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THAWING OF DEEP-FROZEN BULL SEMEN

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There is no unanimous opinion about the best method of thawing deep-frozen bull semen and little work has been done to establish a relationship between the rate of cooling and the rate of thawing bull semen. In the present experiments 80 samples of bull semen were tested. The semen was deep-frozen in polyethylene ampoules by fast and slow methods of deep-freezing. The frozen semen was thawed by four methods: (1) in air at room temperature (2) in ice water (3) in a water bath at + 40°C and (4) at + 75°C. Analysis of the data shows that the highest values as regards the quality of the semen deep-frozen by slow method was obtained after thawing it in ice water at 0°C and the most effective rate of thawing the bull semen deep-frozen by fast was noticed methad when thawed in a water bath at + 75°C. Thus semen deep-frozen by slow method should be thawed in ice water and that frozen by fast method in water bath at + 75°C.

INTRODUCTION

The livestock industry needs improvement by the application of modern techniques of breeding, feeding and management. The art of artificial insemination has played a pivotal role in animal breeding. It provides means for bringing about considerable genetic progress in the herds by extending the use of proven sires over a very large population. The success of artificial insemination depends mainly on the efficient and economical techniques of semen preservation which enable its storage over a long period and its transportation from one place to another without loss in its fertilizing ability. The semen may be stored in fluid state for a short-term but it can be preserved for a long term in frozen state. During the last 15 years, the semen preserved in fluid form is gradually being replaced by deep-frozen semen. The bull semen may be deep-frozen by slow or by fast methods of freezing in ampoules, straws or pellets and kept in liquid Nitrogen (-196°C) or dry ice (-79°C). Before use it has to be warmed up to a fluid state by a

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process called thawing. As there are different methods for deep-freezing bull semen, similarly different techniques are used for its thawing.

At present there is no unanimous opinion about the method of thawing deep-frozen bull semen stored at -79°C or -196°C. Habibulin (1958) studied the effect of different temperatures of water for thawing deep-frozen semen. The higher rate of motility of spermatozoa was observed in samples thawed slowly over a period of 1 to 1.5 hour in a water bath at 0°C. In another series of experiments the best results were obtained in samples thawed in air at room temperature. Lopatko (1962) recommended the thawing of ram semen in air at room temperature. The bull semen deep-frozen by slow and fast method of freezing was thawed by Polge and Jakobsen, (1959) in a water bath at 30°C.

Salisbury and VanDemark (1961) recommended that the deep-frozen bull semen may be warmed without any precautions to body temperature before insemination. However, if the semen is not to be used immediately after thawing, it should be warmed only to 5°C and kept at that temperature till insemination (Van-Demark *et al.* 1957). For this reason, some workers prefer to thaw ampoules of semen frozen at -79°C by immersing them in water at + 5°C or at +20°C (Miller and Van Demark 1954; Mixner, 1955 and Bruce, 1956). Boyd and Hafs (1968) preferred thawing of deep-frozen semen in ice water at 0 to 5°C and used it for insemination within 5 minutes after thawing. They noticed that thawing semen in air at room temperature rapidly decreased fertilizing ability of spermatozoa. Aamdal and Andersen (1968) observed a higher percentage of alive spermatozoa from the samples of deep-frozen semen in straws by thawing in boiling water for 6 seconds than those thawed in water at +35°C. However, water at +75°C proved better for thawing straws. They also studied the conception rate in cows inseminated by deep-frozen bull semen in ampoules and straws, thawed in ice water at 0° and in a water bath at +75°C respectively. They observed an increase of 4.2% in 30-60 days N.R. rate of cows inseminated by semen deep frozen in straws and thawed in water bath at +75°C. The present study was conducted to study the effect of different methods of thawing on the quality of bull semen deep-frozen by slow and fast methods in liquid nitrogen

MATERIALS AND METHODS

Ten ejaculates from cow bulls were collected by artificial vagina and

diluted with glucose-egg yolk–citrate extender with glycerine. This extender has been widely used for deep-freezing bull semen. The extended semen was equilibrated for 6-8 hours at 4-5°C packed in polyethylene ampoules of 1.2 ml capacity sealed by a hot iron and stored at 4 to 5°C for freezing.

The ampoules filled with semen from each ejaculate, were randomly divided into two groups. One group of ampoules was deep-frozen in liquid nitrogen by the slow method according to Ostashko and Bugrov (1968). The other group of ampoules were held in vapours of liquid nitrogen at a height of 7 cm from its surface for 6-7 minutes and then dipped in it (fast methods of deep-freezing semen). The ampoules of each ejaculate deep-frozen by the two methods were stored separately in liquid nitrogen for at least 24 hours before thawing in this study.

Four ampoules from each batch were taken out of liquid nitrogen. One ampoule was placed on the table for thawing in air at room temperature, the second ampoule was dipped in ice water at 0°, the third and fourth ampoule were thawed in a water bath at +40°C and +75°C respectively. While thawing the ampoules in water at +40°C and +75°C, the ampoules were taken out of the water baths when the temperature of the thawing semen was only a few degrees above 0°.

Immediately after thawing, the semen was evaluated on the basis of rate of motility of spermatozoa under the microscope and the ampoules with thawed semen were corked and placed in the incubator at +37.5°C for determining the longevity of the spermatozoa. The absolute index of longevity of spermatozoa was calculated according to Milavanof (1962).

RESULTS AND DISCUSSION

The average percent motility of sperms, longevity in hours and absolute index of longevity of semen deep-frozen by slow or fast method and thawed at different temperatures have been presented in Table 1.

Analysis of the data showed that in bull semen deep-frozen by slow method the poorest quality was obtained by thawing in air at 18° to 20°C. The rate of motile spermatozoa was 37.5 per cent, longevity of spermatozoa 5.7 hours and absolute index of longevity 9.0. The quality of semen was improved by thawing in water. The higher the temperature of the water bath, the better the quality of the thawed semen. The rate of motility, longevity and absolute index obtained by thawing semen in water bath at

+75°C was 42.5 per cent, 7.4 hours and 14.2 respectively as against 41.5 per cent, 7.0 hours and 12.9 respectively after thawing in water bath at +40°C. However, the best results were obtained from samples thawed in water at 0°C where the rate of motility was 43.0 per cent, longevity 7.4 hours and absolute index of longevity 15.0.

The results obtained from samples of semen deep-frozen by fast method and thawed by the same four methods showed (Table 1) that the poorest quality of semen was obtained by thawing in air at room temperature. There was no appreciable difference in the quality of semen thawed in a water bath at 0° and +40°C. However, the maximum values of rate of motility (47.8 per cent), longevity (9.4 hours) and absolute index (22.7) were obtained when thawing of semen was done in a water bath at +75.5°C.

The analysis of variance of the data (Table 2) revealed no significant difference in the rate of motility of the semen samples frozen either by slow or by fast methods. However, highly significant differences ($P<0.01$) were observed in the rate of motility of these samples after thawing in different temperatures. Highly significant interaction between the method of freezing and thawing was revealed by data on motility rate. On comparison by Duncan's Multiple Range Test it was found that samples of semen frozen by fast method and thawed in water bath at +75°C (FT_4) gave the highest motility. There was no significant difference between ST^1, ST_4, FT^1, FT_3 and ST_3 samples. Similarly, FT_2 samples of semen showed the lowest rate of motility.

The analysis of variance of the data obtained on longevity of the spermatozoa at 37.5°C showed that both freezing methods and thawing temperatures had highly significant effect on the longevity ($P<0.01$). However, there was no interaction between freezing x thawing methods. Duncan's Multiple Range Test showed that thawing in air significantly lowered (5.70 hours) the longevity as compared to other thawing temperatures (7.35 to 8.40 hours).

TABLE 1. *Comparative effect of different thawing methods on quality of bull semen**

Method of thawing	Frozen by slow method			Frozen by fast method		
	Rate of motility (per cent)	Longevity of spermatozoa in hours (at 37.5°C)	Absolute index of longevity	Rate of motility (per cent)	Longevity of spermatozoa in hours (at 37.5°C)	Absolute index of longevity
In air at +18-+20°C	37.5	5.7	9.0	30.0	5.7	6.3
In ice water at 0	43.0	7.4	15.0	42.5	7.8	14.8
In water bath at +40°C	41.5	7.0	12.9	42.5	7.7	13.5
In water bath at +75°C	42.5	7.4	14.2	47.8	9.4	22.7

*based on an average of 10 observations.

The analysis of variance of the data regarding absolute index of longevity of the spermatozoa showed that freezing methods, thawing temperatures and freezing x thawing are highly significant ($P<0.01$). The Duncan's Multiple Range Test showed that absolute index of longevity of FT_4 samples was significantly higher from all other treatments while the absolute index of longevity of ST_1 samples was significantly different from FT_3, ST_2 at 1 per cent level and ST_3 at 5 per cent level while this treatment was not significantly different from FT_1, ST_4 and FT_3 samples. The value of absolute index of longevity of FT_1 samples was again significantly different from FT_2 and ST_2 samples. Moreover, the semen samples ST_4 were significantly different from ST_2 and FT_2 samples. FT_3 is again significantly different from FT_2 and ST_2 samples. ST_3 are again significantly different from from ST_2 and FT_2 samples.

TABLE 2. *Analysis of variance of the effects of thawing on rate of motility, longevity and absolute index of longevity of spermatozoa*

S.O.V.	D.F.	M.S R.M.	Lon.	A.I.L.
Freezing methods	1	0.03N.S	12.00**	50.24**
Thawing temperature	3	4092**	25.10**	406.04**
Fr. x Th.	3	1042**	3.77N.S	117.10**
Error	72	0.148	1.68	4.09

R.M–Rate of motility

FT_4	ST_1	ST_4	FT_1	FT_3	ST_3	ST_2	FT_2
4.78	4.30	4.25	4.25	4.25	4.15	3.75	3.02

Long. - Longevity

T_4	T_1	T_3	T_2
8.40	7.60	7.35	5.70

A.I.L – Absolute index of longevity

FT_4	ST_1	FT_1	ST_4	FT_3	ST_3	ST_2	FT_2

R.M. = Rate of motility
Lon. = Longevity
A.I.L. = Absolute Index of Longevity
FR. = Freezing methods
Th. = Thawing methods
N.S = Non-significant
** = Significant at 1 per cent level
FT_1 = Fast freezing method, thawing in ice water
FT_2 = Fast freezing method, thawing in air
FT_3 = Fast freezing method, thawing in water bath at + 40°C
FT_4 = Fast freezing method, thawing in water bath at + 75°C
ST_1 = Slow freezing method, thawing in ice water
ST_2 = Slow freezing method, thawing in air
ST_3 = Slow freezing method, thawing in water bath at + 40°C
ST_4 = Slow freezing method, thawing in water bath at + 75°C
T_1 = Thawing in ice water at 0°
T_2 = Thawing in air at 18+20°C
T_3 = Thawing in water bath at + 40°C
T_4 = Thawing in water bath at + 75°C

Therefore, on the basis of the above data, it may be recommended that to get the maximum quality of bull semen, the thawing should be carried out

in ice water if the semen is deep-frozen by slow method and in a water bath at + 75°C when deep-frozen by fast method of freezing.

The results obtained show that there is a definite relationship between the rate of cooling semen during its freezing and rise in temperature during its thawing. If this relationship is disturbed, the quality of the semen obtained after thawing is affected adversely. The best quality of semen was obtained when it was frozen by fast method and thawed in a water bath at +75°C Similar results were obtained by Aamdal and Anderson (1968) by thawing semen frozen in straws (fast method of freezing) in water bath at +75°C than at +35°C. Such research workers as Hafs and Elliot, 1955; Torbin, 1966 and many others who prefer thawing of semen frozen by fast method in water bath at +40°C, in fact, had no chance of thawing it at higher temperatures.

On the other hand, when the same samples of semen were deep-frozen by slow method, the quality of semen was considerably improved after thawing it in ice water. These results agree to the findings of (Boyd and Hafs 1968) who consider that semen frozen in ampoules by slow method of freezing was better thawed in ice water than in a water bath at +40°C. A number of research workers like Miller and Van Demark (1954); Hafs and Elliot (1955); Foote and Dunn (1955); Mixner (1955); Polge (1957); and many others who recommend that semen frozen by slow method should be thawed in water bath at a temperature ranging from +5 to +40°C, in their work, had not compared these ranges of water temperatures with that of ice water for thawing semen.

The results obtained in the present investigation showed that the semen deep-frozen by either of the two methods yields poorest results when thawed in air at room temperature and contradict the recommendations made by Habibulin (1958) to thaw semen in air at room temperature.

LITERATURE CITED

Aamdal, I. and K. Anderson. 1968. Fast thawing of semen in straws. Zuchthygiene. 3 (1): 22-24.

Boyd, L. J. and H. D. Hafs. 1968. Motility and fertility after rapid and slow thawing of semen in ice water. A.I Digest, 15 (12): 8-9 and 11.

Bruce, W. 1956. The application of the low temperature storage of bull semen for artificial insemination. Proc. III Int. Congr. Ani. Reprod. Cambridge, Sec. 3; 27-29,

Foote, R. H. and H. O. Dunn. 1955. Buffers extenders and methods of freezing semen, Mimeo Routine Lab. Procedure, Cornell Univ. and N.Y.A.B.C. 11.

Habibulin, H. H. 1958. Storage of bull semen in deep-frozen state (Trans). Malochnoia I Misnoia Zivotnovodstvo, 3 (2).

Hafs, H. D., and F.I. Elliott. 1954. Effect of thawing temperature and extender composition on the fertility of frozen bull semen. J. Animal Sci. 13: 958-965.

Hafs, N.D. and F.I. Elliott. 1955. The effect of methods of adding egg yolk and monosaccharides on the survival of frozen bull spermatozoa, J. Dairy Sci. 38:811-815.

Lopatko, M.I. 1962. Method of deep-freezing ram semen at -183° to -196°C. (Trans) Zivotnovodtvo 10.

Milavanof, V.K. 1962. Biology of Reproduction and Artificial Insemination of Animals. Selskokhayaesvinis Litratyri, Zyrnolof i Plakatof, Moscow pp. 451-455.

Miller, W.J. and N.L. Van Demark. 1954. The influence of glycerol level, various temperature aspects and certain other factors on the survival of bull spermatozoa at sub-zero temperature. J. Dairy Sci. 37: 45–51.

Mixner, J. P. 1955. Processing, storing and shipping frozen bull semen. N.J. Agri. Exp. Stat. Circ. No. 573 : 3–16.

Ostashko, F. I and A. D. Bugrov. 1968. Recommendation for deep-freezing and storage of bull semen at -196°C. (Trans.) Kharkov. 'PRAPOR' U.S.S.R.

Polge, C. 1957. Low temperature storage of mammalian spermatozoa. Proc. Roy. Soc. B. 147 : 498–508.

Polge, C. and K. Jakobsen. 1959. Techniques for freezing bull semen. Vet. Rec. 71 (44): 928–932.

Salisbury, G.W. and N.L. Van Demark. 1961. Physiology of Reproduction and Artificial Insemination of cattle. W.H. Freeman and Company, San Francisco and London pp. 380-402.

Torbin, V.F. 1966. Method of deep-freezing bull semen, Zivotnovodstvo, 3.

Van Demark, N.L., W.J. Miller, W.C. Kinney, C. Podriguez and M.E. Friedman. 1957. Preservation of bull semen at sub-zero temperatures, Illinois Agr. Exp. Sta. Bull, 621.