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Effect of different thawing procedures on the quality and fertility of the bull spermatozoa

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ABSTRACT

Objective: To improve the indicators of motility, survival and fertilizing ability of spermatozoa by optimizing temperature factors and the duration of exposure at unfreezing straws. **Methods:** Straws by volume 0.25 mL were thawed at water bath temperatures at 65 ℃, 67 ℃ and 70 ℃ for 6-7 seconds and at 75 ℃ for 4-6 seconds. Impact of exposure time and temperature thawing in the water bath on motility and survival of spermatozoa were studied. **Results:** Studies indicate that for the procedure of defrost water bath straws in seven seconds for temperature conditions of 65 ℃, 67 ℃ and 70 ℃, indicators of progressive motility and absolute survival rate were significantly higher than for the control group an average on 11.4 % (P<0.01). Optimum exposure time (6-7 seconds) and temperature range (65-70 ℃) defrosting semen doses were defined. **Conclusions:** Owing obtained the positive result, method of thawing was developed which increases the indicators of motility, survival and fertilizing capacity of bull semen.

1. Introduction

Frozen semen in straws has become the universally accepted unit of storage and transfer of bovine genetics to cattle procedures which depends on preserve the functional activity of spermatozoa (viability and fertilizing ability)[1]. High viability and motility of spermatozoa are important factors for successful artificial insemination (AI) because a significant correlation between post-thawing sperm viability and subsequent conception rate has been reported[2]. The freezing and thawing of semen inevitably reduces the proportion of motile spermatozoa and causes ultra structural, biochemical and functional damages[3]. It has been shown that an increase in post-thaw viability will result in increased fertility of the semen[4]. Thawing procedure is just as important as the freezing procedure in terms of its impact on the survival of spermatozoa [5]. Defrosting of sperm should be at maximum speed. Increasing the speed of thawing frozen semen increases the number of sperm that restore maximum motility[6]. The rapid thawing of semen decreases the harmful effects of recrystallization processes and hydration, preventing damage to sperm membrane and cytoplasm. In this case, when passing through the temperature danger zone (-50~-30 ℃ and -30~0 ℃) ice crystals do not have time to be formed and sperm switches directly from the glassy state to the liquid state[1, 6-8]. Various factors of interaction with thawing procedures which affect the post thawing motility of sperm such as type of extender, concentration of glycerol, method of semen packing, cooling rate, semen handling during cryopreservation procedure [9, 10] and experimental conditions, such as available facilities, tools and chemicals, vary among countries and areas [11, 12]. Thus the methods of freezing and thawing frozen spermatozoa should be examined in

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each country and area[13]. Many researches have been conducted to determine the optimal thawing temperature, duration and increased to know the adequate thawing rate that may give highest percentage of viable spermatozoa after post thawing process[2, 14, 15]. However, a number of studies have shown that thawing temperatures as high as 60-80 ℃ could further improve post-thaw motility[3, 16-18]. In some countries, pellets of sperm thawed at +55 ℃. Some researchers propose to use for thawing frozen semen of bulls higher temperatures - from +50 ℃ to +75 ℃ or even 100-150 ℃ [19, 20]. Many studies have been conducted to assess the influence of high thawing temperatures on sperm survival and motility, using different thawing rates, for bulls[4, 5, 21-23], boars, rams and dogs[24-26].

Improving defrost modes of sperm bulls and improve sperm quality indicators are relevant and has important theoretical and practical significance. The aim of this research is to improve the performance of motility, survival and fertilizing ability of spermatozoa by optimizing temperature factors and the duration of exposure at unfreezing straws. This study was consisted of two stages: 1) to determine the optimum thawing procedures in order to know the adequate thawing rate that may give highest percentage motility and viability of spermatozoa after thaw process; 2) to evaluate the relationship between this technique of thawing and fertilizing ability of spermatozoa.

2. Materials and methods

Studies were performed using cryopreserved of sperm bulls, frozen in French straws by volume 0.25 mL, in the laboratory of the company of "Progress" (Ukraine).

2.1. Thawing procedures

Straws by volume 0.25 mL were thawed at water bath temperatures at 65 ℃, 67 and 70 ℃ for 6-7 seconds and at 75 ℃ for 4-6 seconds. Under instructions from AI of cattle, straws by volume at 0.25 mL that are recommended to thaw in a water bath at 35 ℃ for 20 seconds was used as the control[27].

2.2. Semen evaluation

Immediately after thawing, the content of each straw was emptied in a 2 mL Eppendorf tube at 38 ℃. The sperm suspension was incubated at 38 ℃ and was evaluated for post thaw motility indicators and viability of spermatozoa through every hour to cell death. For AI 295 heads of Ukrainian red-white dairy cattle breeding in the farm "RVD-Agro" (Ukraine) were used. Straws were thawed immediately before insemination at 65-70 ℃ for 6-7 sec. Fertilization in control group was conducted after thawing sperm in a water bath at 35 ℃ for 20 seconds.

2.3. Motility

Indicators of progressive motility and the dynamic characteristics of sperm movement were assessed with a computer-assisted sperm motility analyzer (Sperm Vision) (CASA) and microscope Olympus CX-31. The dynamic characteristics and progressive motility were analyzed immediately after thawing (0 hour) and every hour of incubation at 38 ℃. Then the absolute survival rate of spermatozoa (ASR) was calculated. Among the dynamic characteristics of sperm movement mean velocity (VAP, μm/s), straight-line velocity (VSL, μm/s), the average distance of movement (DAP, μm/s) and distance in a straight line (DSL, μm/s) were studied[28].

2.4. Statistical analyses

Materials of researches were calculated by methods of mathematical statistics means of the software package Statistica[29].

3. Results

3.1. Motility

Studies indicate that for the procedure of defrost water bath straws in seven seconds at temperature conditions of 65 ℃, indicators of progressive motility (PM) were significantly higher than for the control group by 5.4 % (P<0.05). At the same time, the indicators of PM obtained with six seconds exposure were lower than for the control group by 2.6 % (P>0.05) (Table 1). It was found that by the thaw rate straws in six and seven seconds for temperature at 67 ℃, PM values were significantly higher than for the control group by 5.4 % and 6.5 % (P<0.01), accordingly. The procedure of the thawing straws six and seven seconds for the temperature regime of 70 ℃, PM values were significantly higher than the control group by 5.1 % and 10.5 %, accordingly (P<0.01) (Table 1).

Table 1

Indicators of progressive motility spermatozoa for temperature by thawing 65-70 ℃, after thawing frozen semen (0 hour of incubation) (Mean±SEM, %).

Temperature (℃)	*n*	Thawing rate in a water bath, second		
		20 seconds	6 seconds	7 seconds
35	90	62.7±0.8	-	-
65	20	-	65.3±2.3	68.1±2.1[a]
67	60	-	68.1±1.4[b]	69.2±1.4[c]
70	60	-	67.8±2.2[a]	73.2±2.1[a]

Note: [a]P<0.05; [b]P<0.01; [c]P<0.001 levels significantly to control.

At temperature of 75 ℃ and the procedure of thawing straws 4-6 seconds indicators of progressive motility and absolute survival rate were lower and not statistically

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