Original Article

Lower temperature (27°C) Decreased Total Motile Sperm Count but not DNA Fragmentation Index in Sperm Preparation with Swim-Up Method in Male Infertility

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ABSTRACT

Background

Temperature has a complex impact and is likely to influence the early stages of spermatogenesis. This study aimed to determine the effect of differential temperature in sperm preparation using the swim-up method on total motile sperm count (TMSC) and DNA fragmentation index (DFI) of male infertility.

Methods

This experimental study recruited 37 infertile male patients who attended the Sekar Fertility Clinic, Dr. Moewardi General Hospital, in November 2020, and we added more participants in December 2022. Before collecting semen samples, all infertile males signed informed consent. The collected sample is divided into 1 ml for sperm preparation in groups A (27°C) and B (37°C). The Wilcoxon test analyzed the data; $p < 0.05$ was considered significant.

Results

The mean TMSC result in Group A was 17.00 ± 13.06 x106 versus the mean TMSC in Group B at 21.19 ± 17.20 x106; p=0.004, indicating a lower TMSC in Group A. The mean DFI result in group A was 13.74 ± 10.54% versus group B at 13.35 ± 11.78%; p=0.261, also lower in group A.

Conclusion

Sperm preparation using the swim-up at 27°C significantly lowers TMSC in infertile males compared to 37°C, but the difference in DFI is not significant compared to 37°C.

Keywords

Male infertility; Temperature; DNA fragmentation; Sperm motility

INTRODUCTION

Infertility rates are increasing globally, affecting 50-80 million reproductive-age couples [1]. Male infertility is predominantly linked to intrinsic testicular disorders, which impact semen quality and reproductive outcomes [2]. DNA fragmentation is a common paternal DNA abnormality that can be transmitted to the next generation and is frequently identified in subfertile and infertile sperm [3]. This fragmentation negatively impacts semen parameters, including concentration, motility, morphology, and nuclear maturity. Total motile sperm count (TMSC), which combines volume, concentration, and motility [4], is a key indicator used in intrauterine insemination (IUI) to assess the likelihood of achieving pregnancy [5].

Effective sperm preparation methods, such as the

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"swim-up" technique, are crucial for isolating motile sperm during IUI [6]. These techniques help separate sperm from seminal fluid components and reduce the risk of uterine contractions during the procedure [6,7]. The swim-up method is commonly employed for sperm preparation due to its cost-effectiveness, practicality, and high efficiency in achieving superior sperm quality [8]. It also demonstrates a higher predictive value for IUI success compared to gradient techniques, even when sperm DNA fragmentation index (DFI) is elevated [8,9].

Temperature regulation during sperm preparation is a critical factor affecting sperm quality [10]. Maintaining an optimal temperature helps to minimize oxidative stress and DNA damage, which can lead to increased DNA fragmentation [11,12]. Additionally, as discussed in recent studies, obesity-induced reproductive complications, including decreased sperm viability and DNA integrity, are exacerbated by inflammation, oxidative stress, and hormonal imbalances [13]. Despite this, some fertility clinics employ higher incubation temperatures, aligning with the human body's internal temperature, to minimize disruptions to assisted reproductive technologies and reduce sperm quality decline [9]. This temperature variation poses a question of whether room temperature could serve as an efficient alternative during sperm preparation. Using room temperature could improve the efficiency of sperm preparation processes, particularly in resource-limited settings like Indonesia. Therefore, this study aims to explore the impact of different temperatures on sperm quality during preparation using the swim-up method, specifically examining effects on TMSC and DFI in male infertility cases.

MATERIALS AND METHODS

Study Population and Semen Sample

This experimental study recruited 37 infertile male patients of the Sekar Fertility Clinic, Dr. Moewardi General Hospital. According to the Vishwakarma study, we calculated this study's sample size and got at least 100 research participants [14]. However, the COVID-19 pandemic decreased the number of infertile couples who visited the clinic. Only 37 infertile males were eligible for this study. The eligibility criteria are undergoing the IUI program, early TMSC $\geq 5x10^6$, and sexual abstinence 2-7 days before the day of collecting their sperm. Sperm were prepared using swim-up for analysis 1-3 hours after ejaculation. Characteristic data of research participants were collected using a questionnaire, and anthropometric data were taken from their medical records.

Total Motile Sperm Count Calculation

All collected samples were divided into 1 ml for sperm preparation at 27°C and the other for preparation at 37°C. Calculation of sperm concentration used the Makler Chamber (Sefi Medical, Israel) and the detailed protocol referred to in a previous study [15]. Briefly, sperm concentration was obtained by counting the total sperm in 10 boxes in a Makler chamber. The outcome relates to the number of spermatozoa per unit volume of semen in milliliters (10^6). Progressive motility was counted manually by comparing the sperm moving actively with the total sperm observed and multiplying by 100%. Therefore, the TMSC was calculated using a formula: (volume of semen x sperm concentration x progressive motility) /100% [5].

DNA Fragmentation Index

DNA fragmentation index was assessed using a SpermFunc® DNAf (Fertitech, Canada) and followed the Sperm Chromatin Dispersion test. Initially, semen samples were diluted using normal saline to reach 5-10x10^6 final concentration. Next, the diluted sample was applied to a pre-coated slide and dipped into liquid A at 20-28°C for seven minutes. Afterward, the pre-coated slide was allowed to dry at room temperature, dip into liquid B, and then incubated precisely at 20-28°C for 25 minutes. After staining with liquid B, the pre-coated slide was washed with distilled water, 70% ethanol, 90% ethanol, and 100% ethanol for several minutes. Once the pre-coated slide had dried, it was observed under a light microscope with 400x magnification. The intact DNA was recognized by the halo formation of at least 33.3% in the sperm head. Therefore, the DFI was obtained by comparing the amount of fragmented DNA with total-observed sperm DNA and then multiplied by 100%. The average sperm usually had <30% DFI [16].

Sperm Preparation Using the Swim-up Method

In brief, 1 ml of semen sample was added to the culture medium on a sterile tube and then incubated at 37°C for 45 minutes with the tube's position tilted 45° [17]. Before that, the sperm pellet was pre-washed by 1500 rpm centrifugation for 5 minutes using Thermo Scientific Heraeus Labofuge® 300. The supernatant was removed, and the pellet was resuspended in 3 mL sperm wash media. Finally, the sperm samples were equally divided into two parts. The first part was kept in an incubator

at 27°C and the other at 37°C, each incubated for 20 minutes before insemination. We chose an incubator at 27°C because it is representative of the daily mean temperature in Surakarta, Indonesia. All year, climate and weather averages in Surakarta are between 24-34°C. This condition will vary depending on the environment and weather.

Statistical Analysis

All data obtained in this study were analyzed using the software Statistical Product and Service Solution (SPSS) Base Subscription for Mac Version 28.0.1, SPSS Inc., Chicago, Illinois, USA (SPSS). Categorical data were presented as frequency and percentage, while numerical data were presented as mean ± standard deviation. Because the data did not follow a normal distribution, the Wilcoxon test was used to compare the means of TMSC and DFI in both groups. The significance level was set at 0.05.

Ethical Clearance

The current study protocol was received for review and approval from the Research Ethics Committee at Dr. Moewardi General Hospital, Surakarta, Indonesia (as indicated by the approval number 951/ VII/ HREC/ 2020, dated July 23, 2020). Additionally, informed consent was obtained from all participants before their study enrollment.

RESULTS

Subjects in this study were dominated by men ≥30 years old, infertility period of ≥2 years, 165-179 cm tall, weight ≥70 kg, and falling into the overweight category with a BMI of ≥25-29.9 kg/m^2 (Table 1).

Table 1. Characteristics of the Subjects

Characteristics	Frequency	
	N	%
Age (years)		
<35	21	56.76
≥35	16	43.24
Duration of Infertility (years)		
<3	10	27.03
≥3	27	72.97
Height (cm)		
<165	2	5.41
≥165	35	94.59
Weight (kg)		
<65	8	21.62
≥65	29	78.38
BMI (kg/m^2)		
Underweight (<18.5)	2	5.41
Normal (≥18.5-24.9)	16	43.24
Overweight (≥25-29.9)	14	37.84
Obesity (≥30)	5	13.51
Smoking		
Yes	7	18.92
No	30	81.08

BMI: Body Mass Index

Based on Table 1, most of the study subjects (56.76%) are under 35 years old. A significant portion (72.97%) has been experiencing infertility for three years or longer. Regarding body measurements, most subjects (78.38%) weigh 65 kg or more, and nearly all (91.89%) are 165 cm tall or taller. Smoking habits are present in about one-fifth of the subjects (18.92%).

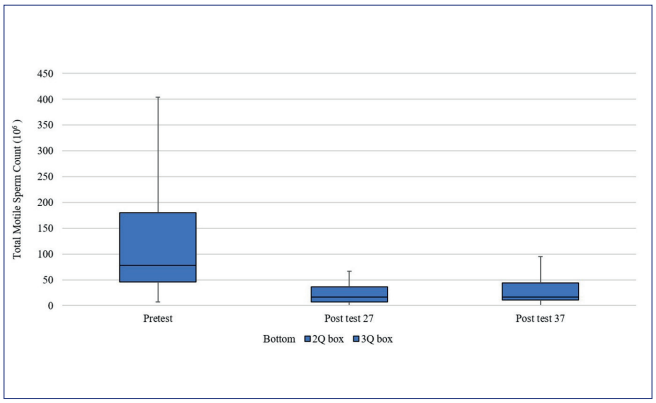

Figure 1. Comparison of TMSC between values pretest (A), post-test 27°C (B), and post-test 37°C (C).

The TMSC was calculated using a formula: (volume of semen x sperm concentration x progressive motility) /100%. The highest TMSC was found in 37 pretest sperm preparation samples, 81.83 ± 61.16.

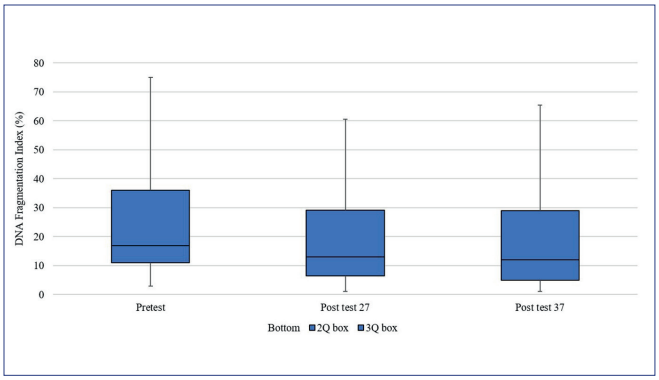

Figure 2. Comparison of DFI Values Pretest (A), Post-test 27°C (B), and Post-test 37°C (C).

The DFI assessment was done by counting the sperm with fragmentation in 500 sperm and multiplying it by 100%. The highest DFI was found in 37 pretest sperm preparation samples, 18.45 ± 11.80.

Figures 1 and 2 showed that a reduction of TMSC and DFI was observed after sperm preparation. Before sperm preparation, TMSC and DFI were 81.83 ± 61.16 x 10^6 and 18.45 ± 11.80%, respectively. In 27°C sperm preparation, TMSC and DFI's mean decreased significantly compared to the mean of TMSC and DFI before preparation ($p<0.001$; $p<0.001$). The same TMSC and DFI reduction patterns were found in the sperm preparation at 37°C ($p<0.001$; $p<0.001$). The mean of TMSC at 27°C sperm preparation significantly differs from 37°C. However, DFI's mean at 27°C sperm preparation did not differ significantly from that of TMSC and DFI at 37°C sperm preparation.

Table 2. Analysis of the Effect of Temperature on the TMSC and DFI Value with Wilcoxon Signed Rank Test.

Group	N	Mean ± Standard Deviation	Incubation Time	p-value
TMSC (x10^6)				
Swim-up 27°C	37	17.00 ± 13.06	20 minutes	0.004*
Swim-up 37°C	37	21.19 ± 17.20	20 minutes	
DFI (%)				
Swim-up 27°C	37	13.74 ± 10.54	20 minutes	0.261
Swim-up 37°C	37	13.35 ± 11.78	20 minutes	

*: significant, DFI: DNA fragmentation index, TMSC: Total Motile Sperm Count

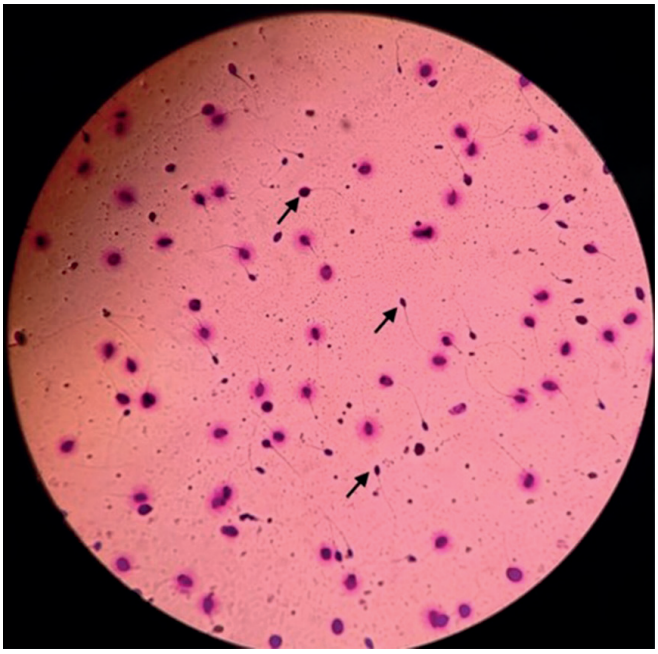

Figure 3. A black arrow is a type of fragmented sperm identified by the lack of a halo formation or a halo smaller than one-third of the diameter of the sperm head.

DISCUSSION

Effect of Sperm Preparation Temperature Swim-Up Method on TMSC

Temperature variations during sperm preparation significantly ($P<0.05$) impacted TMSC scores, with a noted reduction in TMSC post-preparation due to decreased sperm motility. In this study, the limited 1 ml sample volume potentially contributed to false hypothermia, reducing seminal vesicle secretion, which is crucial for sperm motility [18]. A smaller volume of seminal plasma can diminish protective factors for spermatozoa. Additionally, excessive reactive oxygen species (ROS) generated during preparation can damage the sperm membrane and lower motility [19,20]. Consequently, reduced motility adversely affects TMSC.

These findings contrast with Uribe P et al.[21] where TMSC decreased post-preparation, but differences between room temperature (22-25°C) and 37°C incubations were not significant. Progressive motility declined more at room temperature, attributed to sperm conserving energy and reducing motility [19]. Room temperature resulted in sperm resting, unlike hyperactivation seen at 37°C [20,21]. In a recent study, sperm motility at 26°C

was significantly higher than at 35°C [22]. Recent studies found higher motility at 26°C compared to 35°C, while extreme heat (40-42°C) reduced sperm motility and viability due to ROS-induced damage. The molecular response to heat stress involves pathways like Bcl-2, Bax, caspases, cytochrome C, and heat shock proteins, affecting membrane properties and intracellular signaling [22,23].

Effect of Sperm Preparation Temperature Swim-Up Method on DFI

Temperature differences during sperm preparation did not significantly impact sperm DFI scores. Both temperature groups showed effective DFI reduction, likely due to the swim-up method's ability to separate sperm from leukocytes, major ROS sources. As leukocytes produce ROS levels up to 100 times higher than normal, reducing their presence helps limit DNA damage [24,25].

Other factors that can influence DFI during preparation include culture media, centrifugation, light exposure, cryopreservation, incubation duration, temperature, and pH [11,24,26]. Secondary oxidative stress from exogenous ROS may also contribute. For example, samples incubated for 3 hours (10.76%±0.89% DFI) versus 1 hour (6.14%±0.89% DFI) at the same temperature (37°C) showed notable differences [24]. Prior studies found no decline in sperm quality at room temperature (22-25°C) within short incubation periods, though quality decreased at 37°C over 6 hours [21]. In this study, sperm preparation involved incubation under one hour, minimizing ROS exposure.

The results differ from research showing that prolonged incubation at room temperature (24 hours) reduced DFI and preserved sperm quality compared to 35°C [19]. This was due to lower metabolic activity at room temperature, preserving DNA integrity. However, extended incubation increased enzymatic activity, inducing apoptosis [27]. Repalle D et al.[28] also reported that incubating sperm for 30 minutes at room temperature versus 37°C showed minimal impact on DFI.

The limitation of this study is the lack of prior screening for conditions like varicocele or infections in the patients analyzed.

CONCLUSION

The temperature has a complex impact and will likely influence sperm quality during sperm preparation. Higher temperatures cause oxidative stress and induce DNA damage through increased DNA fragmentation. Preparation of sperm using swim-up at 27°C significantly lowers TMSC in infertile males compared to 37°C, but the difference in DFI is not significant compared to 37°C.

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Conflicts of Interest

The authors declare that they have no competing interests.

Contribution of authors:

Conceptualization: LS, AZJ, DI. Data curation: AZJ, MP. Formal analysis: AZJ. Funding acquisition: AZJ. Investigation: AZJ. Methodology: AZJ. Project administration: AZJ. Resources: AZJ, LS, MP. Software: AZJ. Supervision: LS, DI, IQ, YS. Validation: DI, IQ, YS, MP. Visualization: AZJ. Writing – original draft: AZJ. Writing – review & editing: all authors.

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REFERENCES

1. Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, Regional, and Global Trends in Infertility Prevalence Since 1990: A Systematic Analysis of 277 Health Surveys. PLoS Med. 2012;9(12):1–12.
2. Sharma A. Male Infertility; Evidences, Risk Factors, Causes, Diagnosis and Management in Human. Ann Clin Lab Res. 2017;05(03):1–10.
3. García-Ferreyra J. Sperm DNA Fragmentation and Its Relation With Fertility. New Discov Embryol. 2015;3–17.
4. Borges E, Setti AS, Braga DPAF, Figueira RCS, Iaconelli A. Total motile sperm count has a superior predictive value over the WHO 2010 cut-off values for the outcomes of intracytoplasmic sperm injection cycles. Andrology. 2016;4(5):880–6.
5. Nikbakht R, Saharkhiz N. The influence of sperm morphology, total motile sperm count of semen and the number of motile sperm inseminated in sperm samples on the success of intrauterine insemination. Int J Fertil Steril. 2011;5(3):168–73.
6. Seda CK, Özgörgülü A. Evaluation of Semen Samples Before and After 'Swim Up' Technique with Mitotracker. Bangladesh J Med Sci. 2019;18(03):479–83.
7. Henkel RR, Schill WB. Sperm preparation for ART. Reprod Biol Endocrinol. 2003;1:1–22.
8. Jameel T. Sperm swim-up: A simple and effective technique of semen processing for intrauterine insemination. J Pak Med Assoc. 2008;58(2):71–4.
9. Oguz Y, Guler I, Erdem A, Mutlu MF, Gumuslu S, Oktem M, et al. The effect of swim-up and gradient sperm preparation techniques on deoxyribonucleic acid (DNA) fragmentation in subfertile patients. J Assist Reprod Genet. 2018;35(6):1083–9.
10. Agarwal A, Majzoub A. Role of Antioxidants in Assisted Reproductive Techniques. World J Mens Health. 2017;35(2):77.
11. Franken DR, van Wyk R, Stoumann C, Avari K. Temperature controlled centrifugation improves sperm retrieval. Andrologia. 2011;43(3):217–21.
12. 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.
13. Ahmad R, Haque M. Obesity inflicted reproductive complications and infertility in men. Bangladesh J Med Sci. 2023;22(01):7–14.
14. Vishwakarma G. Statistical Basis of Calculation of Sample Size in Nursing Research. In: Nursing Research in 21st Century. First. CBS Publishers & Distributors; 2020. p. 235–47.
15. Boushaba S, Belaaloui G. Sperm DNA Fragmentation and Standard Semen Parameters in Algerian Infertile Male Partners. World J Mens Health. 2015;33(1):1.
16. Le MT, Nguyen TAT, Nguyen HTT, Nguyen TTT, Nguyen VT, Le DD, et al. Does sperm DNA fragmentation correlate with semen parameters? Reprod Med Biol. 2019;18(4):390–6.
17. WHO. WHO Laboratory Manual for the Examination and Processing of Human Semen. World Health. 2010;fifth edit.
18. Gonzales GF, Zapana M. Sperm motility should be assessed in fresh sperm and after a sperm washing procedure. Syst Biol Reprod Med. 1992;28(2):83–9.
19. Thijssen A, Klerkx E, Huyser 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.
20. Marín-Briggiler CI, Tezón JG, Miranda P V., Vazquez-Levin MH. Effect of incubating human sperm at room temperature on capacitation-related events. Fertil Steril. 2002;77(2):252–9.
21. Uribe P, Rojas C, Meriño J, Zambrano F, Villegas J V., Treulen F, et al. Effect of incubation temperature after devitrification on quality parameters in human sperm cells. Cryobiology. 2017;79:78–81.
22. Ostadian C, Mehrafza M, Eftekhari A, Aghajani S, Vahabzadeh H, Gholami M, et al. The Effect of Prolonged Incubation of Sperm at Testis Temperature versus Room Temperature on Semen Parameters: An Experimental Study. J Obstet Gynecol Cancer Res. 2022;7(4):323–8.
23. Zhao F, Whiting S, Lambourne S, Aitken RJ, Sun Y pu. Melatonin alleviates heat stress-induced oxidative stress and apoptosis in human spermatozoa. Free Radic Biol Med [Internet]. 2021;164(August 2020):410–6. Available from: https://doi.org/10.1016/j.freeradbiomed.2021.01.014
24. Agarwal A, Virk G, Ong C, du Plessis SS. Effect of Oxidative Stress on Male Reproduction. World J Mens Health. 2014;32(1):1.
25. Agarwal A, Durairajanayagam D, du Plessis SS. Utility of antioxidants during assisted reproductive techniques: An evidence based review. Reprod Biol Endocrinol. 2014;12(1):1–19.
26. Nabi A, Khalili MA, Halvaei I, Roodbari F. Prolonged incubation of processed human spermatozoa will increase DNA fragmentation. Andrologia. 2014;46(4):374–9.
27. Aboulmaouahib 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. 2016;49(6):1–7.
28. Repalle D, Chittawar PB, Bhandari S, Joshi G, Paranjape M, Joshi C. Does centrifugation and semen processing with swim up at 37°C yield sperm with better DNA integrity compared to centrifugation and processing at room temperature? J Hum Reprod Sci. 2013;6(1):23–6.