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Effect of Various Thawing Times and Temperatures on Frozen Semen Quality of Friesian Bulls in Iraq

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Abstract: This experiment was designed to determine the best practical method of semen thawing which could be applied to frozen semen in straw obtained from Holstein-Friesian produced by Artificial Insemination Center-Iraq. frozen Semen was thawed in the following procedures: 5°C for 30 min, 37°C for 20 sec, 37°C for 30 sec and 60°C for 8 sec and motility, live, morphology and Post-thaw livability of sperm cells was assessed by determining the percentage of progressively motile sperm at 0, 2 and 4 h of incubation at 37°C. Results revealed that motility, live, abnormality, intact acrosoma and Post-thaw livability of sperm cells, were significantly ($p<0.05$) higher for the 37°C for 30 sec and 60°C for 8 sec than the other thawing methods and post-thaw semen can maintained at water bath 37°C until 3 h. Also thawing procedures at 37°C for 30 sec it is recommended to use in Iraq because it was showing good quality of post thawing semen and easy to used in field. In addition to result indicated that thawing at 60°C for 8 sec lead to best results but difficult practically used in field.

Keywords: Friesian bull, frozen semen, temperature, thawing time

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), during preservation several factors may be responsible for the possible (frozen of semen) decrease of fertilizing ability of spermatozoa (Anand, 1979) , in addition to freezing process works to hold all biological activity until thawing and fertilization of frozen sperm depend on thawing technique (Jondet, 1972), thawing step brings the sperm back to life and back to body temperature so thawing must be done carefully to avoid damage to the sperm (Bearden *et al*., 2004). Many researches has 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 (Robbins *et al*., 1976; Pace *et al*., 1981; Dhami and Sahni, 1993). Bearden *et al*. (2004) define that Thawing rates are the process of thawing straws of semen at a specific temperature in a specific amount of time. Various factors 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 (Rodriguez *et al*., 1975; Robbins *et al*., 1976) and experimental conditions, such as available facilities, tools and chemicals, vary among countries and areas (Vishwanath and Shannon, 2000; Thibier and Wagner, 2002). Thus the methods of freezing and thawing frozen spermatozoa should be examined in each country and area (HaYashi and Isobe, 2005). This study was design to found the best practical method of semen thawing which could be applied to all semen production by Iraqi Artificial Insemination Center because it is important to development program of frozen straws and contribute to development livestock in Iraq. Therefore, this study was conducted as attempt to:

- Determine the optimum thawing procedures in order to know the adequate thawing rate that may give highest percentage of viable spermatozoa after post-thaw process.
- Evaluated the relationship between this technique of thawing and livability of sperm after thawing during incubation at 37°C.

MATERIALS AND METHODES

Animals and frozen semen: This study was conducted at the Artificial Insemination center-Abu-Graib. Five Holstein-Friesian bulls were used. According to technique revealed in Artificial Insemination center, ejaculates from the bulls were collected with the aid of an artificial vagina, after collection semen evaluation and used the suitable 8 ejaculates for each bull's, dilution, frozen in 0.25 mL straws and storage 48 h in liquid nitrogen. Hundred and sixty straws (8 ejaculates 5 bulls 4 thawing procedures) straws were used to evaluated (motility, sperm live and abnormality), except study of intact acrosoma and livability parameters eighty straws (4 ejaculates 5 bulls 4 thawing procedures).

Table 1: Effect of thawing temperature on some semen characteristics of bull semen frozen in plastic straws

	Semen characteristics				Livability of sperm incubation at 37°C Water bath after thawing %		
Thawing temperature	Individual motility %	Live sperms %	Abnormality sperm %	Intact acrosome %	0 h	2 h	4 h
5°C for 30 min	41.21±3.64c	50.12±4.94c	40.37±6.22a	26.99±6.47c	38.91±4.04Ac	30.55±5.61Bd	20.39±4.33Cd
37°C for 20 sec	50.11±2.24b	58.48±6.64b	34.87±5.98ab	35.69±6.33b	50.91±3.84Ab	44.11±2.43Bc	33.83±6.72Cc
37°C for 30 sec	65.20±3.21a	77.11±5.40a	30.99±3.66b	61.20±3.21a	60.29±2.66Aa	58.82±3.34Ab	40.89±5.64Bb
60°C for 8 sec	66.33±2.54a	78.77±4.53a	23.65±4.00c	66.77±4.92a	64.77±2.59Aa	62.21±3.56Aa	55.77±5.44Ba

Values are mean±S.E.; Within colum different small letters significant at ($p<0.05$); Within row different capital letters significant at ($p<0.05$)

Thawing procedures: The straws were thawing as following:

- 5°C for 30 min ice water (slow thawing)
- 37°C for 20 sec water bath
- 37°C for 30 sec water bath
- 60°C for 8 sec (rapid thawing)

Assessment of post thawing:

- **Sperm motility:** Spermatozoa motility percentage was assessed subject able using a phase contrast microscope with a warm slide according to Chemineau *et al*. (1991).
- **Sperm live and abnormality:** Percentage of live and abnormality of spermatozoa was evaluated using of Eosin-Nigrosin stain.
- **Intact acrosoma:** The percentage of spermatozoa with intact acrosoma was evaluated according to Brown *et al*. (1982).
- **Livability of sperm:** This characteristics was assessed depends on to evaluated individual motility change of spermatozoa every two hours, by pooled of four straws in test tube and plunged into water bath maintained at 37°C for 0, 2, 4 h by used the same procedure which used by Correa *et al*. (1997).

Statistical analysis: Collected data were analyzed using (SAS, 2001) computer program (ANOVA). Duncan Multiple Rang Test was performed to identify significant difference among the treatment means.

RESULTS

Individual motility of spermatozoa: Table 1 display a changes value of sperm motility, there were gradual increase in individual motility of sperm in parallel with increasing temperature thawing, Table 1 shows that thawing of straw at 37°C for 30 sec and 60°C for 8 sec more significant ($p<0.05$) progressively motile sperms than that if thawing at 37°C for 20 sec and 5°C for 30 min. As depicted in Table 1 individual motility during thawing at 37°C for 20 sec more significantly ($p<0.05$) than thawing at 5°C for 30 min. No differences were found between 60°C for 8 sec and 37°C at 30 sec.

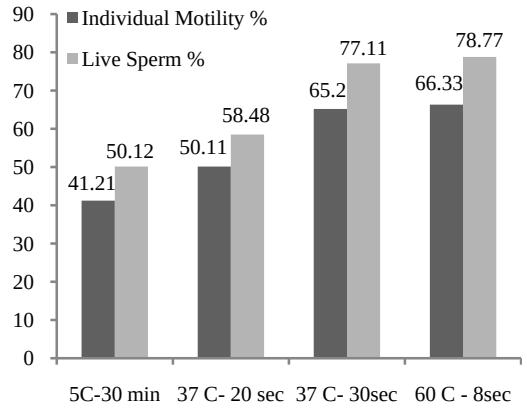

Fig. 1: Effect of thawing temperature on individual motility and live sperms of bull semen frozen in plastic straws

Figure 1 shows that thawing of straws at 60°C for 8 sec gives better individual motility of sperms than 37C° for 20 or 30 sec and 5°C for 30 min.

Percentage of live sperm: Results of live spermatozoa percentage was listing in Table 1, indicated that 37°C for 30 sec and 60°C for 8 sec thawing procedures was superior significant ($p<0.05$) to that at 37°C for 20 sec and 5°C for 30 min thawing. A significant ($p<0.05$) were also appeared between 37°C for 20 sec and 5°C for 30 min, while no significant differences between percentage of spermatozoa live during thawing in 37°C for 30 sec and 60°C for 8 sec. Figure 1 shows that thawing of straws at 60°C for 8 sec give betters live sperms than 37°C for 20 or 30 sec and 5°C for 30 min.

Abnormalities percentage: Data of Table 1 revealed the thawing straws at 60°C for 8 sec decreases the percentage of abnormal sperms significantly ($p<0.05$) when compared with the three thawing procedures 37°C for 20 sec or for 30 sec and 5°C for 30 min, no significant differences between the abnormalities percentage of sperm in both two last thawing methods and between thawing technique 37°C for 20 sec or for 30 sec. Figure 2 showing that thawing of straws at 5°C for 30 min causes more sperm abnormalities when compare with thawing at 60°C for 8 sec and 37°C for 20 or 30 sec .

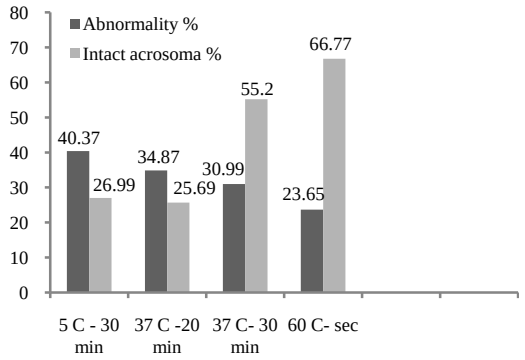

Fig. 2: Effect of thawing temperature on abnormality of sperms and intact acrosoma sperms of bull semen frozen in plastic straws

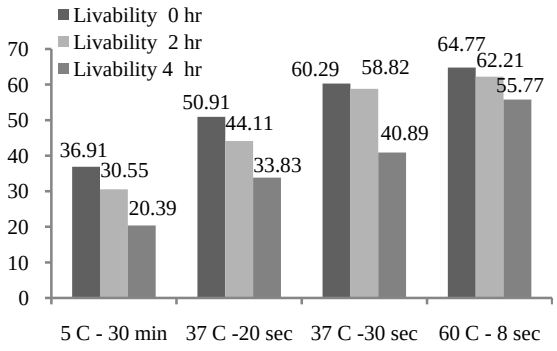

Fig. 3: Effect of thawing temperature on livability of sperms incubation (0, 2 and 4 h at 37°C after thawing frozen semen)

Intact acrosoma: The evaluation of intact acrosoma of frozen bull sperm was shows in Table 1. The results indicated that most intact in acrosoma were found at thawing 37°C for 30 sec and 60°C for 8 sec, no significantly differences between them, but a significantly ($p<0.05$) differences was appeared from thawing at 37°C for 20 sec and 5°C for 30 min, in addition to intact of acrosoma in 37°C for 20 sec more significantly ($p<0.05$) increases than 5°C for 30 min. Figure 2 shows that thawing of straws at 60°C for 8 sec gives more percentage sperm with intact acrosoma than other thawing procedures.

Livability percentage: Table 1 showing the Sperm livability after thawing and storage in the water bath (37°C) during 0, 2 and 4 h, in this parameter can be apparently in two point's:

- **First:** Comparative between livability in different thawing procedures during each period
- **Second:** Comparative study between livability in different period's storage during each thawing technique

First showing that during period (0 h) livability in thawing at 37°C for 30 sec water bath and 60°C for 8 sec was significantly ($p<0.05$) better than 37°C for 20 sec and 5°C for 30 min and the significantly ($p<0.05$) differences between thawing frozen semen in 37°C for 20 sec with 5°C for 30 min, but during 2 and 4 h period the difference was significantly ($p<0.05$) between all thawing procedures (Table 1), on the other hand during 0 h didn't showing significantly ($p<0.05$) differences between 37°C for 30 sec with 60°C for 8 sec. Second: study comparative between livability in different periods storage (0, 2, and 4 h, respectively) during each thawing technique, Table 1 revealed that livability in thawing procedure at 5°C for 30 min and 37°C for 20 sec decrease significantly ($p<0.05$) in all period but 37°C for 30 sec and 60°C for 8 sec maintained the livability until 2 h but during 4 h livability was significantly ($p<0.05$) decreases. Figure 3 showing that livability of post-thaw sperm decrease in all thawing procedures during all duration period of incubation (0, 2 and 4 h, respectively) but least decrease in thawing 60°C for 8 sec and 37°C for 30 sec.

DISCUSSION

Present results are similar to previous publications which demonstrated that while temperatures greater than 35°C result in higher sperm motility, it should be pointed out that the duration of the thawing must be carefully with shortened and carefully timed (Senger, 1980; Ileri and AK, 1993; Blackshaw, 1955; Correa *et al*., 1997; Robbins *et al*., 1973, 1976; Almquist and Wiggin, 1973).

Wiggin and Almquist (1975) demonstration a benefit to post-thaw according retention and motility following rapid thawing. Salisbury *et al*. (1978) reported that Thawing of the semen needs to be rapid, since slow thawing allows re-crystallization of ice within the cells, causing membrane damage (Wiggin and Almquist 1975; Senger, 1980), while Ahmad (1984), Ozkoca (1984) and Nur *et al*. (2005) reported that the post-thaw sperm motility as well as injured acrosoma and live of sperm was higher significant when straws were thawed at 70°C for 5 sec compared to thawing at 37°C for 30 sec and 50°C for 15 sec. The results of present study showed that post-thaw morphological defects were significantly lower when the straws were thawed at 60°C for 8 sec compared to other thawing temperature, Vishwanath and Shannon (2000) revealed that rapid thawing of semen was prevent injury during re-warming. Holt (2000) observed that rapid thawing prevents the possibility of re-crystallization of water molecules and caused injurious to cell membranes, but slow thawing causes higher abnormalities in sperms this results agree with Curry and Watson (1994) whom denoted that the principle problem of slow thawing procedures is the occurrence of more marked osmotic pressure changes. In addition to results revealed that Thawing at 37°C for 30 sec was

the best thawing procedures for frozen bull spermatozoa, Gordon (2002) mentioned that the optimum thawing rate maintained high percentage of motility and viability of spermatozoa during post-thaw is (30-37°C) for 30 sec. Mitchell and Doak (2004) reported that semen is commonly thawed at temperature between 33 and 37°C with a thawing duration of 30 to 40 sec. Pace *et al*. (1981) and Nur *et al*. (2006) showing that thawing straws of bulls in 37°C were resulted higher fertility than those thawed either ambient (10°C) or ice water. Bravo *et al*. (2002) emphasis that straws must be thawed in water bath at 37°C for 30-40 sec or 40°C for 8 sec. Chaiprasat *et al*. (2006) Highest and Lowest sperm progressive motility rate was founded in 37°C for 30 sec and 20°C for 30 sec respectively. Present study revealed that higher percentage of intact acrosoma in thawing 37°C for 20 or 30 sec compared with 5°C for 30 min. A higher normal acrosoma rate and livability can be attained by thawing in water at 35-75°C rather than at 5-20°C (Masuda, 1992). Robbins *et al*. (1973, 1976), Almquist and Wiggin (1973) and Wiggin and Almquist (1975) demonstration a benefit to post-thaw according retention and motility following rapid thawing. The acrosoma damage was reported to occur in sperm thawed at less than 37°C (Robbins *et al*., 1976; Senger *et al*., 1976; Pace *et al*., 1981; Correa *et al*., 1996; Dejarnette *et al*., 2000). Results of present study showed that best livability in 60°C for 8 sec and 37°C for 30 sec for 3 h after post-thaw incubation at 37°C, but Dejarnette *et al*. (2000) found that the latent sperm injury when sperm post-thaw and incubated at 37°C for 3 h, Hayashi and Isobe (2005) revealed that no differences in viability, motility and acrosoma change of sperm during storage at 4°C until 6 h between sperm thawed at 23°C for 30 sec and 37°C for 15 sec.

CONCLUSION

Thawing procedures 60°C for 8 sec and 37°C for 30 sec surpassed others thawing and 60°C for 8 sec surpassed 37°C 30 sec, but thawing procedures 37°C for 30 sec it is recommended used in Iraq because easy used in felid and the results shows that proximally quality of post thaw semen when at 60°C for 8 sec which it is difficult practically used in field.

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