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Impact of seminal attributes of freshly ejaculated semen of Bhadawari bull

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Abstract
Present study was designed to investigate the effect of seminal attributes of freshly ejaculated semen of Bhadawari bull. This study revealed substantial changes of seminal attributes of freshly ejaculated semen of Bhadawari bull. Seminal attributes such as volume, pH, mass motility and sperm concentration. The mean±S.E. values of seminal attributes of bull number BH-51, BH-55, BH-84 and BH-87 were observed a non significant difference (P<0.01) between bulls. From the result obtained in the present investigation it can be concluded that use of antioxidants in semen extender was beneficial to reduce oxidative stress and cryodamage of spermatozoa during cryopreservation.

Keywords: Bhadawari bull, semen, volume, sperm concentration, mass motility

Introduction
Buffalo, a triple purpose animal, provides milk, meat and mechanical power to mankind. Due to its highly nutritious milk, leaner meat and best draught power for wet environments, buffalo offers immense potential for improvement of livelihood. Bhadawari buffalo originated at Bhadawari estate of Agra district and adjoining areas of Gwalior and Etawah and is also scattered in surroundings of Yamuna and Chambal rivers. As bull is half of herd, sire indexing is being utilized to mark out the elite male germplasm. Therefore, maximum utilization of genetically superior bulls through artificial insemination is of prime importance. The key to success of artificial insemination lies in fertilizing capacity of diluted semen, which depends upon quality of semen and suitability of extender to maintain motile life of spermatozoa for maximum period at refrigerator temperature (4 ^{0}C to 5 ^{0}C) as well as at -196 ^{0}C in liquid nitrogen (LN_2). Thus, application of A.I. and frozen semen are recommended for improvement of buffalo production. Technology of deep freezing of semen has remarkably increased preservability and storage of semen for artificial insemination and is contributing enormously for exploitation of proven sire in improving animal productivity and conservation of breed (Benerjee, 1991) [5]. To control level of ROS and promote motility and survival of sperm, numerous antioxidants have proven beneficial in treating male infertility (Sinclair, 2000). Ascorbic acid and vitamin E is naturally occurring free radical scavenger and their presence also assist various other mechanisms in decreasing numerous disruptive free radical processes, including LPO (Bansal and Bilaspuri., 2009) [4]. Vitamin C and E are major antioxidants naturally present in mammalian semen against ROS to protect sperm from lipid peroxidation and to maintain its integrity (Andrabi, 2009; Akhter *et al.*, 2011) [3, 1]. Concentration of antioxidant decreased during freeze-thawing process by dilution of semen with extender and excessive generation of ROS molecules (Andrabi, 2009; Kumar *et al.*, 2011) [3, 7].
Vitamin E includes a group of lipid soluble compounds, tocopherols and tocotrienols that act as antioxidant during stress. Vitamin E is believed to be primary component of antioxidant system of spermatozoa (Surai *et al*., 1998) [13], and is one of the major membrane protectants against ROS and LPO (Akiyama, 1990) [2]. Vitamin E can inhibit LPO reaction in membrane by eliminating peroxyl (ROO-), alkoxyl (RO-), and other lipid-derived radicals (Silva, 2006) [12]. Supplementing vitamin E in feed has been shown to increase total sperm output and sperm concentration in boars (Breininger *et al*., 2005) [6], cattle (Bansal and Bilaspuri, 2009) [4], rabbits (Yousef *et al*., 2003) [14] and rams (Liu *et al*., 2005; Luo *et al*., 2004) [8]. However, there is limited data regarding effect of vitamin C and E alone and together in different extenders on post thaw semen quality. Therefore, to test the hypothesis that vitamin E and vitamin C, if given in combination, may protect buffalo bull spermatozoa from oxidative damage during cryopreservation resulting in higher post thaw sperm viability and motility.

Materials and Methods

Ethical approval

No ethical approval was necessary to pursue this research work.

Animals and sampling

The present study was conducted on four Bhadawari buffalo bulls aged between 3 and 6 years and weighing between 300 kg and 450 kg body weight, reared at the Instructional Livestock Farm Complex, College of Veterinary Sciences and Animal Husbandry, Uttar Pradesh Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura, which is situated in a Semiarid zone of Northern part of India, in the state of Uttar Pradesh. All the experimental bulls were kept in individual pens made up of brick and cement with concrete floor and asbestos roof. The bulls were fed balanced ration as per its availability at farm. Proper vaccination schedule was followed, and animals were vaccinated against important contagious and infectious diseases. Semen collection was made biweekly from each bull with the help of artificial vagina (AV) "Danish model" on dummy animal between 7.00 AM and 8.00 AM during summer and 8.30 AM to 9.00 AM during other seasons using standard protocol. Semen was collected directly into a clean, dry graduated centrifuge tube attached to the latex cone of the AV. Immediately after collection, tube containing semen were marked and placed in the water bath at 37 °C. Ejaculates collected from four Bhadavari bulls were evaluated.

A brief of the methods used for semen evaluation is presented as below:

a) Volume

The volume of semen was directly measured in milliliter (ml) from the graduated centrifuge semen collection tube.

b) Colour and Consistency

Semen sample were also observed for colour and consistency by direct visualization with naked eyes and any abnormalities in colour or consistency were treated as abnormal and the sample were discarded. The normal bull semen was creamy or milky in consistency depending upon the concentration of sperms.

c) Mass motility

It was assessed by placing a small drop of semen of uniform size and thickness over a clean dry glass slide. The semen drop was examined under low power objective (10X) of microscope on a thermostatically controlled warm stage at 37 °C. Motility was rated according to the vigour wave motion on grade scale of 0 to 5 as described by Nazir (1988) [10] in Nili-Ravi buffaloes and is given below.

S. No.	Observation	Mass motility score (0-5 scale)
1.	No motility	0
2.	No wave but sperm movement evident	+1
3.	Slow wave formation	+2
4.	Relatively more wave formation with swirls	+3
5.	Wave with swirls and eddies	+4
6.	Wave with very rapid swirls and eddies	+5

d) Sperm concentration

Sperm concentration was estimated using Haemocytometer (Improved Neubauer's chamber) method (Salisbury *et al*., 1978) [11]. Central primary square is divided into 25 (5x5) secondary squares while each secondary square is further divided into 16 (4x4) tertiary squares. Thus central primary square is divided into 400 (25 x 16) tertiary squares. These total 400 tertiary squares have a total area of 1mm2. When a drop is charged under the cover slip in a Neubauer cell counting chamber, the thickness of the film on chamber is 0.1 mm. Thus the total volume of the semen covering 400 tertiary squares in RBC chamber is 0.1mm3. Sperm concentration estimate was made by diluting semen samples 1000 times with 4 % (NaCl) saline solution. A pinch of eosin (0.05 %) was added to saline solution to give back ground to spermatozoa.

Spermatozoa were counted in five secondary squares of central Primary Square, namely right and left top, right and left bottom and central squares. Those spermatozoa, which was lying in square itself, on top line and right side line of the square, was also counted. Each secondary square contained 16 tertiary squares so total numbers of spermatozoa in 80 tertiary squares were counted. The average of the counts on both side of haemocytometer chamber was taken for calculating the sperm density and the total number of spermatozoa per ml of neat semen was calculated as follows:

Sperm concentration = n x 10 x 10^6 per ml

Here, n = no. of sperms counted in five secondary squares.

Result and Discussion

The study was conducted to assess the influence of vitamin E, vitamin C and its combination (vitamin E+C) as anti-oxidants on semen characteristics and functional integrity during different steps of freezing and thawing in Bhadawari bulls. Four Bhadawari bulls aged between 3-6 years and weighing between 300-450 kg were selected for experiment. Semen was collected from four bulls twice a week with 8 ejaculates per bull. The relevant data has been presented in tables 1 to 6 and illustrated in figures 1 to 5. As no significant difference was observed for various seminal attributes amongst the different bulls. The diluted semen fractions were later subjected to freezing and thawing process. Semen attributes were analyzed at each step during cryopreservation and after thawing to study the effect of vitamin C, vitamin E and its combination on semen quality. The results obtained in the present study were statistically analyzed and are presented as follows:

Seminal attributes of freshely ejaculated semen of bhadawari bull

The mean±S.E. values of seminal attributes evaluated for assessing the quality of freshly ejaculated neat semen from four Bhadawari bull has been presented in Table 1. The seminal attributes of all four bull were well within normal range and were found to be fit for semen cryopreservation. No significant differences were observed between seminal

attributes evaluated for four bulls selected for experiment, indicating that semen quality of bulls were statistically similar, hence pooled for further experimentation. The relevant data has been presented in table 1.

Ejaculated volume (ml)
Perusal of table 1 revealed mean±S.E. semen volume of bull number BH-51, BH-55, BH-84 and BH-87 were 2.80±0.19, 2.90±0.30, 2.34±0.07 and 2.45±0.12 ml respectively. The overall mean value irrespective of bull was found as 2.62±0.10 ml. A non significant difference (P<0.01) was observed between bulls for this parameter (Table 1).

pH
Perusal of table 1 revealed mean±S.E. pH of bull number BH-51, BH-55, BH-84 and BH-87 were 7.04±0.06, 6.95±0.06, 6.99±0.05 and 6.94±0.04 respectively and a non significant difference (P<0.01) was observed between bulls (Table 1). The overall mean value irrespective of bull was found as 6.98±0.03.

Mass motility (0 - 5 scale)
Perusal of table 1 revealed mean±S.E. mass motility of bull number BH-51, BH-55, BH-84 and BH-87 were 3.13±0.08, 3.25±0.09, 3.06±0.06 and 3.06±0.06 respectively. The overall mean value irrespective of bull was found as 3.13±0.04. A non significant difference (P<0.01) was observed between bulls (Table 1).

Sperm Concentration (millions/ ml)
Perusal of table 1 revealed mean±S.E. sperm concentration of bull number BH-51, BH-55, BH-84 and BH-87 were 1806.25±68.20, 1920.00±41.59, 1909.38±38.26 and 1936.88±35.08 millions/ ml respectively and A non significant difference (P<0.01) was observed between bulls (Table 1). The overall mean value irrespective of bull was found as 1893.13±24.42 millions/ ml.

Table 1: Seminal attributes of freshly ejaculated semen of Bhadawari bull.

Bull No.	Volume (ml)	p^H	Mass Motility (0-5 Scale)	Sperm Concentration (million/ml)
BH- 51	2.80± 0.19 (2.4-4.0)	7.04 ± 0.06 (6.8-7.2)	3.13± 0.08 (3-3.5)	1806.25 ± 68.40 (1340-1970)
BH-55	2.90 ± 0.30 (2.4-5.0)	6.95 ± 0.06 (6.8-7.2)	3.25± 0.09 (3-3.5)	1920.00 ± 41.71 (1730-2110)
BH-84	2.34 ± 0.07 (2.0-2.6)	6.99± 0.05 (6.8-7.2)	3.06± 0.06 (3-3.5)	1909.38 ± 38.37 (1760-2120)
BH- 87	2.45 ± 0.12 (2.2-3.2)	6.94 ± 0.04 (6.9-7.2)	3.06± 0.06 (3-3.5)	1936.88 ± 35.18 (1830-2110)
Total	2.62 ± 0.10 (2.0-5.0)	6.98 ± 0.03 (6.8-7.2)	3.13± 0.04 (3-3.5)	1893.13 ± 24.42 (1340-2120)
F-Value	1.994^{NS}	0.722^{NS}	1.333^{NS}	1.535^{NS}

NS: Non-significant, figures in parenthesis indicate range of observed values.

Conclusions
The present investigation was aimed to evaluate the seminal attributes of Bhadawari buffalo bull semen. From the result obtained in the present investigation it can be concluded that use of antioxidants in semen extender was beneficial to reduce oxidative stress and cryodamage of spermatozoa during cryopreservation.

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