ORIGINAL RESEARCH

The success rate of intrauterine insemination in sperm preparation swim-up method at room temperature compared to the incubator temperature

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ABSTRACT

Objective: This study aimed to determine the effect of temperature during sperm preparation on total sperm motile count (TMSC), sperm motility, sperm morphology, DNA fragmentation index (DFI), and pregnancy rate.

Materials and Methods: A quasi-experimental laboratory study with pre- and post-test control group was conducted at Sekar Fertility Clinic, Dr. Moewardi General Hospital, Surakarta, Indonesia. A total of 20 sperm samples from infertile patients were prepared using the swim-up method at 27°C (group 1) and 37°C (group 2). TMSC, motility, morphology, and DFI examinations were performed. In addition, IUI was performed to confirm pregnancy rate. Sperm DNA fragmentation was determined using Sperm Chromatin Dispersion/SpermFunc DNAf test. Sperm DNA fragmentation was characterized by a halo <30% of the volume of the sperm head.

Results: Group 1 had mean TMSC of 13.77 ± 9.30, while group 2 had 14.82 ± 8.82; p=0.218. Group 1 had a motility value 82.25+12.77 and group 2 had 82.55 ± 11.69; p=0.968. The morphological value for group 1 was 11.25 ± 5.15 and group 2 was 11.6 ± 5.34; p=0.626. The mean DFI for group 1 was 17.79 ± 10.88 and group 2 was 18.18 ± 12.95; p=0.765. Pregnancy rate in group 1 was 10% and group 2 was 20%; p=1.000.

Conclusion: There were no significant differences in TMSC, sperm motility, sperm morphology, DFI, and pregnancy rate in sperm preparation using the swim-up method at 27°C and 37°C.

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Highlights:

1. There were no significant differences in TMSC, sperm motility, sperm morphology, and DFI in sperm preparation using the swim-up method at 27°C and 37°C. However, this study provided an overview of the average improvement of DFI at 27°C compared to 37°C.
2. There was no significant difference in the pregnancy rate of IUI in sperm preparation using the swim-up method at 27°C and 37°C.

INTRODUCTION

Malefactors' infertility accounts for half of all infertility cases. Infertility affects 15% of 48.5 million couples globally, and 50% is caused by male factors with a range of 20% to 70%. The leading cause of male infertility is sperm abnormalities, including oligo-zoospermia (low sperm count), asthenozoospermia (poor sperm motility), and teratozoospermia (abnormal sperm morphology).[1–3] Treatment options for male infertility are assisted reproductive technology (ART), including intrauterine insemination (IUI), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI). IUI is the most preferred choice because of the easy method and simple equipment.[4,5] However, the live birth rate in pregnancies with IUI is lower (7.7%) than in the IVF/ICSI program (19.8%). Sperm quality is also affected by sperm preparation technique, preparation temperature, preparation time interval, and preparation medium. It is an essential factor in the success of IUI.[6,7] Low levels of sperm DNA fragmentation during IUI increases pregnancy after IUI.[8]

Incubation at room temperature (23°C) for 24 hour had significantly higher progressive motility and normal morphology. In addition, a decrease in the levels of acrosome reactions, apoptosis, and death of spermatozoa at room temperature reduces the level of DNA fragmentation. On the other hand, incubation at 37°C for 4 hours increased vacuoles in the sperm head.[9] Each 5°C lower temperature was significantly associated with 1.94 × 10^6/ml, 7.12 × 10^6, 0.77%, 0.81%, 6.48 × 10^6, and 5.87 × 10^6 decreases in sperm concentration, total sperm count, total motility, progressive motility, TMSC, and progressively motile sperm count, respectively.[10] Based on those results, a research was conducted to study the effect of differences in temperature of sperm preparation using the swim-up method on total sperm motile count (TMSC), sperm motility, sperm morphology, DFI, and pregnancy rate.

MATERIALS AND METHODS

Sample preparation

A quasi-experimental laboratory test with pre and post-test control group designs was conducted at the Sekar Fertility Clinic, Dr. Moewardi General Hospital, Surakarta, Indonesia. Twenty sperm samples from infertility patients were prepared using the swim-up method at 27°C (group 1) and 37°C (group 2). Sampling used purposive sampling techniques using inclusion and exclusion criteria. The inclusion criteria were as follows: 1) Sperm comes from male infertility patients who a team of fertility doctors had decided to undergo the IUI program, 2) Ejaculate was removed after the patients' abstinence from sexuality for 2-7 days, 3) The volume of ejaculate semen was at least 2 ml, TMSC ≥5 x 10^6 and abnormal sperm morphology <4%. The patients were excluded from the study if they were unable to remove the ejaculate fluid on the day of sampling. Sperm preparation using swim-up method was performed according to WHO guidelines in 2010, where semen undergoes complete liquefaction for 15-60 minutes at incubator temperature of 37°C before processing. Furthermore, the samples were taken as much as 1 ml on each tube and transferred in a cone-shaped centrifugal tube sterilely. At the top of the liquid, the semen was coated with a 2 ml sperm rinse®, then the tube was placed at an angle of 45° and incubated for 60 minutes at a temperature of 23°C and 37°C. Sperm with good quality will actively move out towards the culture media and in aspiration. Sperm that swim farthest have the probability of being sperm with normal motility and morphology.[11]

Total motile sperm count calculation

All samples were divided into 1 ml for sperm preparation at temperatures of 27°C and 37°C. Sperm concentration was calculated using the Makler Chamber (Sefi, Israel). Sperm concentration was obtained by counting the total sperm in 10 boxes in the Makler chamber. The result obtained was the number of spermatozoa per unit volume of semen in milliliters (10^6). The results of TMSC were obtained by multiplying the parameters of semen in the form of volume, and concentration, by the proportion of motile sperm that was progressively divided by 100%.[12]

Sperm motility

Spermatozoa motility is divided into progressive, non-progressive, and immotile. The state in which the spermatozoa move actively, move straight or in large circular motions, and the speed is well measured based on WHO criteria (2010). Sperm motility in semen should be assessed as soon as possible after the sample is thawed (preferably at 30 minutes, maximum within 1 hour after ejaculation) to limit the effects of dehydration, changes in pH, and temperature. The semen sample was mixed evenly. Semen samples were prepared in wet preparations at a depth of 20 μm. The sample was allowed to float for 60 seconds. The slide was checked with contrast optics at 200x or 400x magnification. The assessment was carried out on at least 200 spermatozoa in one field of view and the value of motility. Normal sperm progressive motility value was 40%.[1]

DNA fragmentation index

DNA fragmentation index assessment used SpermFunc® DNAf (Fertitech, Canada) and followed sperm chromatin dispersion test technology. Semen samples were diluted using normal salts to reach a final concentration of 5-10x10^6. Next, a diluted sample was placed on a pre-coated slide and dipped in solution A at 20-28°C for seven minutes. The pre-coated slide was lowered and dried at room temperature, then dipped again in solution B and incubated for 25 minutes at a temperature of 20-28°C. Then, the pre-coated slide was washed with distilled water, 70% ethanol, 90% ethanol, and 100% ethanol for 2 minutes. The pre-coated slide had to be perfectly dry, then 15-20 drops of Wright color over the pre-coated slide was applied and then 30-40 drops Wright buffer solution was given slowly. After 15 minutes, the slide was rinsed with water and dried at room temperature. Observation of 500 spermatozoa under a microscope with 400x magnification was performed and sperm with DNA fragmentation was counted. Normal DNA was recognized to be at least 33.3% of halo formation in the head of the sperm. DFI was obtained by comparing the amount of DNA fragmentation with the total observed sperm count and multiplied by 100%. The average sperm percentage usually has DFI <30%.[13]

Sperm preparation using the swim-up method

Sperm preparation used the swim-up method based on World Health Organization (WHO) protocols. As much as 1 ml of the semen sample was added with a supplemented medium of 1.2 ml on a sterile tube and incubated at 37°C for 45 minutes with an oblique tube position of 45°. Sperm samples were collected with centrifugation of 1500 rpm for 5 minutes with the Thermo Scientific Heraeus Labofuge® 300. The samples were put into storage boxes with sterile techniques to avoid direct light exposure. Incubation was carried out a maximum of 1 hour after ejaculation.[7] Next, the sperm sample was divided into two parts. The first part was stored in a temperature incubator of 27°C and the second part at 37°C, each incubated for 20 minutes before insemination.

Intra Uterine Insemination (IUI)

The IUI step was done to see the pregnancy rate. At the time of IUI, the subject was randomized by the generation of numbers. IUI was performed after the sperm preparation was completed and the female partner of the subject was prepared (all less than 15 minutes). The sperm was inseminated using a Gynaetics catheter by an Obstetrics-Gynaecology specialist. The patients were rested in a lying position for 15-20 minutes after insemination. The use of IUI is recommended when TMSC values range from 3x10^6-19x10^6 motile sperm.[14]

Pregnancy

Sixteen days after insemination, β-HCG was taken. The outcome was categorized as pregnant if the β-HCG level was >5 mIU/ml.

Table 1. Characteristic of research participants

Characteristic	Frequency	Percentage (%)
Age (years)		
<30	6	30
≥30	14	70
Infertility period (years)		
<2	4	20
≥2	16	80
BMI (kg/m^2)		
Underweight	1	5
Normal	7	35
Overweight/Obesity	12	60
Body height (cm)		
<165	1	5
≥165	19	95
Bodyweight (kg)		
<70	8	40
≥70	12	60
Smoking		
Non-smoker	12	60
Smoker	8	40

Std. Dev: Standard deviation; BMI: Body mass index.

Statistical analysis

Normal distribution was tested using Kolmogorov Smirnov. Data were generally distributed if α >0.0 and customarily distributed, presented by mean and standard deviation, and tested using a T-test to sample pairs. The data that were not normally distributed were presented by median and analyzed using Wilcoxon. Categorical data were analyzed by Fischer's Exact test. Data were analyzed using SPSS 23.

Ethics considerations

The institutional review board approved by the Dr. Moewardi General Hospital, Surakarta, Indonesia (IRB No. 1.045/VIII/HREC/2022). The informed consent was submitted by all participants when they had enrolled in this present study.

RESULTS AND DISCUSSION

The participant's characteristics are shown in Table 1. The age of the study participants was mostly ≥30 years as much as 70%, and they experienced infertility ≥2 years since they married as much as 80%. The BMI of overweight/obesity was as much as 60%, height was mostly ≥165 cm in as much as 95%, and weight ≥70 as much as 60%. Most of the participants (60%) were non-smokers.

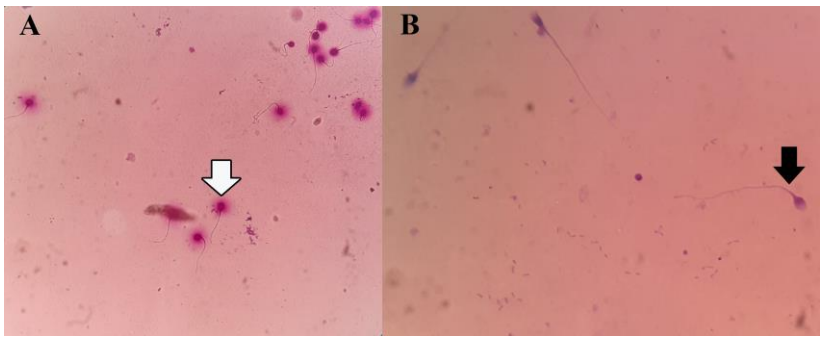

Figure. 1. Sperm DNA fragmentation following Sperm Chromatin Dispersion (SCD) test. (A) The white arrow indicates sperm with fragmented DNA. (B) The black arrow showed sperm with normal morphology.

Table 2. Effect of swim-up temperature on concentration, TMSC, motility, normal morphology, and DNA fragmentation index

Parameters	N	Mean ± Std. Dev	p
Concentration (million/mL)			
Before swim-up	20	66.25 ± 41.67	
After swim-up at 27°C	20	19.05 ± 14.33	0.797
After swim-up at 37°C	20	19.97 ± 13.06	
TMSC ($x10^6$)			
Before swim-up	20	90.16 ± 46.90	
After swim-up at 27°C	20	13.77 ± 9.30	0.218
After swim-up at 37°C	20	14.82 ± 8.82	
Motility (%)			
Before swim-up	20	60.10 ± 18.07	
After swim-up at 27°C	20	82.25 ± 12.77	0.968
After swim-up at 37°C	20	82.55 ± 11.69	
Normal morphology (%)			
Before swim-up	20	7.15 ± 3.07	
After swim-up at 27°C	20	11.25 ± 5.15	0.626
After swim-up at 37°C	20	11.60 ± 5.34	
DFI (%)			
Before swim-up	20	22.69 ± 11.39	
After swim-up at 27°C	20	17.79 ± 10.88	0.765
After swim-up at 37°C	20	18.18 ± 12.95	

TMSC: Total sperm motile count; DFI: DNA fragmentation index;
Std. Dev: Standard deviation

Swim-up at either 27°C or 37°C reduced the number of TMSC and DFI but increased motility compared to pre-swim-up. However, those changes were not statistically significant ($p>0.05$). P-value for each parameter were 0.218, 0.968, 0.626, and 0.765 respectively. This indicated that temperature during swim-up did not affect sperm quality. Table 3 shows that the pregnancy rate in 27°C was 1 (10%) and 37°C was 2 (20%). There were no significant results between IUI at a temperature of 27°C and 37°C ($p=1.000$). Most of the subjects were ≥30 years old.

A decrease in reproductive capacity is associated with age, gonadotropin levels increase, testosterone levels decrease, Leydig cell count, Sertoli cells, and germ cells decrease with age.[15]

Table 3. IUI and pregnancy outcome

IUI	Pregnancy				p
	Negative		Positive		
	n	%	n	%	
Sperm 27°C	9	90	1	10	1.000
Sperm 37°C	8	80	2	20	

IUI: Intrauterine insemination

BMI also affects infertility, in particular by altering the physical structure and molecules of gametes in the testes and sperm.[16] Obesity may cause sperm morphological abnormalities in the form of head abnormalities.[17] In

addition, overweight in men can cause endocrine disorders associated with decreased sex hormone binding globulins and decreased total testosterone levels.[18] The content of cigarettes such as nicotine, cadmium, lead, and benzopyrene negatively affects the integrity of DNA.[19] Cigarettes contain high levels of reactive oxygen species (ROS) (superoxide anions, hydrogen peroxide, and hydroxyl radicals). ROS is produced mainly in seminal fluids, increasing leukocyte levels and causing oxidation stress that leads to sperm DNA damage.[20]

The study showed significant differences between the sperm before and after preparation. Sperm preparation was capable of removing immotile sperm and immature cells.[21] The results showed that the highest DFI occurred in the sperm of the pretest group that had not undergone sperm preparation. Sperm preparation at 27°C had a lower DFI when compared to sperm preparation at 37°C, but showed no significant statistical results. These results were in line with previous research that DFI values increased in processed samples at 37°C compared to room temperature, although the difference was not statistically significant.[22]

This study showed the results that sperm preparation increases motility and morphological levels. Sperm motility and morphology results at 27°C lower than 37°C. This did not confirm the results of the study by Thijssen et al., who reported that sperm quality is better incubated at room temperature (23°C) when compared to 35°C. In addition, the sperm morphology also decreased significantly at 35°C incubation.[7]

Consistent with previous studies, this study showed a pregnancy rate of 15%. In those studies, mean pregnancy rate after IUI treatment was 10-20.5% The pregnancy rate in our study was based on chemical pregnancy, and the result was not statistically significant. Several factors in female and male partners influence the pregnancy rate in ART programs. In females, the increase in clinical pregnancy is affected by age, body mass index, FSH levels, estradiol levels, pre-ovulation follicles, and endometrial thickness. Males are affected by TMSC values and the ratio of sperm to progressive motility.[23,24]

CONCLUSION

There were no significant differences in TMSC, sperm motility, sperm morphology, DFI, and pregnancy rate in sperm preparation using the swim-up method at room temperature (27°C) and body temperature of 37°C. It is necessary to conduct studies with larger sample size, considering the ovary stimulation method, the number of follicles obtained, and observing the outcome until further clinical pregnancy.

DISCLOSURES

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Conflict of interest

The authors have nothing to disclose.

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Author Contribution

Conceptualization: EM, RKD, AMT, AZJ. Data curation: EM, RKD, AMT, AZJ. Formal analysis: EM, URB, MP, LS, MS. Funding acquisition: EM, URB, TP, AAR. Investigation: AL, D, AAR, CH. Methodology: MP, TP, AL, D, CH. Project administration: LS, MS. Resources: EM, URB, RKD, AMT, AZJ. Software: EM, AAR. Supervision: TP, AL, CH. Validation: EM, URB, MP, TP, AAR. Visualization: D, RKD, AMT, AZJ. Writing – original draft: EM. Writing – review & editing: all authors.

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