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Comparative evaluation of the effects of different thawing methods on bull sperm characteristics with computer-assisted semen analysis

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Abstract: Postthaw survival of spermatozoa with high motility and vitality is required for successful artificial insemination. The aim of this study was to compare the effects of two different thawing methods on motility, morphology, kinematic parameters, and viability of spermatozoa with a computer-assisted semen analyzer (CASA). Frozen bull spermatozoa were thawed by using two different thawing procedures: 1) Water bath; straws were thawed in a water bath at 37 °C for 30 s. 2) Dry thawing system; straws were thawed in a dry thawing device at 37 °C for 30 s. A total of 10 straws were used for each thawing procedure. There were significant differences between the thawing methods for acrosome defects ($P < 0.05$), head defects ($P < 0.0001$), middle part defects ($P < 0.05$), and total abnormal spermatozoa rates ($P < 0.05$). There was no difference between the thawing methods for total and progressive sperm motility determined with CASA. There were significant differences ($P < 0.05$) between the thawing methods for STR and ALH values which were kinematic parameters. The STR (66.37%) and ALH (3,28 µm) values of dry system were higher ($P < 0.05$) than the STR (57.88%) and ALH (2.78 µm) values of water bath. In conclusion, the present study demonstrated the possibility of using dry thawing system as alternative to water bath for thawing bull sperm because some postthaw sperm values obtained when dry thawing system was used were better than those obtained when water bath was used.

Key words: Bull sperm, dry thawing, sperm characteristics

1. Introduction

Postthaw survival of spermatozoa with high motility and vitality is required for successful artificial insemination (AI). Senger [1] reported that freezing and thawing of sperm certainly cause a decrease in sperm motility and an increase in morphological, biochemical, and functional damages.

The membrane integrity of spermatozoa is affected by freezing or thawing temperatures. Thawed spermatozoa are more vulnerable than fresh spermatozoa due to a number of damages caused by freezing or thawing processes. Postthaw viability of spermatozoa is notably affected by thawing procedure such as thawing medium (water, air) and thawing temperature [2]. The sperm must be thawed at maximum speed because the rapid thawing of sperm increases sperm motility [3]. Numerous studies have been conducted to determine an ideal thawing temperature for optimum rate of the highest percentage of viable spermatozoa [2,4,5]. In general, frozen bull sperm in straws can be thawed at 33–35 °C in a water bath for 30–40 s if there is no specific recommendation or the diluent type and freezing procedure are not taken into account [6,7]. However, it was also stated that frozen bull semen in a 0.25 mL straw could be thawed at 37 °C for 30 s [8].

Comparison of the results of studies conducted with a computer-assisted semen analyzer (CASA) may be objectively possible because CASA is more objective and reliable than subjective evaluation [9]. In addition to quantitative evaluation of sperm motility, CASA determines the data for the rates of total and progressive motility of spermatozoa and records every spermatozoon track. Therefore, sperm parameters can be individually retrieved. In addition, a CASA system is a useful tool for determining the effects of various in vitro procedures on sperm motility, as well as parameters that study the phenomenon of sperm hyperactivation. Forward progressive motility (FPM) along with certain velocity parameters are required for fertilization of spermatozoa. Spermatozoa kinematic parameters such as average path velocity (VAP), straight-line velocity (VSL), percentage linearity (LIN), percentage straightness (STR), percentage oscillation (WOB), amplitude of lateral head displacement (ALH), beat cross frequency (BCF) are easily calculated with CASA and positively correlated with bull fertility [10–12].

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Dry thawing system has some advantages. It is more practical than thawing sperm in a water bath because there are some difficulties of thawing sperm in water (risk of mixing sperm with water and difficulty of maintaining water temperature in cold weather). Dry thawing system is portable and can be easily used everywhere such as farms and barns. It keeps the required temperature for thawing in it for a certain time (about 10 min) and has a heating section for warming the catheter before AI. There is no necessity for wiping the water from the straw. Therefore, this study was conducted to compare the effects of two different thawing methods (water bath and dry thawing system at 37 °C for 30 s) on motility, morphology, kinematic parameters, and viability of spermatozoa with CASA.

2. Materials and methods

Frozen bull spermatozoa collected at the same date from the same bull and frozen in straws in volumes of 0.25 mL that were obtained from a commercial company, and stored under the same conditions were used and two different thawing procedures were compared with each other in the study. 1) Water bath; 0.25-mL straws were thawed in water bath at 37 °C for 30 s. 2) Dry thawing system; 0.25-mL straws were thawed in a newly developed device at 37 °C for 30 s (Figure). There are four round holes on the top surface of the dry thawing device. One of the holes is used for warming the catheter before AI. One of them is for 0.50-mL straws . The last two holes are for 0.25-mL straws. Since the device is portable and works with 12–13.6 V, it can be used by lighter socket of vehicle. It keeps the required temperature for thawing in it for a certain time (about 10 min). A total of 10 straws were used for each thawing procedure.

2.1. Motility and kinematic characteristics

Total motility and progressive motility were examined with a CASA (Sperm Class Analyzer®, version 6.3.0.59, Microptic, Barcelona, Spain). The slide (Leja 20 µm) was placed onto a stage warmer set at 37 °C. A minimum of five microscopic fields and 500 spermatozoa were analyzed for each sample. For kinematic characteristics of sperm movement; VSL (straight-line velocity, µm/s), VCL (curvilinear velocity, µm/s),VAP (average path velocity, µm/s), ALH (amplitude of lateral head displacement, µm), LIN (linearity, VSL/VCL × 100), WOB (wobble, VAP / VCL × 100), STR (straightness, VSL/VAP × 100), and BCF (beat-cross frequency, hertz) were determined with the software system.

2.2. Sperm morphology

Spermatozoa were morphologically evaluated by using Spermac® (Stain Enterprises, Wellington, South Africa). Sperm samples were smeared across on a clear slide and allowed to air dry. After the samples dried, smears were stained with Spermac® stains, following the manufacturer's recommendations.

Figure. Dry thawing system working with 12–13.6 V, at adjusted temperature of 37 °C, with holes for 0.25 and 0.50 mL straws and for catheter.

2.3. Plasma membrane functionality test

The hypoosmotic swelling test (HOST) was used to assess pos-thaw functionality of the sperm plasma membrane [13]. In brief, 30 µL of semen and 300 µL of hypoosmotic solution [fructose (0.05 M) + sodium citrate (0.023 M) in distilled water, osmolality = 100 mOsm/kg] were mixed. This mixture was incubated at 37 °C for 60 min and then 0.2 mL of the mixture was put on a warm slide and covered with a cover slip. Using a phase-contrast microscope, 200 spermatozoa were assessed and spermatozoa having coiled tail were evaluated to be intact and recorded [14].

2.4. Sperm viability

Eosin–nigrosin stain was used for assessment of sperm viability as described by Raseona et al. [15]. After staining, the slides were dried and covered with a cover slip before evaluation by using CASA at 60× magnification. The rates (%) of live (white sperm heads) and dead (pink sperm heads) sperm were determined by counting a total of 200 spermatozoa per each stained slide.

2.5. Statistical analysis

The data were described as means ($\bar{X}$) ± standard error of means ($S_{\bar{X}}$). Comparisons of the groups were made with the least square method. All statistical analysis and

evaluations were made using SAS (2009) statistic suits [16].

3. Results

Motility and kinematic parameters belonging to bull spermatozoa thawed in water bath and dry thawing system are given in Table 1. There was no difference between thawing methods for total and progressive sperm motility determined with CASA. The rates of total motility of postthaw sperm in a water bath and dry thawing system were 49.25 ± 4.89% and 57.15 ± 4.31%, respectively. The percentages of progressive motility of postthaw sperm in a water bath and dry thawing system were found as 29.02 ± 2.84% and 36.21 ± 3.53%, respectively. Dry thawing of bull spermatozoa significantly increased ($P < 0.05$) STR and ALH kinematic values compared to water bath thawing.

The rates of HOST of postthaw sperm in a water bath and dry thawing system were 76.00 ± 0.77% and 74.00 ± 1.66%, respectively. The percentages of viability of postthaw sperm in a water bath and dry thawing system were found as 48.40 ± 3.51% and 55.60 ± 3.87%, respectively Table 2.

4. Discussion

Thawing procedure of sperm is as crucial as freezing procedure because of its impact on the survival of spermatozoa [17]. It has been known that an increase in postthaw viability will result in increased fertility of sperm [18].

There were no differences between thawing in a water bath and dry thawing system for postthaw sperm motility and viability. Tanghe et al. [19] stated that postthaw total motility, postthaw progressive motility, and morphology of sperm are reliable for predicting in vitro fertilization results for bulls. Although there was no significant difference between the two thawing methods in the current study, total motility and progressive motility of postthaw sperm in dry thawing system were higher than those of sperm thawed in a water bath. It has been shown that while thawing at different thawing temperatures or with different methods, it should be pointed out that the duration of thawing should be carefully timed and shortened [1,2,18]. Tekin et al. [20], who conducted a study comparing spermatological parameters of bull semen using subjective assessment and CASA system, reported that the motility values of postthaw bull semen were 49.2% and 52.8%, respectively. In the present study, after thawing sperm with two different methods, the motility values were 49.25% for water bath and 57.15% for dry thawing system. Whereas the result for motility rate of sperm thawed in water bath in our study is compatible with that of Tekin et al. [20], postthaw motility rate of sperm thawed in dry thawing system is higher than theirs [20]. Postthaw sperm values of dry thawing system were different from both those of water bath in our study and those of other studies and this result may be attributed to differences in thawing procedures.

Recently, there has been a growing interest in evaluating sperm kinematic parameters with CASA to determine sperm motility properties more accurately and objectively than subjective assessment [9–11, 20]. Several researchers [21,22] reported that there were correlations between fertility and sperm kinematic characteristics evaluated with CASA, and VCL, VSL, and VAP may be used for estimation of in vivo fertility. In our study, since there was no difference for velocity parameters between two different thawing methods, it can be said that the effects of dry thawing and water bath thawing on sperm motion traits were the same with each other. An indicator of fertility in bulls is the ability of sperm to transform into a hyperactive stage. The most commonly used parameters to identify hyperactive sperm are high VCL and ALH values and low LIN values. However, it is not beneficial for the spermatozoa to become hyperactive before reaching the oviducts; therefore, VCL, ALH, and LIN kinematic values should be low in sperm samples to be frozen before AI [23]. In our study, thawing methods did not affect some motility kinematic values but the spermatozoa STR and ALH values changed. The results obtained from the study indicated that ALH and STR values of sperm thawed in dry thawing system were significantly higher than those of sperm thawed in a water bath. Some studies [24– 27] reported that spermatozoa with ALH higher than 7 μm/s and VCL higher than 70 μm/s were considered as indicative of hyperactivation. ALH value of our study was lower than 7 μm/s. Although VCL value of our study was a little bit higher than 70 μm/s, it was not affected by thawing methods. In this study, kinematic motility values of dry thawing system and water bath thawing were found to be lower than the average kinematic motility values of the frozen-thawed bull semen of the study conducted by Muino et al. [28]. It has been said that if spermatozoa are progressive, STR value of spermatozoa is higher than spermatozoa in circular swimming pattern [29]. In light of this information, dry thawing significantly increased STR value compared to water bath thawing. This result was in agreement with the statement by Ratnawati et al. [29].

Postthaw defective acrosome rate as well as some other morphological defects when straws were thawed in dry system were lower ($P < 0.05$) than those of straws thawed in water bath. Especially, there was a difference ($P < 0.0001$) for abnormal head rate of spermatozoa when straws were thawed in dry thawing system. The reason for the positive effect of dry thawing system on morphological defects in this study is unknown. However, Nur et al. [17] reported that thawing method affected morphological damages. The results for the effects of thawing methods

Table 1. Values (mean ± standard error of means) for semen motility and kinematic characteristics in different thawing methods.

Parameters	Dry system	Water bath	P-value
	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	
TM (%)	57.15 ± 4.31	49.25 ± 4.89	>0.05
PM (%)	36.21 ± 3.53	29.02 ± 2.84	>0.05
VCL (μm/s)	76.33 ± 3.34	75.20 ± 2.96	>0.05
VAP (μm/s)	44.20 ± 2.71	51.23 ± 3.29	>0.05
VSL (μm/s)	34.21 ± 2.62	35.54 ± 2.48	>0.05
STR (%)	66.37 ± 2.21	57.88 ± 2.17	<0.05
LIN (%)	42.65 ± 2.61	38.37 ± 2.21	>0.05
WOB (%)	58.53 ± 2.30	60.00 ± 2.43	>0.05
ALH (μm)	3.28 ± 0.11	2.78 ± 0.13	<0.05
BCF (Hz)	6.67 ± 0.37	6.25 ± 0.35	> 0.05

TM: Total motility, PM: Progressive motility, VCL: Curvilinear velocity, VAP: Average path velocity, VSL: Straight-line velocity, STR: Straightness (VSL/VAP × 100), LIN: Linearity (VSL/VCL × 100), WOB: Wobble (VAP / VCL × 100), ALH : Amplitude of lateral head displacement, BCF : Beat-cross frequency.

Table 2. Values (mean ± standard error of means) for spermatological parameters in different thawing methods.

Parameters		Dry system	Water bath	P-value
		$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	
Abnormal spermatozoa (%)	Acrosome	2.50 ± 0.26	3.40 ± 0.26	<0.05
	Head	5.00 ± 0.36	8.10 ± 0.43	<0.0001
	Middle part	1.20 ± 0.24	3.10 ± 0.75	<0.05
	Tail	5.10 ± 0.52	4.60 ± 0.70	>0.05
	Total	14.70 ± 0.80	18.50 ± 1.03	<0.05
Host (%)		74.00 ± 1.66	76.00 ± 0.77	>0.05
Viability (%)		55.60 ± 3.87	48.40 ± 3.51	>0.05

on morphological damages were in agreement with those reported by Senger [1] and Nur et al. [17].

Whereas the viability stains are used to assess physical plasma membrane damage, HOST evaluates plasma membrane's biochemical activity but intact plasma membrane does not imply that it is functional [30]. In our study, there was no difference for postthaw HOST values of spermatozoa thawed with different methods. Mishra et al. [31] stated that postthaw HOST values belonging to different breeds and evaluated after holding at more than 35 °C were between 68.70% and 72.20%. The results of the present study were a little bit higher than those of Mishra et al. [31].

In conclusion, the present study demonstrated the possibility of using dry thawing system as alternative to water bath for thawing bull semen because some postthaw sperm values obtained from dry thawing system were better than those obtained from water bath thawing. In other words, thawing in dry system may be useful for successful artificial insemination. However, the results of this study need to be supported by in vivo studies.

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