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Qualitative Changes of Frozen Bull Semen under Field Conditions in Bangladesh

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Abstract: The duration of storage of frozen bull semen with acceptable quality without pouring any liquid nitrogen into 2-liter container was investigated under field conditions. One frozen straw of each of the 3 donor bulls was evaluated daily for 15 days with regard to sperm motility and normal sperm morphology with respect to acrosome, mid-piece and tail. When frozen semen straws were stored within 2-liter liquid nitrogen (LN) container without pouring any LN into the container up to 15 days of storage, the sperm motility and normal sperm morphology decreased gradually up to 10 days of storage and then the sperm motility and the normal sperm morphology decreased significantly ($P< 0.001$). The motility did not differ significantly between bulls. However, the proportion of normal sperm morphology varied significantly between bulls ($P< 0.001$). In conclusion, frozen bull semen straws can be stored in 2-liter liquid nitrogen (LN) container with acceptable quality for 10 days without any LN under field conditions in Bangladesh.

Key words: Acrosome, frozen bull semen, sperm motility, morphology

Introduction

The use of frozen semen is one of the spectacular developments in modern-day artificial insemination programs. The artificial insemination (AI) programs, particularly in cattle, have been greatly enhanced by the development of frozen semen. The aim of the AI fields services is to maximize the number of viable offspring's per breeding animal per unit time.

Moreover, failure of cows to become pregnant and the need for repeated AI are the usual causes of frustration and economic loss to the dairy farm owners (Stevenson *et al.*, 1990). So, to bring any AI program into economically success, among others, an AI dose should contain sufficient number of progressively motile spermatozoa without marked reduction in their quality. Thus the small number of frozen thawed spermatozoa in each insemination dose must be of very high quality to ensure acceptable pregnancy rates (Brinsko and Varner, 1993).

The quality of frozen semen can be preserved for years provided it is stored always under liquid nitrogen at -196°C (Foote, 1978). Moreover, maintaining optimum nitrogen level in the container is very much essential during prolonged storage and transportation of semen (Quintin *et al.*, 1997). However it is not always possible to keep the frozen semen straws under adequate amount of liquid nitrogen in field conditions, like Bangladesh because of unavailability of liquid nitrogen in AI sub-centers. Usually the AI technicians working at different AI sub-centers of Bangladesh bring the frozen semen straws containing 2-liter container to the district AI centers and pour liquid nitrogen into the container before it becomes empty. Further, it is not always possible for a single AI technician working in an AI sub-center to bring the container to district AI center for filling in right time. It is likely that the quality of semen straws may deteriorate or even all spermatozoa may die if the liquid nitrogen in right time resulting in lower pregnancy rate following the insemination. Considering these clinical demands the present investigation was carried out to determine the duration of storage of frozen semen without compromising with the quality and without pouring any liquid nitrogen into the 2-liter container.

Materials and Methods

The investigation was conducted at the Reproductive Laboratory of the Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh during the period from January to July 1999.

Fifteen frozen semen straws of each of the bulls were preserved in a 2-liter liquid nitrogen container and the container was filled fully with liquid nitrogen. One frozen semen straws of each of the bulls was evaluated daily for days with respect to sperm motility and morphology with respect to normal acrosome, mid-piece and tail. The frozen semen straws were thawed in a water bath at 37°C for 12 to 15 sec. For thawing operation, the straws were taken out from the container as quickly as possible. To evaluate the sperm motility, a small drop (about 10 μl of frozen- thawed semen was placed on a pre-warmed (37°C) microscope slide, covered with a pre-warmed (37°C) cover slip and examined at a magnification of 400x under phase contrast microscope. The motility was always scored subjectively using blind technique by two examiners at 37°C. The average scores of the motility given by two examiners were recorded. The sperm morphology with respect to normal acrosome mid-piece and tail was evaluated in buffered normal saline-fixed semen samples, using phase contrast microscope (100x). The buffered formal was prepared by dissolving disodium hydrogen phosphate ($2H_2O$, 24.7mmol/L), potassium dihydrogen phosphate (18.7mmol/L), sodium chloride (92.6mmol/L) and formaldehyde (1.54 mols/L) in 100ml distilled water (Hancock, 1957). To prevent any temperature variation-related damage to the spermatozoa during fixation of spermatozoa, the frozen thawed semen and formal saline were always mixed at the same temperature. The abnormality found in the formal saline-fixed spermatozoa phase contrast microscopy were classified as presence of proximal and distal cytoplasmic droplets, abnormal mid-piece, abnormal acrosome, detached head, bent tail, coiled tail and double coiled tail. At least 200 spermatozoa were examined from each sample.

The discrete and proportion data were transformed, respectively, by log and arcsine transformation to near normality before being analyzed (Anonymous, 1996). The difference in sperm motility and morphology between bulls was tested for significance, using one way ANOVA and the difference in sperm motility and morphology between days of storage were tested by using repeated measures of ANOVA (Anonymous, 1996).

Results

When the sperm motility of the day 1 sample was compared with the sperm motility of day 15 sample, the data varied from 47.2 ± 7.1 to 0.0 % during the storage period for 15 days (Table 1). It clearly indicated that when frozen semen was stored within 2-liter liquid nitrogen (LN) container without pouring any LN into the container up to 15 days of storage, the sperm motility decreased gradually up to 10 days of storage and then it decreased abruptly leading to cessation irrespective of the bulls. At day 10 (Day1= day of storage), the recorded sperm motility was 31± 7%. However, upon storage the sperm motility did not differ significantly between bulls (Table 1).

The data on the effect of frozen semen storage on proportion of spermatozoa with respect to normal acrosome , mid piece and tail are shown in Table 2. When the data were pooled over the bulls,

the proportion of spermatozoa with respect to normal acrosome, mid piece and tail varied from 56.3 ± 3.0% to 81.4 ± 4.0% during 15 days of storage. Upon storage, the data about normal acrosome, mid-piece and tail reduced gradually up to 10 days of storage and then the proportion of normal spermatozoa reduced sharply up to 15days of storage. At day 10, the recorded proportion of normal spermatozoa was 77± 5%. Moreover, upon storage, the proportion of normal sperm morphology also varied significantly between bulls; bull 57 contained higher proportion than that of bull 6742 (86.2 to 59.0% vs.77.4 to 53.0%; Table 2; P< 0.001)

Table 1: The data on the effects of duration of storage on sperm motility

Days of storage	Motility %			
	Bull 1 (6742)	Bull 2 (3502)	Bull 3 (57)	Data pooled over the bulls
1	47.0 ± 3.0	43.3± 6.0	52.0± 10.4	47.0± 7.1
2	43.3± 3.0	42.0± 3.0	50.0± 9.0	45.0± 6.1
3	43.3± 3.0	42.0± 3.0	48.3± 8.0	44.4± 5.2
4	43.3± 6.0	42.0± 3.0	43.3± 8.0	44.4± 6.0
5	38.3± 8.0	38.3± 3.0	48.3± 8.0	42.0± 8.0
6	38.3± 8.0	35.0± 5.0	47.0± 8.0	40.0± 8.0
7	38.3± 8.0	37.0± 8.0	42.0± 8.0	39.0± 7.0
8	37.0± 8.0	33.3± 6.0	40.0± 5.0	37.0± 6.1
9	38.3± 8.0	32.0± 13.0	38.3± 8.0	36.1± 9.0
10	30.0± 10.0	30.0± 10.0	32.0± 3.0	31.0± 7.2
11	32.0± 3.0	30.0± 10.0	28.3± 3.0	30.0± 6.0
12	27.0± 6.0	23.3± 6.0	28.3± 3.0	26.1± 5.0
13	8.30± 3.0	7.0± 3.0	8.3± 3.0	8.0± 3.0
14	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
15	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0

Values are mean ± SD
The difference in mean motility between bulls was non significant.

Table 2: The data on the effects of duration of storage on sperm morphology

Days of storage	Normal sperm morphology (%)			
	Bull 1 (6742)	Bull 2 (3502)	Bull 3 (57)	Data pooled over the bulls
1	77.4± 1.2^{b}	81.0± 1.0^{b}	86.2± 2.1^{a}	81.4± 4.0
2	76.3± 2.3^{b}	80.0± 2.0^{b}	86.1± 1.2^{a}	81.0± 5.0
3	75.3± 3.3^{b}	79.0± 2.1^{ab}	85.43.0^{a}	80.0± 5.0
4	74.0± 1.1^{b}	79.2± 1.1^{ab}	86.0± 5.0^{a}	80.0± 6.0
5	74.0± 3.0^{b}	77.0± 2.1^{b}	87.0± 1.4^{a}	79.2± 6.0
6	76.0± 2.0^{b}	78.4± 3.0^{b}	86.1± 2.0^{a}	80.1± 5.0
7	76.0± 0.3^{b}	77.2± 1.1^{b}	86.0± 2.0^{a}	80.0± 5.0
8	73.0± 2.0^{b}	77.0± 1.0^{b}	86.0± 2.0^{a}	78.4± 6.0
9	73.1± 4.4^{b}	76.3± 1.0^{ab}	85.3± 3.0^{a}	78.3± 6.1
10	70.3± 1.2^{b}	79.0± 2.3^{ab}	81.2± 2.0^{a}	77.0± 5.2
11	70.0± 2.4^{b}	78.1± 4.2^{ab}	81.3± 4.0^{a}	76.3± 6.0
12	69.0± 4.0^{b}	73.0± 2.0^{ab}	78.1± 3.0^{a}	73.3± 5.0
13	61.2± 1.4^{b}	67.0± 1.1^{ab}	69.0± 2.0^{a}	66.0± 4.0
14	54.0± 1.1^{b}	60.0± 1.0^{a}	59.4± 2.1^{a}	58.0± 3.1
15	53.0± 1.0^{b}	57.0± 1.0^{a}	59.0± 1.4^{a}	56.3± 3.0

Values are mean ± SD
Means in the same row with different superscripts differ significantly from each other(P< 0.001).

Discussion

The results of the present investigation indicate that the frozen semen straws can be stored up to 10 days (Day 1= day of storage) with acceptable post-thaw quality without pouring any liquid nitrogen (LN) in 2 liter container under field conditions.

Spermatozoa of bull can be stored at liquid nitrogen temperatures (-196°C) for indefinite periods and after thawing retain relatively high rates of quality (Salisbury *et al.*, 1978; Graham *et al.*, 1984). There is no further loss in quality of frozen semen which is continuously stored at -196°C under the surface of liquid nitrogen (Sherman, 1990). Although, frozen semen can be stored successfully for years, certain characteristics of aging of spermatozoa have been reported elsewhere (Salisbury, 1968; Salisbury and Hart, 1970). It is likely that aging of spermatozoa will always cause deterioration of semen quality. It is important to keep frozen semen always immersed in liquid nitrogen. Lack of optimal liquid nitrogen even for a few hours in the container may result in complete destruction of a sperm bank (Hafez, 1993). Usually semen straws start thawing at -130°C, if semen is not preserved under optimal level of liquid nitrogen. Accordingly, in the present investigation, in spite of obtaining gradual reduction in the quality of semen, acceptable quality was obtained up to 10 days perhaps because of maintaining the adequate level of LN in the container. However, the present investigation used only 3 straws daily from a container. This means the 2-liter LN container was carefully opened only for 3 times daily thawing semen. Moreover, it should be remembered that if one use more semen straws for insemination daily the storage duration would be reduced, because of rapid evaporation of LN. Further, duration of storage in field condition is influenced by duration of leaving the container open during insemination (Foote, 1986). According to Bhuiyan (1998), the frozen semen sample having > 30% sperm motility and > 65% normal spermatozoa with respect to acrosome, mid-piece and tail in combination is regarded as good quality semen. Obtaining 31± 7% and 77 ± 5% sperm motility and proportion of normal spermatozoa respectively at day 10 of storage was regarded as acceptable quality of frozen semen in previous studies.

In the present study, upon storage of frozen semen in 2-liter LN container the sperm motility did not vary between bulls. However, the proportion of spermatozoa with respect to normal acrosome, mid-piece and tail varied significantly between bulls upon preservation. This may be due to the variation in age and breed of the bulls although they were reared under same conditions. The bulls used in this study were of different ages and breeds. There are reports of bull age-related variations in semen quality; the quality of semen declined as they become older (Collins *et al.*, 1962; Hahn *et al.*, 1969). Moreover, the frequency of abnormal spermatozoa in semen may increase with advancing age of bulls (Rao, 1971; Foote, 1986). Contrasting to the present findings, Foote (1986) found higher post-thaw sperm motility in semen from young bulls (3-6 years) than that of older ones(6-12 years). The age-related variations are perhaps caused by changes in the composition of seminal plasma, which in turn make the spermatozoa of the older bulls more susceptible to cryoinjury. The trend did not always hold true because many older bulls kept after rigid selection at AI had as good quality semen as younger bulls had (Herman, 1956; Lundgren, 1980). Nevertheless, the quality of preserved semen can vary depending on its breed (Rao and Rao, 1979; Balsare and Deshmukh, 1994). There are also indications of breed variation with respect to sperm morphology; the proportion of normal spermatozoa was higher in Boran than that in Boran X Friesian bulls in Ethiopia (Tegegne *et al.*, 1994). Additionally, the spermatozoa of Holstein bulls had better post-thaw motility than those of Sahiwal bulls, although bulls of both breeds lived in identical environment (Bhupal *et al.*, 1993). This variation may result from differences in composition of seminal plasma due to genetic influences which in turn may make difference in damages and injuries during semen handling irrespective of preservation method (Nandroo *et al.*, 1987; Mohan *et al.*, 1994).

It is concluded that frozen semen straws can be stored in 2-liter liquid nitrogen (LN) container with acceptable quality for 10 days without filling any LN under field conditions in Bangladesh.

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