Semen Production and Semen Quality of Mehsana Buffalo Breed Under Semiarid Climatic Conditions of Organized Semen Station in India

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Abstract

Semen production data comprising of 55071 ejaculates of 144 bulls from Mehsana buffalo breed was analysed. The traits under study were semen volume, sperm concentration, initial sperm motility, post-thaw sperm motility and number of semen doses per ejaculate. The objective of the present study was to assess the effect of various factors affecting semen production traits and measure the semen production potential of Mehsana buffalo bulls. Data collected of semen production traits were analysed using linear mixed model, including a random effect of bull along with fixed effect of various non-genetic factors like farm, ejaculate number, season of birth, period of birth, season of semen collection and period of semen collection. First ejaculation had higher semen volume and sperm concentration resulted in to higher number of semen doses but semen quality was better in second ejaculation. Season of birth of the bull was affecting semen quality traits. As the period of birth advances semen volume increases whereas sperm concentration decreases which reflected in persistent production of number of semen doses per ejaculate. Monsoon and summer were favorable seasons for semen collection because of higher sperm concentration which resulted in to higher semen doses per ejaculate. Additionally, Monsoon collected semen had highest volume. Hence, monsoon followed by summer season would be the favorable season for semen collection. Period of semen collection affecting all the semen production traits under study but it did not have specific trend which means managerial and environmental changes over the period have sizable influence on the traits. Results of the study will help to plan future managemental practices and breeding strategies to improve semen production traits.

Introduction

With an intensive selection for increased milk yield, reproductive performance was declined in many countries, in part due to an unfavourable genetic relationship. The intense selection for production traits in the last decades has led to a decrease in fertility. Sustain or improve reproductive efficiency of dairy cattle along with productivity become one of the major challenges of the dairy industry worldwide. Many factors may account for decline in reproductive performance like physiological, nutritional, environmental and genetic. In this sense, several studies have recognized that there is substantial genetic variation underlying reproductive success in dairy cattle. More emphasis was given for improvement of reproduction traits in females only rather than the males. A deficiency in the breeding ability of the bull has a larger impact on the herd productivity as well as fertility problems of females The contribution of the males either through the natural mating or artificial insemination (AI) cannot be ignored and careful scrutiny of the reproduction traits of bulls should be done before their extensive use in farm and field condition for AI.

In recent years many countries have also implemented genetic evaluation for reproductive traits of the bulls. Thus, the relative emphasis of dairy cattle breeding has gradually shifted from production to functional traits such as reproduction. After extensive implementation of AI technique in field condition, semen stations are working with the objective to maximize the output of good quality semen from bulls. To fulfil the objective they are executing various semen evaluation method and also interested into factors affecting semen production traits of the bulls. This is assessed by aiming on semen volume, sperm concentration and sperm motility of the bull in each ejaculate. Moreover, scrutinizing the source of variations in semen production traits due to various non-genetic factors like farm, ejaculate number, season and period of birth, season and period of semen collection is necessary to ensure sufficient semen production from the bulls.

Studies on semen production traits of buffaloes are very scanty (Singh et al. 2013; Bhakat et al. 2015; Ramajayan 2016: Bhave et al. 2020) as compared to cattle. Most of the studies on semen production traits of buffalo were done using very less number of ejaculates and focused on age of bull and primary managemental practices like semen collectors, time of collection and interval between collections. Location of the farm, season and period of birth, season and period of semen collection like non-genetic factors could have significant effect in variation of semen production traits. These non-genetic factors are associated with environment variation which might be came from feeding and other managemental practices at that time. The variation contributed due to these factors could be rectify after careful evaluation and semen production traits will be improved. The present study analyses semen production traits of two different semen stations of India with state of art facility for buffalo bull semen collection and processing.

Materials And Methods

Data

Evaluation of semen production traits was carried out on Mehsana buffalo bulls of two frozen semen stations of Gujarat viz. Pashu Samvardhan Kendra (PSK), Jagudan and Dama Semen Production Unit (DSPU), Dama. Information on breed characteristics is
available on the national portal of the NBAGR website (National Bureau of Animal Genetic Resources 2021).

Data pertaining to semen production traits are available with two semen stations as database which were utilized for the present study with permission of the semen stations. Details of period of data, number of bulls and ejaculates utilized for the present study is given following table.

**Table 1: Details of period of data, number of bulls and number of ejaculate**

| Breed           | Farm | Period of Data Collection | Number of Bulls | Number of Ejaculates used for the Study |
|-----------------|------|---------------------------|-----------------|----------------------------------------|
| Mehsana Buffalo | PSK  | 2014-2020                 | 112             | 18235                                  |
|                 | DSPU | 2011-2020                 | 132             | 36836                                  |

**Location and climate of farms**

The PSK, Jagudan is located in Gujarat state, India, in the outskirts of Mehsana city (23.5880° N, 72.3693° E) and at an altitude of 114 m above sea level. The region experiences three different seasons (Figure-1), viz. winter (November to February), monsoon (March to June), and winter (July to October).

The DSPU, Dama is located in Gujarat state, India, in the outskirts of Deesa city (24.2585° N, 72.1907° E) and at an altitude of 144.58 m above sea level. The region experiences three different seasons (Figure-2), viz. winter (November to February), monsoon (March to June), and winter (July to October).

**Semen collection and evaluation**

Both the semen stations following standard routine practices for the collection of semen from Mehsana buffalo bulls. The bulls were cleaned properly on the day of semen collection in early morning before semen collection. For each bull semen collectors are specified and that semen collectors performed all the operations of semen collection for that particular bull. In the semen collection operations, Dummy bulls were used for sexual stimulus, and each bull allowed to perform 2 to 3 false mounts before final semen collection ride. The time require for false mounting and actual collection mount varies from bull to bull. Normally one to three semen ejaculates were collected from the bull on the day of semen collection. After collection of semen, Semen volume was recorded and kept in a water bath at 37 °C. The semen stations are using photometer for estimation of sperm concentration per ejaculate (x 10^6/ml). The sperm concentration was recorded per ml for particular ejaculate. The initial motility of the sperm cell was estimated by the semen stations as percentage by examining a drop of diluted semen with Tris buffer placed on a pre-warmed slide covered with a pre-warmed cover slip in a phase contrast microscope with a stage warmer at a magnification of 40x. The sperm cells which exhibit progressive movement were scored on a scale of 0 to 100 percent. The collected semen with poor quality which did not fulfil minimum standard criteria were removed from further process of frozen semen dose production. After completion of initial assessment, frozen semen doses were prepared using 0.25 ml straw which contain 20 × 10^6 sperms per dose (i.e., with the hypothesis that it reaches approximately 10 million motile sperms after thawing per dose), sealed, and printed. Semen straws were cooled at 4°C for approximately 3 hr after that frozen down at around −140°C for 10 min in a programmable freezer followed by storage in liquid nitrogen. Post-thaw sperm motility was then carried out for those stored frozen semen doses after 24 hr using 2–3 straws. Thawing of frozen semen straw was done by removing a straw from the liquid nitrogen container and plunging it in warm water bath at 37°C for 30 seconds. Frozen thawed semen was collected in a small test tube by cutting the ends of the straw and remaining procedure was as per initial sperm motility estimation.

**Semen production traits and influencing factors**

Semen production traits considered to study the effects of various non-genetic factors are semen volume, sperm concentration, initial sperm motility, post-thaw sperm motility and number of semen doses per ejaculate.

Non-genetic factors affecting semen production traits are farm, number of ejaculate, season and period of birth, season and period of semen collection.

There are two farms under study. The bulls were maintained under proper housing, feeding, management and health care. The young bulls were trained for semen collection using artificial vagina. The semen collection was done twice a week from individual bull and
ejaculates were obtained with an interval of 15 - 30 minutes. The nutrition requirement is standardized, so bulls are fed ad-libidum chaffed green and dry fodder mixture as per seasonal availability, concentrate mixture as per requirement based on body weight with area specific mineral mixture. For analysis and description following coding is use.

| Name of Farm                        | Code |
|------------------------------------|------|
| Pashu Samvardhan Kendra, Jagudan   | F1   |
| Dama Semen Production Unit, Dama   | F2   |

Mehsana bulls were grouped as per seasons of birth of bull and season of semen collection as winter (November to February), summer (March to June) and monsoon (July to October) looking to the monthly average environmental conditions observed at farms. For analysis and description following coding is use.

| Month of Birth                  | Code for season of birth | Code for season of semen collection |
|--------------------------------|--------------------------|-------------------------------------|
| November to February            | SB1                      | SSC1                                |
| March to June                   | SB2                      | SSC2                                |
| July to October                 | SB3                      | SSC3                                |

Data collected for Mehsana bulls from their respective semen stations were grouped as per year in which particular bull was born and semen collected. Considering that the every two year environmental conditions show change and accordingly the managemental practises were changing over the particular period, which affect the semen production traits under study. For analysis and description following coding is use.

| Period of Birth      | Code for period of birth | Period of Semen Collection | Code for period of semen collection |
|----------------------|--------------------------|-----------------------------|-------------------------------------|
| 2004 to 2005         | PB1                      | 2011 to 2012                | PC1                                 |
| 2006 to 2007         | PB2                      | 2013 to 2014                | PC2                                 |
| 2008 to 2009         | PB3                      | 2015 to 2016                | PC3                                 |
| 2010 to 2011         | PB4                      | 2017 to 2018                | PC4                                 |
| 2012 to 2013         | PB5                      | 2019 to 2020                | PC5                                 |
| 2014 to 2015         | PB6                      |                              |                                     |
| 2016 to 2017         | PB7                      |                              |                                     |

Semen collection from an individual bull was done two or three times in a day with the time interval of 15-30 minutes, accordingly it was grouped as first (EJ1), second (EJ2) or third ejaculate. To study the effect of ejaculate number on various semen production traits in present study it was classified as such first and second ejaculate as follow. Data pertaining to third ejaculate were limited, hence it was not utilized for the study.

**Statistical Analysis**

Abnormal records in semen production traits i.e. missing data or non-justifiable data were eliminated. The non-genetic factors affecting the semen production traits of Mehsana buffalo bulls evaluated were farm, ejaculate number, season of birth, period of birth, season of semen collection and period of semen collection.

The effects of non-genetic factors on semen production traits like semen volume, sperm concentration, initial sperm motility, post-thaw sperm motility and number of semen doses per ejaculate were studied by multivariate analysis under linear mixed model and restricted maximum likelihood (REML) method considering all non-genetic factors listed above have fixed effect and bull as random
effect to study the within bull and between bull variability. The data were analysed using SAS software version 9.3 and PROC MIXED as base command (2011).

The differences between the least squares means for sub classes under a particular effect were tested by Scheffe test (Scheffe, 1959) to check the significance. The high heterogeneous variances between the subclasses, unequal group size, pairwise and unpairwise comparison lead to use of Scheffe test as other tests find differences between the least squares subclass means. Scheffe test is one of the best adjustments that can used to decrease experiment wise error rates when testing multiple comparisons. Scheffe test is a very conservative adjustment that why it is the safest method. The F-ratio used in the calculation is unique in that the mean square (MS) for only two groups being compared is used in the numerator and the MS for all respective comparison is used in the denominator. This means that each pairwise comparison has to have the same significance as the variance for all comparisons when using Scheffe test.

\[
TS: Fs = \frac{(\bar{x}_i - \bar{x}_j)^2}{S_w^2 \left( \frac{1}{n_i} + \frac{1}{n_j} \right)}
\]

Where,

- **TS** = Test of significance
- **Fs** = F for scheffe
- **Xi** = Mean of ith group
- **Xj** = Mean of jth group
- **Sw** = Within group variance
- **ni** = Total number of observation of ith group
- **nj** = Total number of observation of jth group

The semen production trait (expressed in percentages) such as initial sperm motility was adjusted after angular transformation of the percentages as per Snedecor and Cochran (1987). While expressing the means and standard errors, angles were reconverted to percentages to a precision of two decimals.

Statistical model is designed to estimate least squares means of semen production traits for the random effect of bulls and fixed effect of non-genetic factors i.e. farm, ejaculate number, season of birth, period of birth, season of semen collection and period of semen collection

\[
Y_{rahbcfgx} = \mu + R_r + S_a + Z_h + T_b + U_c + X_f + Y_g + e_{rahbcfgx}
\]

Where,

- **Y_{rahbcfgx}** = Semen production trait of xth individual observation belonging to rth bull random effect, ath farm, hth ejaculate, bth season of birth, cth period of birth, 5th season of semen collection and 7th period of semen collection
- **\mu** = Overall mean
- **R_r** = Random effect of rth bull (r = 1 to 244)
- **S_a** = Effect of ath farm (a = 1 and 2)
- **Z_h** = Effect of hth ejaculate number (h = 1 and 2)
- **T_b** = Effect of bth season of birth (b = 1 to 3)
- **U_c** = Effect of cth period of birth (c = 1 to 7)
Results And Discussion

The least squares means (LSMs) for semen production traits i.e. semen volume, sperm concentration, initial sperm motility, post-thaw sperm motility and number of semen doses per ejaculate with the random effect of bull and fixed effects of non-genetic factors such as farm, ejaculate number, season of birth, period of birth, season of semen collection and period of semen collection are given in Table 2. The results of type-3 tests of non-genetic factors and their interactions are given in Table 3.
Table 2
Least-squares means of semen production traits for non-genetic factors in Mehsana buffalo bulls

| Non-genetic factors | n   | Semen volume (ml) | Sperm concentration (millions per ml) | Initial Sperm motility (per cent) | Post-thaw motility (per cent) | Number of semen doses per ejaculate |
|---------------------|-----|------------------|---------------------------------------|-----------------------------------|-----------------------------|-----------------------------------|
| Overall             | 55071 | 3.34 ± 0.18       | 1238.19 ± 75.57                       | 70.55 ± 0.12                      | 60.82 ± 0.16                | 181.61 ± 11.82                   |
| Farm                | **   | **               | 1394.10 ± 83.89                       | 70.96 ± 0.14                      | 51.48 ± 0.18                | 190.46 ± 13.19                   |
| F1                  | 18235 | 3.07 ± 0.20^b     | 1082.29 ± 77.64                       | 70.14 ± 0.12                      | 70.17 ± 0.16                | 172.75 ± 12.13                   |
| F2                  | 36836 | 3.61 ± 0.19^a     | 1234.23 ± 74.08                       | 70.61 ± 0.12                      | 60.96 ± 0.16                | 184.44 ± 11.60                   |
| Ejaculate number    | **   | **               | 1473.28 ± 75.62                       | 70.53 ± 0.12                      | 60.75 ± 0.16                | 248.15 ± 11.83^a                 |
| EJ1                 | 31875 | 3.91 ± 0.18^a     | 1003.11 ± 75.65                       | 70.58 ± 0.12                      | 60.89 ± 0.16                | 172.75 ± 12.13                   |
| EJ2                 | 23196 | 2.77 ± 0.18^b     | 1033.11 ± 75.65                       | 70.14 ± 0.12                      | 70.17 ± 0.16                | 172.75 ± 12.13                   |
| Season of birth     | ns   | ns               | 1199.78 ± 77.70                       | 70.41 ± 0.13                      | 60.73 ± 0.16                | 173.12 ± 12.14                   |
| SB1 (Winter)        | 17947 | 3.29 ± 0.19       | 1199.78 ± 77.70                       | 70.41 ± 0.13                      | 60.73 ± 0.16                | 173.12 ± 12.14                   |
| SB2 (Summer)        | 5295  | 3.34 ± 0.24       | 1280.58 ± 100.44                      | 70.63 ± 0.16                      | 60.77 ± 0.16pendicular      | 187.26 ± 15.69                   |
| SB3 (Monsoon)       | 31829 | 3.38 ± 0.18^a     | 1234.23 ± 74.08                       | 70.61 ± 0.12                      | 60.96 ± 0.16                | 184.44 ± 11.60                   |
| Period of birth     | **   | **               | 1394.10 ± 83.89                       | 70.96 ± 0.14                      | 51.48 ± 0.18                | 190.46 ± 13.19                   |
| PB1 (2004-05)       | 684   | 4.22 ± 0.37^ab    | 1060.12 ± 152.22^ab                   | 70.86 ± 0.25^ab                   | 60.86 ± 0.32^ab             | 199.05 ± 23.93                   |
| PB2 (2006-07)       | 10669 | 4.09 ± 0.21^a     | 1040.54 ± 86.74^c                     | 70.36 ± 0.14^ab                   | 60.51 ± 0.18^bc             | 186.04 ± 13.63                   |
| PB3 (2008-09)       | 5798  | 3.49 ± 0.24^abc   | 1130.05 ± 100.26^bc                   | 70.22 ± 0.16^b                    | 60.34 ± 0.21^c              | 163.98 ± 15.66                   |
| PB4 (2010-11)       | 20775 | 3.06 ± 0.18^b     | 1286.26 ± 74.62^b                     | 70.42 ± 0.12^b                    | 60.56 ± 0.16^c              | 176.75 ± 11.70                   |
| PB5 (2012-13)       | 5993  | 2.95 ± 0.22^bcd   | 1244.72 ± 93.12^bcd                   | 70.56 ± 0.15^ab                   | 60.37 ± 0.20^c              | 169.96 ± 14.67                   |
| PB6 (2014-15)       | 9722  | 2.98 ± 0.20^cd    | 1266.51 ± 81.92^bc                    | 70.52 ± 0.14^ab                   | 61.07 ± 0.18^b              | 175.56 ± 13.04                   |
| PB7 (2016-17)       | 1430  | 2.59 ± 0.25^d     | 1639.16 ± 102.75^a                    | 70.92 ± 0.17^a                    | 62.02 ± 0.22^a              | 199.90 ± 16.41                   |
| Season of semen collection | **   | **               | 1195.98 ± 75.67^b                     | 70.55 ± 0.12                      | 60.82 ± 0.16                | 172.69 ± 11.85^b                 |

Note: Values with different superscript letters are significantly different (P < 0.05).
## Table 3

Type-3 tests for effects of non-genetic factors and their interactions on semen production traits in Mehsana buffalo bulls

| Source of variation | Degree of freedom | Semen production traits | Semen volume (ml) | Sperm concentration (millions per ml) | Initial Sperm motility (percent) | Post-thaw motility (percent) | Number of semen doses per ejaculate |
|---------------------|-------------------|--------------------------|-------------------|--------------------------------------|---------------------------------|-------------------------------|-------------------------------------|
|                      |                   |                          | F Value           | P Value                              | F Value                        | P Value                          | F Value                             |
| Farm                | 1                 |                          | 14.77             | 0.0001                               | 29.59                          | <.0001                         | 23037.7                            | 3.77                                | 0.0523                             |
| Ejaculate number    | 1                 |                          | 5802.66           | <.0001                               | 5908.64                        | <.0001                         | 10756.6                            | <.0001                              | 1.063                              |
| Season of birth     | 2                 |                          | 0.34              | 0.7132                               | 0.6                            | 0.5468                         | 3.07                               | 0.0466                              | 1.41                               | 0.2432                             |
| Period of birth     | 6                 |                          | 11.77             | <.0001                               | 7.99                           | <.0001                         | 16.45                              | <.0001                              | 1.46                               | 0.1874                             |
| Season of semen collection | 2 |                          | 20.91             | <.0001                               | 44.15                          | <.0001                         | 45.38                              | <.0001                              | 1.04                               | <.0001                             |
| Period of semen collection | 4 |                          | 399.5             | <.0001                               | 44.97                          | <.0001                         | 44.97                              | <.0001                              | 1.06                               | <.0001                             |

Overall LSMs of semen volume per ejaculate was found to be 3.34 ± 0.18 ml in the present study, which was higher as compared to semen volume reports of 2.66 and 2.71 ml by Bhakat et al. (2015) and Shakya et al. (2018) in Murrah buffalo respectively. Similarly, semen volume per ejaculate in other buffalo breeds reported to be 3.04, 3.34 and 3.12 ml in Surti buffalo by Dhami et al. (2016), Chaudhary et al. (2017) and Pathak et al. (2018); 2.31 ± 0.46 ml in Tarai buffalo by Tiwari et al. (2009); 2.10 (In young bulls) and 2.60
Semen volume per ejaculate found in the present study was lower than reported value of 3.67 ml (Saini et al., 2017) and 4.48 ml
(Bhave et al., 2020) in the Murrah buffalo; 4.68 ml (Bhave et al., 2020) in the Surti buffalo; 5.11 ml (Ghodasara et al., 2016), 5.13 ml
(Parmar et al., 2020) and 5.10 ml (Bhave et al., 2020) in the Jaffarabadi buffalo; 4.63 ml (Hameed et al., 2017) and 4.7 ml (Ahmed et
al., 2018) in the Nili-Ravi buffalo; 4.09 ml (Bhave et al., 2020) in the Banni buffalo; 4.11 ml (Bhave et al., 2020) in the Bhadawari
buffalo and 4.79 ml (Bhave et al., 2020) in the Pandharpuri buffalo.

The semen volume per ejaculate was significantly (P ≤ 0.01) higher in farm-2 (3.61 ± 0.19 ml) as compare to farm-1 (3.07 ± 0.20 ml).
Hameed et al. (2017) reported significant effect of districts on the semen volume per ejaculate in Nili-Ravi buffalo which was in
correspondence to the present study that effect of two farms at different locations was significant.

The finding of the present study revealed that effect of seasons was not found on semen volume per ejaculate. The hypothesis behind
the study on effect of season of birth on semen volume per ejaculate was to find the effect of environment of particular season on
growth of the bull which lead to development of reproductive organs to achieve pubertal age and ultimately it reflect to the semen
volume per ejaculate.

Bulls born during period-1 (2004 to 2005) produced higher semen volume per ejaculate of 4.22 ± 0.37 ml, thereafter it did not differ
significantly up to period-3 (2008 to 2009) however semen volume per ejaculate was found to be lower (2.59 ± 0.25 ml) in the period-7
(2016 to 2017) born bulls. Effect of period of birth on the semen volume per ejaculate was found significant (P ≤ 0.01) in the present
study which might be due to changes in the environmental condition and managemental practices over the periods.

Monsoon season of semen collection was found favorable and significantly (P ≤ 0.01) highest semen volume per ejaculate (3.40 ±
0.18 ml) was recorded, which was followed by summer (3.34 ± 0.18 ml) and winter (3.28 ± 0.18 ml) seasons of semen collection.
Present findings were at par with those of Ravimurugan et al. (2003) and Bhat et al. (2004) in Murrah; Bhave et al. (2020) in Banni,
Bhadawari, Jaffarabadi, Murrah, Pandharpuri and Surti buffaloes pooled data reported significant effect of season of semen
collection on semen volume per ejaculate. Whereas, Bhakat et al. (2015) in Murrah; Koonjaenak et al. (2007) in Swamp and Hameed et
al. (2017) in Nili-Ravi buffalo reported non-significant effect of season of semen collection on semen volume per ejaculate. Highest
semen volume per ejaculate was produced during monsoon followed by summer and winter seasons in present study. The higher
semen volume was reported in monsoon season of semen collection was due to physiological effect of breeding season of buffalo.

Monsoon season of semen collection was reported as favorable season of semen collection by Ravimurugan et al. (2003) and Bhat et
al. (2004).

Semen collected during the ten years’ time span was considered for the present study. During the period of semen collection 1 to 3
(years 2011 to 2016), semen volume per ejaculate increased significantly (P ≤ 0.01) from 2.57 ± 0.19 ml to 3.68 ± 0.18 ml. Thereafter,
during the remaining periods of semen collection 4 to 5 (2017 to 2020), semen volume per ejaculate decreased significantly (P ≤ 0.01)
as compared to production during period-3 (2015 and 2016). Significant effect of period of semen collection on the semen volume per
ejaculate was found in the present study. Similar to the present study Mir et al. (2016) and Bhave et al. (2020) also reported significant
effect of period of semen collection on semen volume per ejaculate in Murrah and pooled data of Banni, Bhadawari, Jaffarabadi,
Murrah, Pandharpuri and Surti buffaloes respectively.

The semen collected in the first ejaculate number gave significantly (P ≤ 0.01) higher semen volume (3.91 ± 0.18 ml) compared to
second ejaculate number (2.77 ± 0.18 ml). Significantly higher semen volume was produced in the first ejaculate collection in the
present study. Lower semen volume in the second ejaculation was due to physiological effect which was always bound to occur in
subsequent collection after first collection. Similar findings were also reported by Ramajayan (2016) in Murrah and Bhave et al. (2020)
in pooled data of Banni, Bhadawari, Jaffarabadi, Murrah, Pandharpuri and Surti buffaloes.

Overall LSMs of sperm concentration was found as 1238.19 ± 75.57 million per ml in the present study, which was higher as compare
to reported values of 766.69 ± 5.50, 1016.68, 1164 and 978.9 million per ml by Bhakat et al. (2011), Bhakat et al. (2015), Saini et al.
(2017) and Shakya et al. (2018) respectively in Murrah buffalo. Finding of the present study was also higher than the sperm
concentration reports of 838.30, 1219.98 and 1180 million per ml in Jaffarabadi buffalo by Ghodasara et al. (2016), Parmar et al.
(2020) and Bhave et al. (2020); 1100 to 1200 million per ml in Swamp buffalo by Koonjaenak et al. (2006); 990 and 854.27 to
1023 million per ml in Nili-Ravi buffalo by Sajjad et al. (2007) and Hameed et al. (2017); 920.0 ± 71.09 million per ml in Tarai buffalo
by Tiwari et al. (2009); 930 million per ml in Pandharpuri by Bhave et al. (2020); 963.05 and 846.30 million per ml in Surti buffalo by Dhami et al. (2016) and Chaudhary et al. (2017) respectively and 1160 million per ml in Bhadawari by Bhave et al. (2020).

The LSM of sperm concentration found in the present study was lower than the earlier reports of 1610.23 ± 142.07 million per ml (Selvaraju et al., 2008), 1343 million per ml (Pathak et al., 2018) and 1310 million per ml (Bhave et al., 2020) for the Murrah buffalo; 1365.15 ± 120.23 million per ml (Dhami and Sahni, 1994) for the Jaffarabadi buffalo; 2335.7 to 3550.5 million per ml (Ahmed et al., 2018) for the Nili-Ravi buffalo; 1246 million per ml (Pathak et al., 2018) and 1270 million per ml (Bhave et al., 2020) for Surti buffalo and 1310 million per ml (Bhave et al., 2020) for Banni buffalo.

The sperm concentration was significantly (P ≤ 0.01) higher in farm-1 (1394.10 ± 83.89 million per ml) as compare to farm-2 (1082.29 ± 77.64 million per ml). The finding of the present study on sperm concentration was in accordance with study conducted by Hameed et al. (2017) in Nili-Ravi buffalo who reported significant effect of districts on the sperm concentration.

The finding of the present study revealed no significant (P > 0.05) effects of season of birth on sperm concentration. Similar to the semen volume, the hypothesis behind the study of effect of season of birth on sperm concentration was to find the effect of environment of particular season on growth of the bull which lead to development of reproductive organs to achieve pubertal age and ultimately it reflect in the sperm concentration.

Sperm concentration was significantly low in the semen produced by 2004 and 2005 born bulls (1060.12 ± 152.22 million per ml) compared to 2016 and 2017 born bulls (1639.16 ± 102.75 million per ml) but sperm concentration in semen was at par in the bulls born during 2004 to 2015. Effect of period of birth on the sperm concentration was found significant (P ≤ 0.01) in the present study which might be due to changes in the environmental conditions and managemental practices over the periods. As the semen volume reported in the present study was gradually decreased over the period of birth of the bulls from 2004–05 to 2016–17, contrary increase in the sperm concentration was evident. As the semen volume decreases sperm concentration is likely to increase, which might be the reason behind increasing trend in the sperm concentration of the bulls born during 2004–05 to 2016–17.

Summer and Monsoon seasons of semen collection were found favorable with significantly (P ≤ 0.01) higher sperm concentration of 1265.08 ± 75.75 and 1253.52 ± 75.69 million per ml respectively as compared to winter season (1195.98 ± 75.67 million per ml). Higher sperm concentration found during summer season of semen collection which did not differ with sperm concentration of monsoon season of semen collection in the present study. Similar to the present study Bhakat et al. (2015), Hameed et al. (2017) and Bhave et al. (2020) also reported significant effect of season of semen collection on sperm concentration. Whereas non-significant effect of season of semen collection reported by Koonjaenak et al. (2007). In accordance to the present study Bhat et al. (2004) also reported higher sperm concentration during the summer season of semen collection. Contrary to this, Ravimurugan et al. (2003) has reported higher sperm concentration during winter season of semen collection.

During 2011 and 2012 to 2017 and 2018 sperm concentration decreased significantly (P ≤ 0.01) from 1284.82 ± 77.39 to 1180.34 ± 75.63 million per ml but during the years 2019 to 2020 significant (P ≤ 0.01) rise was found in sperm concentration (1267.30 ± 76.02 million per ml). Significant effect of period of semen collection on the sperm concentration was found in the present study. Bhave et al. (2020) also reported significant effect of period of semen collection on sperm concentration during the study based on pooled data of Banni, Bhadawari, Jaffarabadi, Murrah, Pandharpuri and Surti buffaloes.

The semen collected in the first ejaculation gave significantly (P ≤ 0.01) higher sperm concentration (1473.28 ± 75.62 million per ml) as compare to the second ejaculation (1003.11 ± 75.65 million per ml). Sperm concentration was significantly affected by ejaculate number and significantly higher sperm concentration was found in the first ejaculate number in the present study. Similar findings were also reported by Ramajayan (2016) in Murrah and Bhave et al. (2020) in pooled data of Banni, Bhadawari, Jaffarabadi, Murrah, Pandharpuri and Surti buffaloes.

Overall LSMs of initial sperm motility (70.55 ± 0.12 %) in the present study was higher as compared to initial sperm motility reports of 68.40 ± 1.30, 68.1, 60.64 and 68.4% by Shakya (2013), Khatun at el. (2013), Bhakat et al. (2015) and Shakya et al. (2018) in Murrah buffalo respectively. Finding of the present study was also higher than the initial sperm motility reports of 61.37 % in Mehsana buffalo (Patel and Dhami, 2016), 59.44 ± 3.05 and 61.80 % in Jaffarabadi buffalo by Shelke and Dhami (2001), Patel and Dhami (2016) respectively and 49.72 to 66.02 % in Nili-Ravi buffalo by Hameed et al. (2017).
Initial sperm motility found in the present study was lower than 76.58\% (Shukla, 2002), 71.56 ± 0.37 % (Rana, 2012), 90 % (Saini et al., 2017), 84.38 \% (Pathak et al., 2018) and 74.05 \% (Bhave et al., 2020) reported for the Murrah buffalo; 78.90 ± 1.22 \% (Patel et al., 2012), 79.41 \% (Ghodasara et al., 2016), 79.53 \% (Parmar et al., 2020) and 74.70 \% (Bhave et al., 2020) for the Jaffarabadi buffalo; 79.00 ± 3.82 \% (Tiwari et al., 2009) for Tarai buffalo; 75 \% (Dhami et al., 2016), 80.76 \% (Chaudhary et al., 2017), 84.58 \% (Pathak et al., 2018) and 73.21 \% (Bhave et al., 2020) for Surti buffalo; 72.1 to 72.2\% (Ahmed et al., 2018) for the Nili-Ravi buffalo; 74.14 \% (Bhave et al., 2020) for Banni buffalo; 71.57 \% (Bhave et al., 2020) for Bhadawari buffalo and 73.22 \% (Bhave et al., 2020) for Pandharpuri buffalo.

The initial sperm motility was significantly (P ≤ 0.01) higher in farm-1 (70.96 ± 0.16 \%) as compare to farm-2 (70.14 ± 0.12 \%). Hameed et al. (2017) reported significant effect of districts on the initial sperm motility in Nili-Ravi buffalo which supports present findings of significant effect of two farms at different locations.

Season of birth of bull significantly (P ≤ 0.05) affect initial sperm motility. Higher initial sperm motility was found in summer (70.63 ± 0.16 \%) and monsoon (70.61 ± 0.12 \%) season born bulls as compare to winter (70.41 ± 0.13 \%) born bulls. The higher initial sperm motility of 70.92 ± 0.17 \% was observed in the 2016 to 2017 born bulls, whereas it was the lower (70.22 ± 0.16 \%) in 2008 to 2009 born bulls.

Effect of period of birth on the initial sperm motility was found highly significant (P ≤ 0.01) in the present study which might be due to changes in the environmental condition and managemental practices over the periods. Effect of age of bull at first semen collection on initial sperm motility was significant (P < 0.05) in the present study. Higher initial sperm motility of 70.61 ± 0.36 to 70.73 ± 0.07 \% were found in the bulls with 1 to 3 years of age at first semen collection which was at par with initial sperm motility found in the semen of 5 to 7 years of age of bull at first semen collection.

Initial sperm motility was not affected significantly by season of semen collection in the present study. The present findings are similar to those of Koonjaenak et al. (2007) and Hameed et al. (2017). They reported non-significant effect of season of semen collection on Initial sperm motility. Contrarily significant effect of season of semen collection on initial sperm motility was reported by Ravimurugan et al. (2003), Bhakat et al. (2015) and Bhave et al. (2020).

Highly significant (P ≤ 0.01) effect of period of semen collection was found on initial sperm motility. Highest initial sperm motility of 70.69 ± 0.12 \% was found in the semen collected during the period of 2019 to 2020 whereas lowest initial sperm motility of 70.44 ± 0.12 \% was found in the semen collected during the period of 2013 to 2014. It revealed that change in managemental and environmental condition during semen collection may contribute to differential initial sperm motility.

The semen collected in the second ejaculation gave significantly (P ≤ 0.01) higher initial sperm motility of 70.58 ± 0.12 \% as compare to the first ejaculation (70.53 ± 0.12 \%). Lower initial sperm motility in the first ejaculation was due to physiological effect which was always bound to occur as the first ejaculation contains more non-viable sperms. Similar to the present study, Ramajayan (2016) in Murrah and Bhave et al. (2020) in pooled data of Banni, Bhadawari, Jaffarabadi, Murrah, Pandharpuri and Surti buffaloes also reported significant effect of ejaculate number on initial sperm motility.

Overall LSMs of post-thaw sperm motility was found to be 60.82 ± 0.16 \% in the present study, which was higher as compare to post-thaw sperm motility reports of 59.58 and 48.45 \% by Pathak et al. (2018) and Bhave et al. (2020) in Murrah buffalo respectively. Finding of the present study was also higher than the post-thaw sperm motility of 34.30 \% in Mehsana buffalo (Patel and Dhami, 2019), 33.20, 58.71, 57.60 and 48.37 \% reported in Jaffarabadi buffalo by Patel and Dhami (2016), Ghodasara et al. (2016), Parmar et al. (2020) and Bhave et al. (2020) respectively; 58.33 and 48.34 \% reported in Surti buffalo by Pathak et al. (2018) and Bhave et al. (2020) respectively; 43.25 \% reported in Kundhi buffalo by Kaka et al. (2012); 48.47 \% reported in Banni buffalo by Bhave et al. (2020); 43.8 to 46.6 \% reported in Nili-Ravi buffalo by Ahmed et al. (2018); 47.80 \% in Bhadawari buffalo and 48.93 \% in Pandharpuri buffalo reported by Bhave et al. (2020) respectively.

Post-thaw sperm motility found in the present study was lower than reported values of 72.45 ± 2.22 \% (Purohit et al., 2000) and 61.75 \% (Dhami et al., 2016) for Surti; 67.64 \% (Samo et al., 2005) and 64.53 ± 0.76 \% (Rehman et al., 2012) for Kundhi and 75.85 ± \% (Alavi-Shoushtari and Babazadeh-habashi, 2006) for Azarbaijani buffalo.

The post-thaw sperm motility was significantly (P ≤ 0.01) higher in farm-2 (70.17 ± 0.16 \%) as compare to farm-1 (51.48 ± 0.18 \%).
Effect of season of birth was significant ($P \leq 0.05$) on post-thaw sperm motility. Post-thaw sperm motility of 60.96 ± 0.16% was observed in the winter born bulls which significantly differed with monsoon born bulls' post-thaw sperm motility (60.73 ± 0.16%).

Effect of period of birth was highly significant ($P \leq 0.01$) on post-thaw sperm motility. Higher post-thaw sperm motility of 62.02 ± 0.22% was observed in the 2016 to 2017 born bulls but it was lower (60.34 ± 0.21%) in 2008 to 2009 born bulls which was at par with 2004 to 2007 and 2010 to 2013 born bulls. Effect of period of birth on the post-thaw sperm motility was found highly significant ($P \leq 0.01$) in the present study which might be due to changes in the environmental conditions and managerial practices developed and adopted over the periods. Younger bulls produced semen with higher post-thaw sperm motility as compare to adult and older bulls in the present study.

Post-thaw sperm motility was not affected significantly by season of semen collection in the present study. Contrarily to the present study significant effect of season of semen collection on post-thaw sperm motility was reported by Bhave et al. (2020) in the pooled data of Banni, Bhadawari, Jaffarabadi, Murrah, Pandharpuri and Surti buffaloes.

Highly significant ($P \leq 0.01$) effect of period of semen collection was found on post-thaw sperm motility. Post-thaw sperm motility of 61.04 ± 0.16% was found to be significantly highest in the semen collected during the period of 2019 to 2020 as compare to post-thaw sperm motilities during periods 1 to 4. Post-thaw sperm motilities of semen collected during period 1 to 4 were at par with each other. As the period of semen collection advanced post-thaw sperm motility increased showing better handling practices adopted by the semen stations with time.

The semen collected in the second ejaculation gave significantly ($P \leq 0.01$) higher post-thaw sperm motility of 60.89 ± 0.16% as compare to the first ejaculation (60.75 ± 0.16%). Post-thaw sperm motility was significantly affected by ejaculate number and significantly higher post-thaw sperm motility was found in the second ejaculation in the present study. Lower post-thaw sperm motility in the first ejaculation was due to physiological effect which was always bound to occur as the first ejaculation contains more non-motile sperms due to gap between semen collection days and sperm production cycle in the reproductive organ as sperms get produce, mature and become non-motile till next semen collection. Similarly significant effect of ejaculate number on post-thaw sperm motility were reported by Ramajayan (2016) in Murrah and Bhave et al. (2020) in pooled data of Banni, Bhadawari, Jaffarabadi, Murrah, Pandharpuri and Surti buffaloes.

The overall LSM of number of semen doses per ejaculate was 181.61 ± 11.82. Ejaculate number, season of semen collection and period of semen collection contributed significantly ($P \leq 0.01/0.05$) to the variation in number of semen doses per ejaculate. Whereas, farm, season of birth and period of birth were non-significant sources of variation for number of semen doses per ejaculate.

Number of semen doses per ejaculate did not differ significantly between farm-1 and 2. Semen production traits like semen volume, sperm concentration, initial sperm motility and post-thaw sperm motility under present study were significantly differed between farm-1 and 2 but number of semen doses per ejaculate was not affected. Number of semen doses per ejaculate mainly depend on semen volume and sperm concentration. From the data of the present study it was observed that farm-1 has lower semen volume compared to farm-2 but sperm concentration was higher in farm-1 as compare to farm-2 which might have resulted in overall at par production of semen doses per ejaculate.

Effect of season of birth was non-significant ($P > 0.05$) on number of semen doses per ejaculate. This indicate overall adoption of bulls to the particular environment to achieve pubertal age and have well developed reproductive organs without influence of seasonal variation. Number of semen doses per ejaculate was not affected significantly by period of birth of bull.

Number of semen doses per ejaculate were significantly ($P \leq 0.01$) affected by season of semen collection. Semen collected in the monsoon season has higher number of semen doses per ejaculate (186.89 ± 11.85) which was followed by 185.23 ± 11.87 in summer, however difference between both of them was non-significant. Significantly lower number of semen doses was observed in the winter season of semen collection (172.69 ± 11.85). Number of semen doses per ejaculate were significantly affected by season of semen collection in the present study. Significantly higher number of semen doses per ejaculate were produced from the semen collected during summer and monsoon seasons compared to winter season. Semen characteristics like semen volume and sperm concentration were higher during the summer and monsoon seasons’ collected semen. Hence, the higher number of semen doses per ejaculate were produced during summer and monsoon season of semen collection. Contrary to the present finding, Bhosrekar (1988) reported highest frozen semen doses in the winter season but he also narrated that rainy season seemed to be better for semen
freezability and lower discard rate. Similar to the present study significant effect of season of semen collection on number of semen doses per ejaculate was also reported by Bhosrekar (1988) and Bhosrekar et al. (1992) in Surti buffalo.

Highly significant ($P \leq 0.01$) effect of period of semen collection was found on number of semen doses per ejaculate. Higher number of semen doses per ejaculate ($196.26 \pm 11.86$) was observed from the semen collected during 2015 to 2016 whereas lowest number of semen doses per ejaculate of $151.51 \pm 12.32$ was produced from the semen collected during 2011 to 2012. Significant ($P \leq 0.01$) effect of period of semen collection on the number of semen doses per ejaculate was found in the present study. Relatively higher number of semen doses per ejaculate produced during 2015 to 2016 and 2019 to 2020 periods which might be due to better environmental and managerial practices during the periods.

The semen collected in the first ejaculation produced significantly ($P \leq 0.01$) higher number of semen doses per ejaculate ($248.15 \pm 11.83$) as compare to the second ejaculation ($115.06 \pm 11.84$).

In conclusion, Monsoon and summer were favorable seasons for semen collection because of higher sperm concentration which resulted in to higher semen doses per ejaculate in Mehsana buffalo bull. Additionally, Monsoon collected semen had highest volume. Hence, monsoon followed by summer season would be the favorable season for semen collection. Mature Mehsana buffalo bulls of 3 to 5 years of age or bulls having more than 700 kg body weight or the bulls, where semen collection was done after 2012 produced higher semen volume leading to higher semen doses per ejaculate. This indicates that bulls maturing at the age of 3 years (approx.) or having body weight of 700 kg or more produced more semen. First ejaculation had higher semen volume irrespective of age and season of semen collection resulting in to more semen doses per ejaculate in Mehsana buffalo bulls.

Declarations

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Conflicts of interest

The authors declare that they have no conflict of interest.

References

1. Ahmed, S., Khan, M. I. R., Ahmad, M. and Iqbal, S. (2018). Effect of age on lipid peroxidation of fresh and frozen-thawed semen of Nili-Ravi buffalo bulls. Italian Journal of Animal Science, 17: (3), 730–735.
2. Alavi-Shoushtari, S. M. and Babazadeh-habashi, B. (2006). Seasonal variation in the characteristics of the Azarbaijani buffalo (Bubalus bubalis) semen. Iranian Journal of Veterinary Research, 7(1/14): 49–54.
3. Bhakat M.; Mohanty, T. K.; Raina, V. S.; Gupta, A. K. and Khan, H. M. (2011a). Effect of age and season on semen quality parameters in Sahiwal bulls. Tropical Animal Health and Production, 43: 1161–1168.
4. Bhakat, M., Mohanty, T.K., Gupta, A.K., Prasad, S., Chakravarty, A.K. and Khan, H.M., 2015. Effect of season on semen quality parameters in Murrah buffalo bulls. Buffalo Bulletin, 34(1), 100–112.
5. Bhat, V., Honnappa, T. G. and Dubey, B. M. (2004). Seasonal effects on seminal attributes in Murrah bulls under Bangalore agro climatic condition. Indian Journal Animal Reproduction, 25(1): 23–24.
6. Bhave K. G., Thilak Pon Jawahar, K., Venkataramanan, R., Sontakke, S., Joshi, G. and Ducrocq, V. (2020). Semen production and semen quality of indigenous buffalo breeds under hot semi-arid climatic conditions in India. Tropical Animal Health and Production, 52(6): 2529–2539.
29. Ravimurugan, T. and Sindhu, C. (2020). Effect of age and body weight on semen volume of Murrah bulls. International Journal of Chemical Studies, 8(5): 33–35.

30. Saini, J., Dhande, P. L., Gaikwad, S. A., Shankhapal V. D. and Shelar R.R. (2017). Physico-morphological characters of semen of murrah buffalo bull. Indian Journal of Animal Reproduction, 38(1): 54–55.

31. Sajjad, M., Ali, S., Ullah, N., Anwar, M., Akhter, S. and Andrab, S. M. H. (2007). Blood serum testosterone level and its relationship with scrotal circumference and semen characteristics in Nili-Ravi buffalo bulls. Pakistan Veterinary Journal, 27: 63–66.

32. Samo, M. U., Brohi, N. A., Kaka, I., Qureshi, T. A. and Memon, M. M. (2005). Study on sexual behavior and seminal quality characteristics of Kundhi buffalo bulls. Pakistan Journal of Biological Sciences, 8(11): 1628–1629.

33. SAS Institute Inc. (2011). SAS 9.3 Help and Documentation, PROC MIXED, PROC GLM Cary, NC: SAS Institute Inc.

34. Scheffé, H. (1959). The Analysis of Variance. Wiley, New York. (Reprinted 1999, ISBN 0-471-34505-9)

35. Selvaraju, S., Reddy, I. J., Nandi, S., Rao, S. B. N and Ravindra, J. P. (2008). Influence of IGF-I on buffalo (Bubalus bubalis) spermatozoa motility, membrane integrity, lipid peroxidation and fructose uptake in vitro. Animal Reproduction Science, 113 (4): 60–70.

36. Singh, A.P., Singh, R., Singh, A.K., Gupta A.K. and Raina, V.S., 2013. Influence of microclimate modification on sexual behaviour and semen characteristics of Murrah buffalo bull during the hot humid period in India. Indian Journal of Animal Sciences, 83(4), 431–434.

37. Shakya, V. (2013). Studies on scrotal biometrics and seminal attributes in relation to chromosomal profile in buffalo breeding bulls. M.V.Sc Thesis (Veterinary Gynaecology and Obstetrics), Nanaji Desmukh veterinary Science University, Jabalpur, M.P.

38. Shakya, V., Joshi, S., Shivhare, M., Nema, S. and Gupta, V. K. (2018). Studies on seminal attributes in relation to chromosomal profile in Murrah buffalo bulls. Buffalo Bulletin, 37: 65–70.

39. Shelke, V. B. and Dhami, A. J. (2001). Comparative evaluation of physico-morphological attributes and freezability of semen of Gir cattle (Bos indicus) and Jaffarabadi buffalo (Bubalus bubalis) bulls. The Indian Journal of Animal Science, 71(4): 319–324.

40. Shukla, M. K. (2002). Studies on semen additives to improve cryopreservation of Murrah buffalo (Bubalis bubalis) semen. M.V.Sc. thesis (Animal Reproduction, Gynaecology and Obstetrics), Govind Ballabh Pant University of Agriculture and Technology, Pant Nagar, India.

41. Snedecor, G. W. and Cochran, W. G. (1987). Statistical Methods. (5th edn.) Iowa State University Press, Ames, Iowa. pp.xx + 503.

42. Tiwari, M., Parsad, R. B. and Gupta, H. P. (2009). Physico-morphology and in vitro fertility semen / spermatozoa of Tarai buffalo semen. Indian Journal of Animal Physiology, 1: 11–14.

**Figures**

![Figure 1](image-url)

Figure 1

Average monthly high and low temperature at PSK, Jagudan during the period of study
Figure 2

Average monthly high and low temperature at DSPU, Dama during the period of study