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Effect of Various Thawing Times and Temperatures on Frozen Semen Quality in Plastic Straws of Artificial Insemination of Bulls in Iraq

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Abstract: The present study was designed to determine the best practical method of semen thawing which could be applied to frozen semen of bulls in straw produced by Artificial Insemination Center, Iraq. Frozen semen was thawed at 15°C for 30 sec, 40°C for 10 sec and 50°C for 5 sec. individual motility, live, and dead sperm cells was assessed using light microscope. Results revealed that number, motility, and live of sperm cells, were significantly higher at the 50°C for 5 sec than other thawing temperatures In conclusion, it is recommended to use a temperatures of 50°C for 5 sec and to dissolve the frozen semen because it gave the best quality semen after thawing and easy to utilize in field.

Keywords: Frozen sperm, Temperature, Thawing time.

Introduction

Frozen semen in straws has become the accepted unit of storage and transfer of genetics factors to cattle which depends on preserve the viability and fertilizing ability of spermatozoa (Anand, 1979). During frozen of semen, several factors may be responsible for the decrease of fertilizing ability of spermatozoa (Brito *et al*., 2002). In addition to frozen of semen, biological activity until thawing and fertilization of frozen sperm depend on thawing technique (Jondet, 1972). Thawing step brings the sperm back to life and to body temperature so thawing must be done carefully to avoid damage to the sperm (Bearden *et al*., 2004). Previous studies have been carried out to determine the optimal thawing temperature and duration to know the adequate thawing rate that may give highest percentage of viable spermatozoa post-thawing process (Robbins *et al*., 1976; Pace *et al*., 1981; Dhami and Sahni, 1993). Various factors interaction with thawing procedures which affect the motility of sperm include extender, concentration of glycerol, method of semen packing, cooling rate and semen handling during cryopreservation procedure (Rodriguez *et al*., 1975; Robbins *et al*., 1976), and experimental conditions including available facilities, tools and chemicals, vary among countries and areas (Vishwanath & Shannon, 2000; Thibier & Wagner, 2002). Thus the methods of freezing and thawing

frozen spermatozoa should be examined in each country and area (Hayashi and Isobe, 2005). Therefore, this study aimed to found out the best practical method of semen thawing which could be applied to get the highest percentage of viable spermatozoa post-thawing process.

Materials and Methods

Frozen semen

This study was conducted at the College of Veterinary Medicine, University of Basrah. The straw of frozen semen of bulls were obtained from Artificial Insemination Center in Abu Ghraib, Iraq.

Experimental design:

Thirty- six artificial insemination straws were used capacity (0.1 ml) and the straw were stored in a bottle of nitrogen that are specific for straw of artificial insemination underscore (-196°C). That straw were used to evaluate the motility, live, and dead sperm. They were divided into three groups each of 12. The straws were thawed as following: 50°C for 5 sec, 40°C for 10 sec and 15°C for 30 sec in water bath.

Assessment of post- thawing

Sperm count

Spermatozoa number was quantified using a Hemocytometer under a light microscope as described by Evans and Maxwell (1987). Briefly, a drop of thawed semen was placed on a haemocytometer chamber. The sperms than were measured in five squares: four of them in the corner and the fifth one in the middle was down and then multiply by 10000.

Sperm motility

Spermatozoa motility percentage were assessed using a light microscope as described by Evans and Maxwell (1987).

Live and dead sperms: Percentage of live and dead spermatozoa were evaluated using Eosin-Nigrosin stain. Briefly, a drop of thawed semen was placed on a slide, stained with Eosin-Nigrosin, dried at room temperature and examined under light microscope. The dead sperm appear pink in colour while the live sperm colourless.

Statistical analysis: Collected data were analyzed using SPSS (1996), one way ANOVA test was performed to identify significant difference among the treatment means.

Results

Sperm count:

Analysis of the Sperms number data were revealed a marked increase in number of sperm in parallel with increasing temperature thawing (Table 1). The current study revealed that thawing of straw at 50°C for 5 sec increase the sperms number significantly compared to the thawing at 40°C for 10 sec and at 15°C for 30 sec.

Individual motility of spermatozoa

Analysis of the Sperm motility data revealed a marked increase in the individual motility of sperm in parallel with increasing of the temperature decreasing the time of thawing (Table 1). The current study revealed that thawing of straw at 50°C for 5 sec increase the percentage of sperm motility significantly compared to the thawing at 40°C for 10 sec and at 15°C for 30 sec.

Live sperms

Analysis of the live sperm data revealed a marked increase in Percentage of live sperm in parallel with increasing of temperature and decrease the time of thawing (Table 1). The present study revealed that thawing of straw at 50°C for 5 sec decreased sperms significantly compared to the thawing at 40°C for 10 sec and at 15°C for 30 sec.

Dead sperms

Analysis of the dead sperms data revealed a marked decrease in dead sperms in parallel with increasing temperature and decreasing the time of thawing (Table 1). The current study revealed that thawing of straw at 50°C for 5 sec decrease dead sperms percentage significantly compared to the thawing at 40°C for 10 sec and at 15°C for 30 sec.

Table (1): Effect of thawing temperature and time on some semen characteristics of stored in plastic straws (Mean± SD, n=12).

Thawing temperature and time	Semen characteristics			
	Sperm count $\times 10^6$	Sperm motility %	Live sperm %	Sperm dead%
15°c for 30 sec	4.16±0.131 a	77.17±2.5 c	79.10±2.28 c	21.02±1.49 a
40°c for10 sec	4.02±0.012 b	84.35±1.23 b	86.00±1.27 b	14.20±1.32 c
50°c for5 sec	4.20±0.55 a	86.22±1.99 a	87.80±1.39 a	12.20±1.35 b
LSD	0.010	0.026	0.012	0.050

Different letters within column mean significant differences at p≤0.05

Discussion

The current study showed that thawing of straw at 50°C for 5 sec increase the sperms number compared to the other thawing temperature. This finding is in agreement with previous study (Ostashko,.1978), in which the rapid thawing of semen leads increase sperm counts and restore its activity and vitality. This finding may be attributed to the fact that rapid thawing leads to the preservation of sperm membrane and cytoplasm, which leads to an increase in the sperm count(Pace *et al*., 1981).

Present study also showed that thawing of straw at 50°C for 5 sec increase the percentage of sperm individual motility compared to the other thawing temperature. These finding is in agreement with previous studies (Ileri and Ak, 1993; Correa *et al*., 1997), in which the temperatures greater than 35°C result in higher sperm motility. This finding may be attributed to the fact that thawing of the semen should be rapid, since slow thawing allows re-crystallization of ice within the cells causing membrane damage (Wiggin and Almquist 1975; Senger, 1980).

The current study also showed that thawing of straw at 50°C for 5 sec was decreased the percentage of dead sperms compared to the other thawing temperature. This finding is in agreement with previous study (Vishwanath and Shannon, 2000), in which the rapid thawing of semen prevents injury during re-warming. This finding may be attributed to the fact that thawing of the semen should be rapid, because slow thawing allows causes higher percentage of dead sperms due to the occurrence of more marked osmotic pressure changes (Curry and Watson 1994).

Conclusion

Thawing of frozen semen at 50°C for 5 sec is recommended to be used because it gave good quality of semen post thawing.

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