, but more samples had not enough sperm for SCA analyzing after 48 hours. Thus, 40 other men with higher total sperm count ($>40 \times 10^6$) were examined for DFI, and the results showed that DFI in sperm incubated at 26°C is significantly lower than that at 35°C. In contrast, sperm DFI showed a significant increase with increasing incubation time.

The effects of prolonged incubation time on sperm DNA breaks, chromatin condensation, and subsequently, fertilization rate was evaluated in a study by Ahmed *et al.* (24). The Pure Sperm Gradient centrifugation method was used to prepare the semen. The samples were then divided into two parts, with the first part used immediately for ICSI, whereas the second one was kept in the incubator for 5 hours at 37°C, 5%

CO₂, and 90% humidity. Their results showed that sperm incubation for about 5 hours could cause a significant increase in chromatin condensation and DNA double strands break, while no influence on fertilization rates was observed.

To evaluate the effects of incubation and preparation conditions on sperm DNA integrity, Matsuura *et al.* (12) showed that although the DFI increased in a time-related manner by incubation at room temperature and 37°C, DFI following 24 hours of incubation at room temperature was significantly lower than that at 37°C. A study by Jackson *et al.* (9) showed that short-term storage of sperm (4 hours) at room temperature did not affect sperm DNA fragmentation. However, storage for 24 hours significantly increases fragmentation. A study that aimed at assessing the rate of DNA fragmentation using the sperm chromatin dispersion method followed by the swim-up at different time intervals (0, 1, 2 and 3 hours) showed that long-term incubation of normozoospermic samples was shown to be associated with a higher rate of sperm DNA fragmentation. Therefore, sperm samples planned for ART procedures should be used following incubation for 2 hours at body temperature (25).

One of the by-products of sperm metabolism in sperm culture medium is reactive oxygen species (ROS). While small amounts of ROS, at the physiological level, play an important role in controlling sperm function, exposure to very high levels of ROS is harmful to sperm (26). Enzymes responsible for phosphorylation/dephosphorylation of tyrosine proteins are potential targets of ROS. As a result, mild oxidative conditions increase tyrosine phosphorylation and acrosomal reaction. Lipid peroxidation due to a low level of ROS leads to sperm binding to zona pellucid, which may release non-esterified fatty acids from the plasma membrane of sperm (27). However, high levels of ROS are caused by abnormal leukocytes or sperm that can overcome the seminal plasma antioxidant defense barrier, leading to the peroxidation of plasma membrane unsaturated fatty acids and DNA damage (28).

A study conducted by Tvrdá *et al.* (29) in 2019 investigated the effects of adjustment of sperm concentration of ejaculates on the quality of sperm DNA and longevity. After centrifugation and resuspension of the sperm pellet in PBS, the samples were adjusted into different concentrations ranging from 200, 100, 50, 25, 12, and 6×10^6 /mL. Each sample was incubated at 37°C for 24 hours. The results showed that a lower sperm concentration (below 25×10^6 /mL) should be used for spermatozoa incubation for ART purposes to avoid a higher sperm DNA fragmentation rate. They conclude that sperm DNA fragmentation of the pellet will increase following 24 hours in higher concentration at 35°C regardless of the status of the cell. In that study, a rise in DFI rate was observed in samples with higher sperm concentration (regardless of viability).

In our study, we performed a test for living cells by using the swim-up method following centrifugation. We showed that there is a considerable rise in DFI in living sperm cells during 48 h. We also showed that sperm incubation at room temperature could lead to sperm DNA longevity.

Conclusion

The results of this study indicated that when the prepared sperm samples are incubated for 24 hours at 26°C compared to 35°C, they show significantly better quality. However, further research is required as to whether these results can also improve pregnancy rates. If long-term incubation of sperm in the laboratory is

possible without compromising its quality, this could be a significant opportunity for the physician and laboratory staff to improve the treatment of patients and provide more power of choice. It seems that this might be considered only for samples with a concentration of more than 40 million.

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The authors declared no acknowledgment.

Conflict of Interest

The authors declared no conflict of interest.

References

- Ilacqua A, Izzo G, Emerenziani GP, Baldari C, Aversa A. Lifestyle and fertility: the influence of stress and quality of life on male fertility. *Reprod Biol Endocrinol*. 2018;16(1):115. [PMCID] [DOI:10.1186/s12958-018-0436-9] [PMID]
- Ricci G, Perticarari S, Boscolo R, Montico M, Guaschino S, Presani G. Semen preparation methods and sperm apoptosis: swim-up versus gradient-density centrifugation technique. *Fertil Steril*. 2009;91(2):632-8. [DOI:10.1016/j.fertnstert.2007.11.068] [PMID]
- Marín-Briggiler CI, Tezón JG, Miranda PV, Vazquez-Levin MH. Effect of incubating human sperm at room temperature on capacitation-related events. *Fertil Steril*. 2002;77(2):252-9. [DOI:10.1016/S0015-0282(01)02982-X]
- Franken DR, van Wyk R, Stoumann C, Avari K. Temperature controlled centrifugation improves sperm retrieval. *Andrologia*. 2011;43(3):217-21. [DOI:10.1111/j.1439-0272.2010.01136.x] [PMID]
- Thijssen A, Klerkx E, Huysen C, Bosmans E, Campo R, Ombelet W. Influence of temperature and sperm preparation on the quality of spermatozoa. *Reprod Biomed Online*. 2014;28(4):436-42. [DOI:10.1016/j.rbmo.2013.12.005] [PMID]
- Boomsma CM, Heineman MJ, Cohlen BJ, Farquhar C. Semen preparation techniques for intrauterine insemination. *Cochrane Database Syst Rev*. 2004;17(3):CD004507. [DOI:10.1002/14651858.CD004507.pub2]
- Bui AD, Sharma R, Henkel R, Agarwal A. Reactive oxygen species impact on sperm DNA and its role in male infertility. *Andrologia*. 2018;50(8):e13012. [DOI:10.1111/and.13012] [PMID]
- White DR, Phillips DM, Bedford JM. Factors affecting the acrosome reaction in human spermatozoa. *J Reprod Fertil*. 1990;90(1):71-80. [DOI:10.1530/jrf.0.0900071] [PMID]
- Jackson RE, Bormann CL, Hassun PA, Rocha AM, Motta EL, Serafini PC, et al. Effects of semen storage and separation techniques on sperm DNA fragmentation. *Fertil Steril*. 2010;94(7):2626-30. [DOI:10.1016/j.fertnstert.2010.04.049] [PMID]
- Hosseini A, Khalili MA. Improvement of motility after culture of testicular spermatozoa: the effects of incubation timing and temperature. *Transl Androl Urol*. 2017;6(2):271-6. [PMCID] [DOI:10.21037/tau.2017.03.43] [PMID]
- Çok T, Aytaç PÇ, Şimşek E, Haydardedeoğlu B, Kalaycı H, Özdemir H, et al. The effect of preserving prepared sperm samples at room temperature or at 37 C before intrauterine insemination (IUI) on clinical pregnancy rate. *Turk J Obstet Gynecol*. 2015;12(1):6-10. [DOI:10.4274/tjod.31644] [PMID] [PMCID]
- Matsuura R, Takeuchi T, Yoshida A. Preparation and incubation conditions affect the DNA integrity of ejaculated human spermatozoa. *Asian J Androl*. 2010;12(5):753-9. [DOI:10.1038/aja.2010.46] [PMID] [PMCID]
- Organization WH. WHO laboratory manual for the examination and processing of human semen. 2010.
- Cohen J, Fehilly CB, Walters DE. Prolonged storage of human spermatozoa at room temperature or in a refrigerator. *Fertil Steril*.

- 1985;44(2):254-62. [DOI:10.1016/S0015-0282(16)48747-9]
15. Villarroya S, Scholler R. Lateral diffusion of a human sperm-head antigen during incubation in a capacitation medium and induction of the acrosome reaction in vitro. *J Reprod Fertil.* 1987;80(2):545-62. [DOI:10.1530/jrf.0.0800545] [PMID]
 16. Osheroff JE, Visconti PE, Valenzuela JP, Travis AJ, Alvarez J, Kopf GS. Regulation of human sperm capacitation by a cholesterol efflux-stimulated signal transduction pathway leading to protein kinase A-mediated up-regulation of protein tyrosine phosphorylation. *Mol Hum Reprod.* 1999;5(11):1017-26. [DOI:10.1093/molehr/5.11.1017] [PMID]
 17. Peer S, Eltes F, Berkovitz A, Yehuda R, Itsykson P, Bartoov B. Is fine morphology of the human sperm nuclei affected by in vitro incubation at 37° C? *Fertil Steril.* 2007;88(6):1589-94. [DOI:10.1016/j.fertnstert.2007.01.069] [PMID]
 18. Aboulmaouhib S, Madkour A, Kaarouch I, Saadani B, Sefrioui O, Louanjli N, et al. Effect of semen preparation technique and its incubation on sperm quality in the Moroccan population. *Andrologia.* 2017;49(6):1-7. [DOI:10.1111/and.12688] [PMID]
 19. Petrella C, Hsieh J, Blake E, Thrift K, Zacur H, Zhao Y. Human sperm can survive at room temperature for weeks: Measured by motility and viability of sperm maintained under various conditions. *Fertil Steril.* 2003;80:210. [DOI:10.1016/S0015-0282(03)01468-7]
 20. Lachaud C, Tesarik J, Canadas ML, Mendoza C. Apoptosis and necrosis in human ejaculated spermatozoa. *Hum Reprod.* 2004;19(3):607-10. [DOI:10.1093/humrep/deh130] [PMID]
 21. Yavas Y, Selub MR. Intrauterine insemination (IUI) pregnancy outcome is enhanced by shorter intervals from semen collection to sperm wash, from sperm wash to IUI time, and from semen collection to IUI time. *Fertil Steril.* 2004;82(6):1638-47. [DOI:10.1016/j.fertnstert.2004.04.061] [PMID]
 22. Fauque P, Lehert P, Lamotte M, Bettahar-Lebugle K, Bailly A, Diligent C, et al. Clinical success of intrauterine insemination cycles is affected by the sperm preparation time. *Fertil Steril.* 2014;101(6):1618-23. [DOI:10.1016/j.fertnstert.2014.03.015] [PMID]
 23. Jansen CH, Elisen MG, Leenstra CW, Kaaijk EM, van Stralen KJ, Verhoeve HR. Longer time interval between semen processing and intrauterine insemination does not affect pregnancy outcome. *Fertil Steril.* 2017;108(5):764-9. [DOI:10.1016/j.fertnstert.2017.08.003] [PMID]
 24. Ahmed I, Abdelateef S, Laqqan M, Amor H, Abdel-Lah M, Hammadeh M. Influence of extended incubation time on human sperm chromatin condensation, sperm DNA strand breaks and their effect on fertilisation rate. *Andrologia.* 2018;50(4):1-8. [DOI:10.1111/and.12960] [PMID]
 25. Nabi A, Khalili MA, Halvaei I, Roodbari F. Prolonged incubation of processed human spermatozoa will increase DNA fragmentation. *Andrologia.* 2014;46(4):374-9. [DOI:10.1111/and.12088] [PMID]
 26. Aitken RJ. Free radicals, lipid peroxidation and sperm function. *Reprod Fertil Dev.* 1995;7(4):659-68. [DOI:10.1071/RD9950659] [PMID]
 27. de Lamirande E, Jiang H, Zini A, Kodama H, Gagnon C. Reactive oxygen species and sperm physiology. *Rev Reprod.* 1997;2(1):48-54. [DOI:10.1530/ror.0.0020048] [PMID]
 28. Aitken RJ, Clarkson JS. Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. *J Reprod Fertil.* 1987;81(2):459-69. [DOI:10.1530/jrf.0.0810459] [PMID]
 29. Tvrdá E, Arroyo F, Ďuračka M, López-Fernández C, Gosálvez J. Dynamic assessment of human sperm DNA damage II: the effect of sperm concentration adjustment during processing. *J Assist Reprod Genet.* 2019;36(4):799-807. [DOI:10.1007/s10815-019-01423-y] [PMID] [PMCID]

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