A Study of Sperm Quality Characteristics Changes in Different Storage Temperatures above Freezing Point

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ABSTRACT

Introduction: Sperm is very sensitive to temperatures changes as it is capable of changing the sperm characteristics. Therefore, the aim of this study was to identify the optimum storage temperature above freezing point for sperm transportation in order to maintain the good sperm characteristics.

Objective: Sperm characteristics such as motility, viability and morphology were evaluated after 1, 2 and 3 hour(s) stored at different temperatures (4˚C, 25˚C and 37˚C).

Method: The fresh semen bovine were collected using artificial vagina at Institut Biodiversiti Veterinar Kebangsaan, Jerantut (Pahang). Sperm motility and viability were carried out using computer-assisted sperm analysis (CASA) while sperm morphology was carried out using scanning electron microscope (SEM).

Result: The results obtained demonstrated that 25°C has high sperm progressive movement as well as sperm viability compared to 4°C and 37°C. Sperm stored at 25°C showed normal morphological structure whereas there were morphological alteration at storage temperature of 4°C and 37°C.

Conclusion: In conclusion, 25˚C is the best storage temperature above freezing point to maintain sperm characteristics during sperm transportation.

Keywords: Sperm Quality, Temperature, Motility, Viability, Morphology

Introduction

The development of laboratory diagnostic tests in the past is still in use again until now. Improvement of diagnostic tests with conventional semen profile provides a more accurate assessment of the level of male fertility [1].

However, the main sources of laboratory tests difficult to proceed are the result of different interpretations for all species or individuals and are only available in certain research laboratories. Moreover, fresh semen begins to decline in quality as well as in sperm concentration when sperm are located outside the body [2]. Sperm transport process from a collection of fresh semen sample to the laboratory requires a proper semen storage technique to ensure the sperm viability and fertilization capacity are maintained [3].

Sperm are very sensitive to changes in environmental temperature. Techniques of sperm storage in the laboratory and transport processes must be in satisfactory condition. However, this study offers an alternative for the storage of sperm to be more convenient and practical to be adopted. Sperm storage has been tested at 4˚C, 25˚C and 37˚C. These techniques are easy to be practiced if it is proven to help maintain the characteristics of sperm quality during the sperm transport process.

Through this present study, the best storage temperature is expected to maintain the characteristics of sperm quality during the sperm transport process can be achieved even help the health field especially to review the status of the male reproductive cells.

Materials and Methods

Chemicals and Equipments

In this study, chemicals are prepared including phosphate buffered saline (PBS), Eosin Y and Nigrosin were purchased from Sigma-Aldrich Chemie (Steinheim, Germany), sodium chloride (NaCl) was purchased from Sigma Chemical Company (St. Louis, USA), disodium hydrogen phosphate (Na2HPO4) and hydrogenated sodium phosphate (NaH2PO4) were purchased from Merck (Germany). Equipments are required for this study such as electronic balance (Fisher Scientific B-4200), temperature-controlled centrifuge (Mikro 200R Hettich Zentrifugen, Germany), magnetic stirrer (Torrey Pines Scientific), thermometer (Japan), water bath temperature of 37°C (Yihder Bu-420), Makler counting chamber (depth 10 μm; Sefi Medical Instruments Ltd. Haifa, Israel), Olympus CX31 light microscope (Olympus Optical Co., Ltd. Japan), CASA (computer assisted sperm analysis) Hamilton Thorne Motility-CEROS version 12.1c (Hamilton Thorne Research Inc., Beverly Mass.) equipped with phase contrast Olympus CH30 microscope and GC system 7890A equipped with a 7683B injector, and scanning electron microscopy.

Sampling and Semen Collection

The fresh semen samples of bovine Bali were selected and collected using artificial vagina at Institut Biodiversiti Veterinar Kebangsaan, Jerantut (Pahang). Samples were then delivered to the laboratory at 25˚C within 10 minutes of collection. During this time, semen was allowed to liquefy.

The initial fresh sample of progressive motility was evaluated subjectively (200 X magnification) by computer assisted semen analysis (CASA) and morphology was evaluated using phase contrast microscopy (at 1000 X magnification). Fresh sample with progressive motility (≥ 70%) and normal morphology (≥ 80%) were included in this study to determine the sperm characteristics in different storage temperatures.

In this study, sample were diluted with phosphate buffered saline (PBS) at a final concentration 25 x 10^6 spermatozoa/ml. Samples were incubated for 1, 2 and 3 hour(s) at different temperature (4˚C, 25˚C and 37˚C). Sperm quality analysis (motility and viability) were carried out using CASA while sperm morphology was carried out using scanning electron microscope after 3 hours incubation.

Sperm Motility Analysis

Bovine semen was evaluated for motility parameters based on guideline [15] using a CASA. The motility characteristics of the sperm cells were classified into three groups as progressive, non-progressive and non-motile. 10 µl of sample was loaded on slide microscope and observed through CASA. Sperm cells (n = 200 as minimum cells) per slide was examined. Motility parameters were collected and recorded as the percentage of motility by CASA (at 200 X magnification) with at least 10 non-consecutive fields.

Sperm Viability Analysis

Sperm viability was determined using one step eosin-nigrosin staining technique [9]. One drop of semen sample was mixed with one drop of eosin-nigrosin mixture. After 30 seconds, a drop of mixture was loaded on slide microscope, smears were made, air dried and examined using a CASA (at 400 X magnification). Sperm cells (n = 200 as minimum cells) per slide was examined with at least 10 non-consecutive fields. For assessment of sperm viability, spermatozoa with red or dark pink heads are considered dead while spermatozoa with white heads or light pink heads are considered alive. Viability parameters were collected and recorded as the percentage of viability.

Sperm Morphology Analysis

Sperm morphology was observed under scanning electron microscope (at 10000 X magnification). After 3 hours, the samples were sent to Institute Medical Research (IMR) to observe and compare the changes in sperm morphology at different storage temperatures.

Statistical Analysis

All analyses were run in triplicates. Data were analyzed using one-way analysis of variance (ANOVA) and the differences were considered to be significant at $P < 0.05$. All statistical analyses were performed with SPSS.

Result and Discussion

The studies conducted by researchers are still seeking the other alternatives to minimize the decline in sperm quality during transport process. Therefore, best storage temperature is determined that can maintain the sperm characteristics during the sperm transportation. Among the parameters to be determined were such as motility, viability and sperm morphology.

The comparison in sperm motility parameters (progressive, non-progressive and non-motile) stored for 1, 2 and 3 hour(s) at different storage temperatures shown in Table 1. There was significant different in time incubation ($P < 0.05$). Sperm storage at 25°C has high progressive movement percentage followed by temperature at 4°C and 37°C after 1 hour ($P < 0.05$). However, the progressive movement was significantly reduced after 2 and 3 hours storage compared to 1 hour storage ($P < 0.05$). Based on previous study [12], this is due to the absence of preservative medium that provides nutrients to the sperm to enable to move progressively. Sperm do not receive enough nutrients to survive and move. Thus, the progressive movement of sperm has a very significant decline and contributes to increase the non-progressive and non-motile sperm which are seen in this study.

The percentage of progressive movement at 4°C was significant lower than 25°C ($P < 0.05$). The reason that low temperature less suitable used in storage temperature are still unknown. However, sperm still can move at low temperatures as long as no ice crystal formation occurs based on previous study [8]. The same study demonstrates sperm storage with preservative at 4°C showed about 50% reduction in sperm progressive movement on the first day and no movement were seen after three days. On the other hand, sperm samples stored at 37°C has the lowest in motility parameters compared to 4°C and 25°C ($P < 0.05$). The same reported findings [8] show that only 10% of spermatozoa are still moving after stored at 37°C on the first day and no movement is possible on the second day. This data supports in this study when the percentage of progressive movement at 37°C was the lowest compared to other temperatures ($P < 0.05$). This is indicates the enzymes aldose reductase (AKR1B1) and sorbitol dehydrogenase (SODH), which responsible to control sperm movement, are denatured at 37°C and led to decline in motility [5]. These enzymes are presence in epididymis of bovine [10], rat [6] and human [4].

One step eosin-nigrosin staining technique used in this study to distinguish between live sperm and sperm dead [7-9]. Based on the Table 1, the results showed sperm viability at 25°C was significantly higher compared to 4°C and 37°C. However, all three temperatures gave lower sperm viability with increasing time of storage. This is indicates there was changes in structure of the sperm membrane. The dead sperm has eosin-nigrosin staining appears red in their head indicates the membrane integrity have been denatured (at 37°C) or altered (4°C) whereas live sperm do not has staining in their head indicates the membrane integrity remain intact [7].

Based on the Figure 1, 2 and 3, sperm morphology were observed under scanning electron microscope (SEM) at three different storage temperatures after three hours. Sperm storage at

25°C found to be providing no significant morphological changes as shown in Figure 2. The head, middle and tail are in normal condition as reported in the findings [13]. However, there was morphological alteration at 4°C, in which the sperm head was separated from the tail as shown in Figure 3. Drop in temperature lead to a reduction in disruption of sperm membrane consisted of protein and phospholipid [14]. Thus, disruption of sperm structure eventually led to sperm morphology changes.

37°C is the body temperature. Changes in sperm morphology is impossible to represent the sperm in the human body as shown in Figure 1. Storage temperature of 37°C showed that the sperm head separated completely from the tail. Sperm storage at high temperature is changing the activities of proteins found in the sperm membrane [11]. Thus, most of the protein contents are denatured at temperature of 37°C and cause disruption of membrane integrity. This contributes to the overall separation of sperm structure between the head and tail.

Conclusion

In conclusion, our study found that 25˚C is the best storage temperature compared 0˚C and 37˚C in this study to maintain sperm characteristics during sperm transportation.

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Table 1: Comparison in sperm motility parameters (progressive, non-progressive and non-motile) as well as sperm viability at different storage temperature with time incubation at 1 hour, 2 hours and 3 hours

Temperature	37°C			25°C			4°C		
Time	1 hour	2 hours	3 hours	1 hour	2 hours	3 hours	1 hour	2 hours	3 hours
Parameter									
Motility									
Progressive	50.27 ± 0.67	22.63 ± 2.70*	2.47 ± 1.39**	88.43 ± 1.31a	71.43 ± 2.11a*	65.30 ± 2.91a*	82.50 ± 1.61a	60.17 ± 2.06b*	54.63 ± 2.90b*
Non-progressive	26.83 ± 1.62	26.77 ± 1.10	27.74 ± 1.99	7.80 ± 1.21a	13.10 ± 0.79a*	18.13 ± 1.31a*	8.83 ± 1.20a	13.40 ± 1.28a*	14.47 ± 0.68a*
Non-motile	22.90 ± 1.65	50.60 ± 2.46*	69.79 ± 2.26**	3.77 ± 1.62a	15.47 ± 2.54a*	16.57 ± 4.18a*	8.67 ± 0.42a	26.43 ± 2.14b*	30.90 ± 2.35b*
Viability									
Live	25.00 ± 2.09	15.20 ± 3.20*	5.00 ± 2.90**	53.27 ± 1.50a	42.60 ± 3.34a*	34.87 ± 2.40a**	37.40 ± 2.65b	27.20 ± 2.79b*	13.53 ± 2.54b**

a, b indicates significant difference between values of storage temperature (4˚C, 25˚C and 37˚C)

*, ** indicates significant difference between values of time incubation at 1, 2 and 3 hour(s)

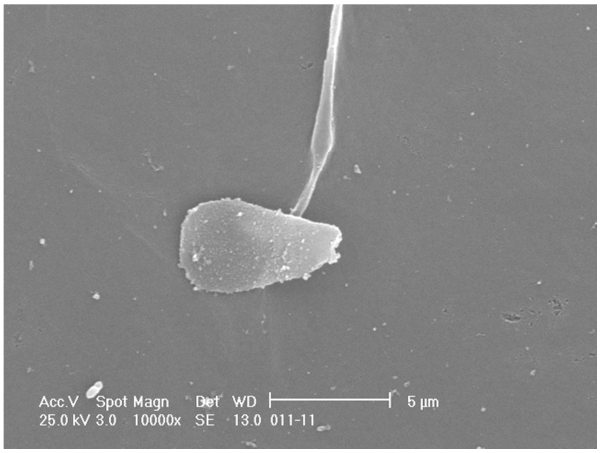

Figure 1: Sperm morphology stored at 37°C

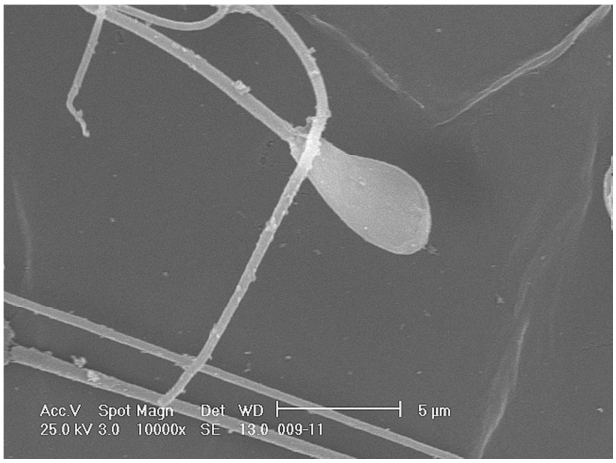

Figure 2: Sperm morphology stored at 25°C

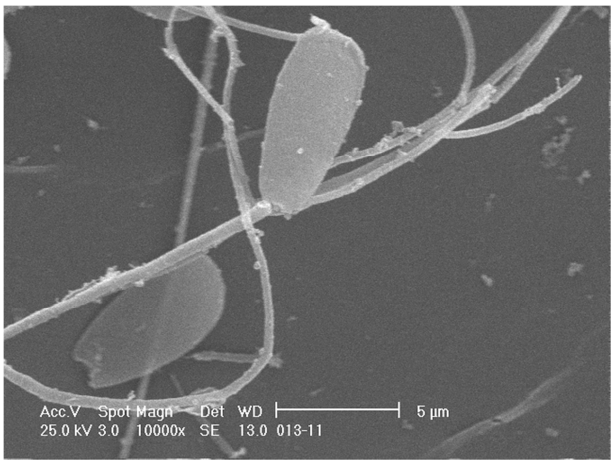

Figure 3: Sperm morphology stored at 4°C