# EVALUATION OF FRESH SEMEN QUALITY AND PREDICTING THE NUMBER OF FROZEN SEMEN DOSES IN JAFFRABADI BUFFALO BULL

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## ABSTRACT

Scientific information on frozen semen characteristics of Jaffrabadi buffaloes (*Bubalis bubalis*) is scanty and centre to this research is to evaluate frozen semen characteristics in Jaffrabadi buffalo bulls. The study included six buffalo bulls with average age 95.83±5.47 months and average body weight of 834.16±47.63 kg maintained at Cattle Breeding Farm, Junagadh Agricultural University, Junagadh. A total of 206 ejaculates were collected during the period of study. Ejaculates collected from the six Jaffrabadi buffalo bulls were clean, dense to very dense (D = 67.2%, DD = 32.8%) and milky white (72.5%) to creamy (27.5%) in colour. During this observatory study average (Mean±SE) semen parameter like, ejaculatory volume, mass activity, sperm concentration, initial progressive sperm motility and total sperm number per ejaculate were 5.11±0.17 ml, +3.43±0.04, 838.30±25.74×$10^6$/ml, 79.41±0.60% and 4053.99±150.56×$10^6$ spermatozoa, respectively. Average dilution rate in 0.5 ml medium straw (40 million sperm/straw) was found to be ideal at 10.48±0.32 ml. In a year expected number of ejaculates that could be frozen from the 6 bulls was 34.34±6.43 and correspondingly, the expected number of frozen doses produced from bulls could be 3546.46±540.30 numbers.

**Keywords:** Jaffrabadi bulls, ejaculates, frozen doses, semen parameter

## INTRODUCTION

Jaffrabadi buffalo is one of the heaviest buffalo breeds of world, inhabitant of Gir forest area in Saurashtra region of Gujarat, India. These buffaloes are known on their higher milk fat per cent (>8%) and larger fat globular size and hence the milk is preferred for Ghee and Khoa making (Thomas and Sastry, 2005). High quality Jaffrabadi frozen semen producing centers in the region are few and studies on semen characters and sexual behavior are scanty. There are many agencies/organizations of government and non government organizations working in the field of breed improvement in the Jaffrabadi buffaloes, but information need to be assessed on the reproductive performance, behavior and semen characteristics of Jaffrabadi bull. Changes in the environmental condition influence sperm output, accessory sex gland secretion and epididymal function, all of which are reflected in the ejaculate as volume, sperm numbers or sperm motility, morphology, viability *etc.* (Koonjaenak *et al*., 2007). The knowledge of sexual behavior and semen evaluation are valuable tools to estimate the reproductive efficiency of a breeding bull (Brohi, 1993).

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## MATERIALS AND METHODS

Six Jaffrabadi buffalo bulls of Cattle Breeding Farm, Junagadh Agricultural University, Junagadh aged 95.83±5.47 months (mean±SE, ranges 75 to 108 months) with live weight of 834.16±47.63 kg (mean±SE, ranges 730-1000 kg) with typical breed characters (Figure 1 and 2) formed the experimental material. Buffalo bulls were kept in individual pens under a loose housing system on a concrete floor with the orientation of its long axis in the east-west direction. The bulls were fed green fodder such as maize, sorgum, sunflower and lucern according to the season and availability along with *ad lib* mature pasture grass hay. Concentrate component of ration comprised of mixture (50:50) of commercial concentrate pellet (Amul power dan) and cotton seed cake at the rate of 4.5 kg per bull/per day along with mineral mixture powder at the rate of 40 g/bull/day. The bulls were drenched with 10 eggs and 500 ml edible oil (cotton seed oil) 3 times a month.

A clinical history of each bull was taken at the start of this study, including previous illnesses, mating behavior and libido. The data were compiled on a total of 206 ejaculates of 6 Jaffrabadi buffalo bull during the period from July 2011 to June 2012.

Bulls were properly cleaned in perpetual area with plenty of clean water and semen was collected using male dummy without giving any false mounting. Samples were collected early in the morning once a week, using artificial vagina (AV) maintaining inner temperature 40-42$^{o}$C. The temperature of semen processing room was maintained at 20-25$^{o}$C during the whole study hours. Immediately after collection, each sample was transferred to laboratory and placed in a water bath at 35$^{o}$C. Sterilization of all items were maintained before the day of collection and kept in an incubator at 45$^{o}$C.

Ejaculates were collected by AV technique by trained person (Figure 3). Two ejaculates with a gap of 20 to 30 minutes were collected (Figure 4). Immediately after collection ejaculate was evaluated for clarity/cleanliness (1 = clean, 2 = dirty or contaminated), colour (1 = watery, 2 =

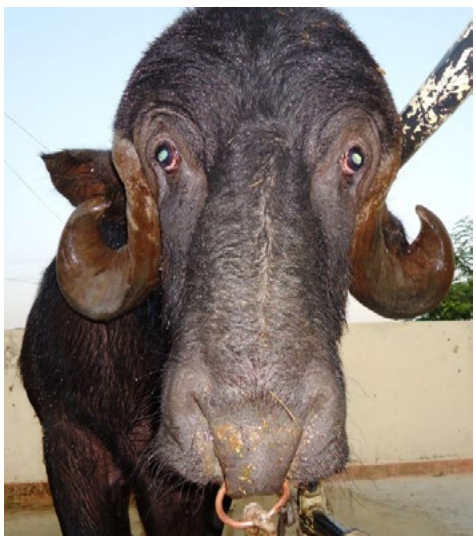

Figure 1. Jaffrabadi buffalo bull.

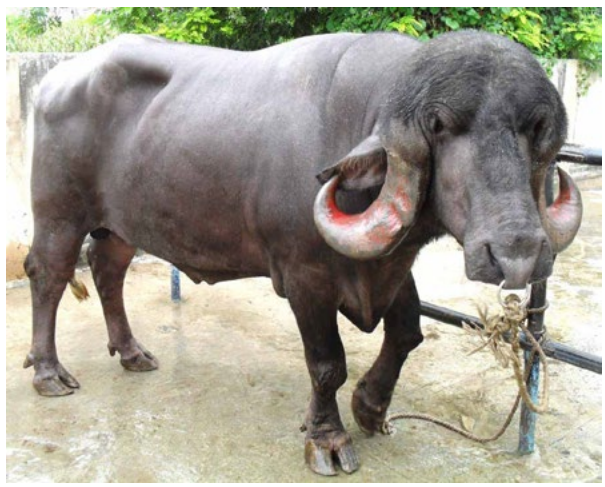

Figure 2. Typical shield like head.

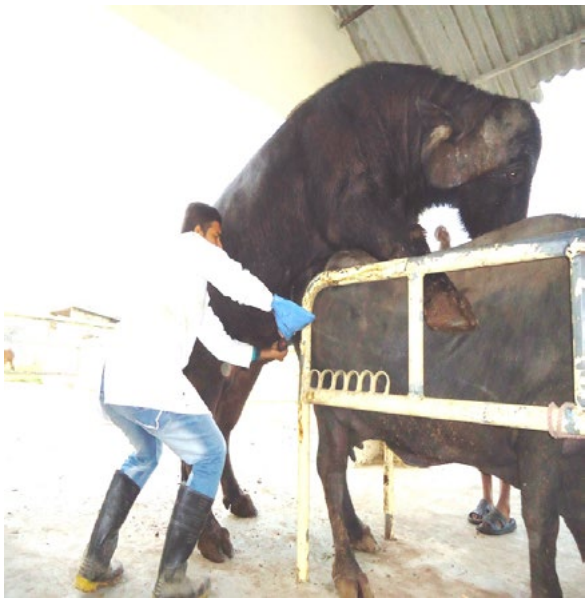

Figure 3. Semen collection in Jaffrabadi buffalo bull.

Figure 4. Thick creamy white semen.

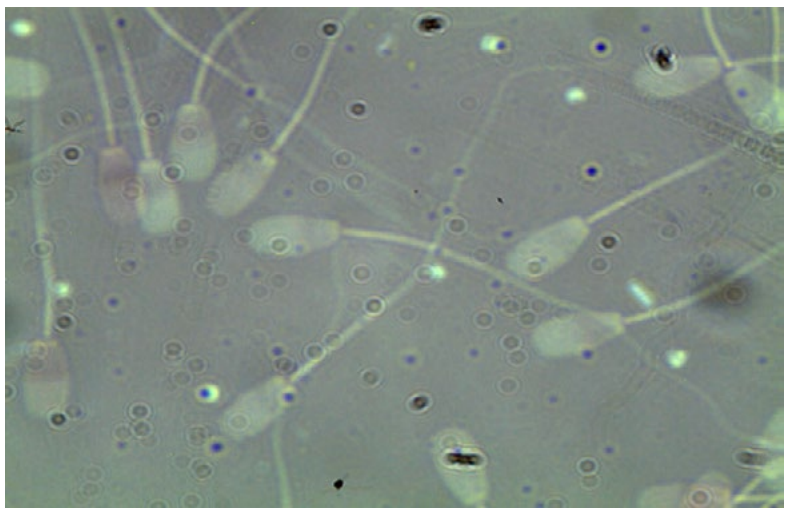

Figure 5. Live and dead count of spermatozoa using eosin and nigrosin staining.

milky, 3 = creamy), density (0 = thin, D = dense, DD = very dense) and Volume (ml, graduated collection tube). Mass activity of spermatozoa was recorded by placing a drop of semen on a warm slide at 100 × magnification under a microscope with attached stage warmer (temperature set at 37°C), camera and LCD screen (0 = no mass activity, +1 = slow waves, +2 = quick waves, +3 = very quick waves, +4 = Waves, churning of whirls and eddies) (Nath, 1988). Motility, as a percentage of individually motile spermatozoa, was estimated by examining a drop of diluted fresh semen (with buffer solution) under a microscope at 200×. Motility percentage was scored on the basis of the percentage of spermatozoa with normal forward progressive movement, while those showing circling movements or those oscillating at one place were regarded as immotile (Ahmad, 1994). Sperm concentration was assessed with Bovine Accucell photometer, (IMV) by diluting 1:100 time neat semen in to Sodium chloride solution 0.9% W/V. Live and Dead spermatozoa count has been carried out using Eosin and Nigrosin staining technique (Figure 5) (Campbell, 1956).

## RESULT AND DISCUSSION

Age of the bulls at the beginning of collection during the study period ranging from 75 months to 108 months, with a mean of 95.83±5.47 months. A total of 217 ejaculates were collected during the period of study. The distribution of collections per bull is shown in Table 1. Eleven ejaculates were considered very thin (watery) or dirty and were therefore discarded from semen processing and freezing. Semen characteristics of the remaining 206 ejaculates are summarized in Table 1.

Colour of semen studied was actually the thickness of the semen together with pigment. The ejaculates collected from the six Jaffrabadi buffalo bulls were clean, dense to very dense (D = 67.2%, DD = 32.8%) and milky white (72.5%) to creamy (27.5%) in colour (Figure 4). Javed *et al*. (2000) reported milky-white caoloured semen in Nili-Ravi and in Swamp, buffalo bulls.

Ejaculate volumes from Jaffrabadi bulls ranged from 2-12 ml (5.11±0.17 ml), similarly, nearly same ejaculatory volume were reported by Tomar *et al*. (1966); Shukla and Mishra (2005) in Murrah bulls and Javed *et al*. (2000) in Nili-Ravi bulls. However, scientists (Bhakat *et al*., 2011; Pant *et al*., 2003; Koonjaenak *et al*., 2007; Rehman *et al*., 2012) reported lower ejaculated volume as compared to Jaffrabadi in Murrah, swamp and Kundhi buffalo breeds, respectively. Differ in the semen volume in various breeds of buffaloes might be due to differences in genetics, reproductive health status of bulls, age of bulls, frequency of collection, pooled volume, nutrition, season and management (Nazir, 1988; Soderquist, 1992). Variations can also be due to skill of semen collector/attendant and temperature of AV.

Mass activity of experimental Jaffrabadi bulls (range from +2.5 to +4 with a mean of +3.43±0.04) is similar to earlier reports of mass activity reported by various researcher (Ram, 1988; Dhami, 1992; Shukla and Mishra, 2005) in Murrah bulls and Rehman *et al*. (2012) in Kundhi buffalo.

Sperm concentration of six Jaffrabadi bulls were ranged from 425 to 2012×$10^6$/ml with mean of 838.30±25.74×$10^6$/ml. Present findings are in agreement with the findings of Ram (1988), whereas higher concentration of spermatozoa/ml of neat semen were recorded by several workers (Prajapati *et al*., 2000; Pratap *et al*., 1999; Rehman,

Table 1. Mean (±SE) of semen parameter/characteristics, dilution rate/ml of semen and Number of doses frozen/year/bull from Jaffrabadi bulls.

| Name or Number of Bulls | Laxman | Bhagro | Moti | Nagraj | Sundar | Raja | Average |
|---|---|---|---|---|---|---|---|
| Age of bulls (Months) | 102 | 96 | 75 | 108 | 89 | 105 | 95.83±5.47 |
| No. of ejaculates | 54 | 46 | 40 | 23 | 25 | 18 | 34.34±6.43 |
| Volume of semen/ ejaculate | 4.59±0.27 | 6.89±0.51 | 4.94±0.24 | 4.82±0.27 | 4.20±0.39 | 4.17±0.36 | 5.11±0.17 |
| Cleanliness of semen (Score: 1-2) | 1 | 1 | 1 | 1 | 1 | 1 | - |
| Colour (Score: 1-3) | 2-3 | 2-3 | 2-3 | 2-3 | 2-3 | 2-3 | - |
| Density (Score: 0-DD) | D-DD | D-DD | D-DD | D-DD | D-DD | D-DD | - |
| Mass activity | 3.39±0.08 | 3.30±0.09 | 3.53±0.08 | 3.48±0.14 | 3.48±0.10 | 3.50±0.12 | 3.43±0.04 |
| Initial progressive sperm motility (%) | 79.43±0.98 | 75.65±1.92 | 81.00±1.15 | 80.43±1.38 | 83.00±1.10 | 79.17±1.52 | 79.41±0.60 |
| Sperm concentration ($10^6$/ml) | 732.00±29.16 | 561.37±41.44 | 960.38±45.02 | 916.17±78.22 | 1212.76±96.86 | 974.00±85.33 | 838.30±25.74 |
| Total sperm number ($10^6$) | 3348.18±227.45 | 3314.45±314.13 | 4817.27±345.80 | 4394.16±486.14 | 5025.76±535.66 | 4069.78±477.79 | 4053.99±150.56 |
| % of Live sperm | 85.17±0.76 | 84.76±1.02 | 85.70±0.99 | 87.09±1.09 | 84.52+1.32 | 85.94±1.44 | 85.38±0.42 |
| Average dilution rate/ ml of semen | 9.15±0.36 | 7.02±0.52 | 12±0.56 | 11.45±0.98 | 15.16±1.21 | 12.18±1.07 | 10.48±0.32 |
| Post Thaw progressive motility | 57.13±0.86 | 57.39±0.94 | 59.26±1.08 | 59.57±1.11 | 60.60±0.99 | 61.94±0.94 | 58.71±0.42 |
| Calculated no. of ejaculate frozen(dose)/ year/bull | 4535.8 | 4449.83 | 4742.4 | 2538.69 | 3183.6 | 1828.46 | 3546.46±540.30 |

*et al*., 2012; Shukla and Mishra, 2005). During the study initial progressive sperm motility ranged from 65 to 95%, with mean of 79.41±0.60 and is in agreement with the findings of Koonjaenak *et al*. (2007) in swamp buffalo. Sahu and Pandit (1997) and Shukla and Mishra (2005) recorded higher initial progressive motility in Murrah bulls. Lower percentage of initial motility than the present findings in Murrah bulls was also reported by Bhakat *et al*. (2011) and Kumar *et al*. (1993). The post thaw motility ranged from 45% to 65% with average mean of 58.71±0.42 during the entire study period. Percentage of live and dead spermatozoa of all the Jaffrabadi bulls was ranged from 75 to 96 percent live spermatozoa with average of 85.38±0.42 percent live spermatozoa.

Total sperm number per ejaculate ranged from 2400 to 10136 million sperm with mean of 4053.99±150.56×$10^6$ spermatozoa. Mean dilution rate was found to be 10.48±0.32, with a range of 4.5 to 25.83 ml. Expected number of ejaculates that could be frozen from the 6 bulls was 34.34±6.43 (ranging from 18 to 54) and correspondingly, expected frozen doses produced from bulls could be 3546.46±540.30 (ranged 1828.46 to 4742.4). Out of six bulls, ejaculates were collected from three bulls for entire year that produced 4576±67.13 frozen doses/year. Two bulls were used for semen collection for only 8 months and one bull was used for 5 months. Each semen doses were of 0.5 ml with 40 million sperm concentration per straw. Bhakat *et al*. (2011) revealed average total sperm output of Murrah buffalo bull was 2,561.05±77.80×$10^6$. Average dilution rate was found to be 12.49±0.13. Expected number of ejaculates that could be frozen per year per bull was 53.27 and correspondingly, the expected frozen doses produced per year per bull could be 6,879.49. Zafar *et al.* (1988) reported yearly production to be 8,412 semen doses per bull in Nili-Ravi buffalo bulls and Roy (2006) produced 5,147.48 doses/year/bull in Murrah bulls which was higher than the estimate for Jaffrabadi bulls in the present study.

In vitro semen evaluation parameters used in the present study are used to determine fresh semen motility in post-thaw samples. Some research workers established a correlation between motility and field fertility; others did not (Christensen *et al.,* 1999; Tardif *et al*., 1999). Variations in semen quality parameters recorded in the present investigation, were well supported by earlier reports, may be due to individual variations (Saxena and Tripathi, 1978), ejaculate frequency (Nath, 1988), differences in age (Bhat *et al*., 2002), genetic makeup of the bulls (Tomar *et al*., 1966), season of study (Tuli, 1984) and agro climatic conditions.

Present study reveled that semen characters of Jaffrabadi bulls are comparable to Murrah and there characteristics can be made use of to meet the high demand for semen from selected Bulls of high genetic merit. Harvesting maximum semen doses per bull was the other approach to increasing the number of inseminations possible per bull. Information on expected semen doses per bull will help in planning the functioning of A. I. center at field level as per the capacity of the semen station running at Cattle Breeding Farm.

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