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Sexual Behaviour and Seminal Attributes Associated with Fertility of Jaffarabadi Buffalo Bulls

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ABSTRACT

Background: The selection of breeding bulls based on the association of sexual behaviour, seminal attributes and conception rate are more important and economical. However, information on association of sexual behaviour and seminal attributes with fertility in buffaloes particularly with reference to Jaffarabadi buffalo bulls is meager.

Methods: The study was carried out to assess the association of sexual behaviour and seminal attributes with first service conception rate (FCR) of Jaffarabadi buffalo bulls. A total of 192 semen ejaculates were evaluated and cryo-preserved. First AI conception rate (FCR) was carried out on 800 Jaffarabadi female buffaloes inseminated with frozen-thawed semen and pregnancy was confirmed by per rectal palpation at 2 months post-insemination.

Result: The results indicated that both sexual behaviour and seminal attributes were associated with fertility in Jaffarabadi buffalo bulls.

Key words: Fertility, Jaffarabadi buffalo bull, Seminal attributes, Sexual behaviour.

INTRODUCTION

Jaffarabadi is a riverine buffalo, native to western Gujarat, well known for its dairy potential, disease resistance and heat tolerant capacity (Patbandha *et al*., 2020). Such characteristics lead to an increase in demand for frozen semen of Jaffarabadi buffalo bulls throughout the country. The breeding soundness of bull is most important to obtain semen of good freezability with higher conception rate. Positive association of sexual behaviour with seminal attributes and fertility was observed in cattle and buffaloes (Anzar *et al*., 1993; Singh *et al*., 2015; Chaudhary *et al*., 2019). Positive association between sexual behavior and seminal attributes reflects the genetic potency of a breeding bull, which is most important in breeding program. It has been reported that high sexual desire and acceptable sexual union are desirable characteristics for a successful bovine artificial insemination (AI) program (Chenoweth, 1981; Ahmad *et al*., 2005). Further, the bulls having higher sexual desire or libido produced more viable spermatozoa through multiple ejaculates in a short period of time. Thus, selection of bulls with good sexual desire could improve reproductive performance in dairy bovines (Ahmad *et al*., 2005). Thus, selection of breeding bulls based on the association of sexual behavior, seminal attributes and conception rate are most important and economical. However, the information on association of sexual behaviour and seminal attributes with fertility in buffaloes particularly Jaffarabadi buffalo bulls is meager. Therefore, the present investigation was conducted to assess the association of sexual behavior, seminal attributes and conception rate from ejaculates of Jaffarabadi buffalo bulls.

MATERIALS AND METHODS

The present study was conducted on ejaculates (n=192) of Jaffarabadi breeding bulls maintained at Cattle Breeding Farm, Junagadh Agricultural University, Junagadh during November, 2017 to June, 2018. Junagadh is located at on 21.29°N latitude and 70.27°E longitudes. It has an average elevation of 107 meters (351 ft.) from mean sea level. It has a tropical climate with maximum temperature 40°C during summer and minimum temperature 10°C during winter. Junagadh receives on an average rainfall of 625 to 750 mm per annum. There are three distinct seasons, a mild winter from November to February, a hot summer from March to June and monsoon from July to October. The bulls are fed as per the Minimum Standard Protocol (MSP) issued by Government of India (2000), which includes feeding @ 0.8 kg concentrate, 1.6 kg dry fodder and 4.16 kg of green fodder per 100 kg of body weight in addition to supplementation of mineral mixture @ 60 g per day. The bulls were kept in sheltered paddocks with access to water ad libitum. The optimum health care like clipping of preputial hair and vaccination against haemorrhagic septicaemia, black quarter and foot and mouth disease were undertaken. Besides this, screening for tuberculosis, Johne's disease, brucellosis, campylobacteriosis and trichomoniasis was also carried out.

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The temperament score (0-5 scale; Hearnshaw and Morris, 1984), libido score (10 points; Chenoweth, 1981) and penile erection, protrusion and intensity of thrust (all 0-5 score; Joshi and Kharche, 1992) were recorded. Reaction time was recorded in seconds. Flehmen reaction score of "1" was given if the bull exhibited Flehmen response, while a score of "0" was given if no response was obtained. Semen was collected from four Jaffarabadi breeding bulls by artificial vagina (25 cm long and 6.5 cm in diameter) maintained at 42-45°C in incubator as per procedure as described by Singh *et al*. (2000) during the morning hours between 7.00 to 8.30 A.M. Then the semen was processed and cryo-preserved at Frozen Semen lab under standard operative procedures. Immediately after collection, the ejaculates were assessed for volume (ml), colour (1=watery, 2=milky, 3=creamy) and sperm motility. A light microscope was used to determine mass activity (0-4 score) and the percentage of individual progressive motile spermatozoa. Sperm concentration as millions per ml of semen was estimated using bovine photometer (Accucel, IMV, France) against 530 nM wave length. The morphological abnormalities of the spermatozoa were studied in the eosin-nigrosin stained slides. The acrosome integrity was evaluated by simplified nigrosine-eosin-Giemsa staining technique as described by Kutty *et al.* (1996), whereas the hypo-osmotic swelling test (HOST) was done using 150 mosm/l HOST solution to evaluate the functional integrity of the sperm membrane (Jeyendran *et al*., 1984). For freezing of semen Andromed extender and French medium straws with conventional wide mouth liquid nitrogen freezer. Then post-thaw seminal attributes like post-thaw motility (PTM), post-thaw live sperm (PLND), post-thaw abnormal sperm (PABN), post-thaw host reactive sperm (PHOST) and post-thaw acrosomal integrity (PACRM) were carried out. The trial on first insemination conception rate was carried out on 800 Jaffarabadi buffaloes inseminated with frozen-thawed semen at Cattle Breeding Farm, JAU, Junagadh as well as under field conditions during study period. The inseminated buffaloes were confirmed for pregnancy by per rectal palpation at 2 months post-insemination.

The association among different variables was carried out using Pearson's correlation coefficient method. The association was considered as significant at $p \leq 0.05$. All the analyses were carried out using Statistical Packages for Social Sciences (IBM® SPSS® statistics, USA, version 20.0) software.

RESULTS AND DISCUSSION

Correlation of sexual behaviour with seminal attributes

Association of sexual behavioural traits with seminal attributes has great importance to predict the fertility potency of breeding bulls. Sexual behaviour traits particularly penile protrusion, thrust and reaction time showed significant association with certain fresh and post-thaw seminal attributes in Jaffarabadi bull (Table 1). Penile protrusion showed significant and positive correlations with MA (r=0.353, $p \leq 0.05$), LND (r=0.363, $p \leq 0.05$), PTM (r=0.365, $p \leq 0.05$) and PLND (r=0.382, $p \leq 0.01$). However, there was negative and significant association of intensity of thrust with ACRM (r=-0.369, $p \leq 0.05$), but significant and positive association with PLND (r=0.350, $p \leq 0.05$).

The significant positive correlation of penile protrusion with MA, PTM and PLND in the Jaffarabadi buffalo bulls were similar to the findings of Rathod (2018) in Kankrej bulls, who also recorded significant (P<0.01) and positive correlation of penile protrusion score and MA, while significant (P<0.05) positive correlation of penile protrusion score with LND, PTM and PLND. There was significant positive correlation of penile protrusion score with VOL and MA, but non-significant positive correlation of penile protrusion score with IM and CON and non-significant negative correlation with LND in Kankrej bull. Kumar (1993)

Table 1: Correlation of sexual behaviour with seminal attributes (fresh and post-thaw) and fertility (n=192).

Seminal attributes	Temperament	Libido	Penile erection	Penile protrusion	Thrust	Reaction time	Flehmen reaction
Volume (VOL)	-0.164	-0.165	0.135	-0.203	0.018	0.011	-0.018
Concentration (CON)	0.069	-0.030	-0.101	0.152	0.055	-0.059	0.162
Mass activity (MA)	0.034	0.026	0.065	0.353*	0.258	-0.360*	-0.024
Initial motility (IM)	0.098	-0.097	-0.044	0.093	0.129	-0.391*	-0.175
Live sperm (LND)	-0.008	0.142	0.054	0.363*	0.119	-0.010	0.024
Abnormal sperm (ABN)	0.214	-0.192	0.182	0.107	-0.161	-0.172	0.043
Host reactive sperm (HOST)	0.043	0.240	0.160	-0.156	-0.059	0.151	0.079
Acrosomal integrity (ACRM)	0.154	0.264	-0.294	-0.131	-0.369*	0.076	0.017
Post-thaw motility (PTM)	0.056	0.321	0.047	0.365*	0.044	-0.128	-0.199
Post-thaw live sperm (PLND)	-0.104	0.037	0.125	0.382*	0.350*	-0.065	-0.120
Post-thaw abnormal sperm (PABN)	0.063	0.073	0.025	0.080	-0.078	0.116	0.078
Post-thaw host reactive sperm (PHOST)	0.029	-0.012	-0.022	0.133	-0.296	-0.230	0.107
Post-thaw acrosomal integrity (PACRM)	0.214	0.109	-0.212	-0.101	0.210	0.198	0.007
First AI conception rate (FCR)	0.361*	0.166	0.376*	0.352*	0.676**	-0.217	-0.074

**Significant at p<0.01 level; *Significant at $p \leq 0.05$ level.

and Singh *et al*. (2015) reported significant positive correlation of penile protrusion score with CON in Sahiwal bulls. A significant (P<0.05) negative correlation of thrust with ACRM was observed in Jaffarabadi buffalo bulls, but Kumar (1993) observed a significant positive correlation of thrust with VOL, CON and LND in Sahiwal bulls. The variations in results might be attributed to genetic makeup of the bulls and season of study. The negative correlation of reaction time with MA and IM in the present investigations is comparable with the results of El-Chahidi *et al*. (1980) who reported negative correlation of reaction time with VOL, LND and ABN in Egyptian buffaloes. Further, reaction time showed positive and significant correlation with VOL in Murrah bulls, but such association was not observed in Jaffarabadi buffalo bulls (Shukla and Mishra, 2005). In consonance with the present study, Singh *et al* (2015) observed significant and negative correlation of reaction time with MA and IM in Sahiwal bulls. However, Rathod (2018) found non-significant positive correlation of reaction time with VOL, HOST and ACRM in Kankrej bulls and similar findings were recorded in Jaffarabadi buffalo bulls of the present study.

Correlation of fresh with post-thaw seminal attributes

Correlation of fresh and post-thaw seminal attributes of Jaffarabadi buffalo bulls are depicted in Table 2. There were positive and significant (p≤0.01) association of VOL, CON, MA and IM of fresh semen with PTM (r=0.249, r=0.409, r=0.470 and r=0.508, respectively). Sperm viability or LND had positive and significant association with PTM (r=0.167, p≤0.05), PLND (r=0.527, p≤0.01) and PHOST (r=0.383, p≤0.05). The ABN and HOST in fresh and frozen semen showed a positive and significant (p≤0.01) association (r=0.512 and r=0.468, respectively). There was positive and significant association of ACRM with PTM (r=0.368, p≤0.05) and PCRM (r=0.369, p≤0.01).

In the present study, bulls producing higher ejaculate VOL showed better IM and PTM, which might be due to higher testicular size and scrotal circumference in these bulls. The ABN had highly significant (P<0.01) positive association with PABN (r=0.512). Similar observations were recorded by, Shukla and Mishra (2005) who observed correlations between ABN with PABN in buffalo bulls. Further, Shukla and Mishra (2005) reported positive correlation between HOST and PHOST in Murrah bulls and same trend was observed in Jaffarabadi buffalo bulls of the present study. Further, in a similar positive correlation between HOST and PHOST has also been reported in crossbred (Sharma *et al.,* 1992) and buffalo bulls (Kumar, 1993). The percent ACRM of sperm had a positive correlation with PTM, which indicated that higher percent of intact acrosomes resulted in better sperm viability and motility. Vyas *et al*. (1992) observed significant positive correlations of IM of semen with post-thaw live sperm and motility but negative correlations with post-thaw sperm abnormalities in crossbred bull. In Jaffarabadi buffaloes bulls the IM of fresh semen was positively correlated with PTM. In a recent study on zebu cattle and buffalo bull, Pathak *et al.* (2018) reported significant and positive interrelationship of motility, live sperm and HOST of fresh semen with post thaw semen (r=0.40 to 0.81). The association of seminal attributes of fresh ejaculate with post-thaw semen indicated that neat semen attributes could be helpful for early evaluation of semen freezability in Jaffarabadi buffalo bulls. However, there are reports that bulls having good fresh semen characteristics have poor freezability. The changes in temperature impart changes on the composition and structure of sperm plasma membrane integrity by adversely modifying sperm function (Krogenaes *et al*., 1994).

Correlation of sexual behaviour and seminal attributes with conception rate

In Jaffarabadi buffalo bulls, sexual behaviour showed significant and positive correlation with first service conception rate (FCR). There was a significant and positive correlation of temperament (r=0.361, p≤0.05), penile erection (r=0.376, p≤0.05), penile protrusion (r=0.352, p≤0.05) and intensity of thrust (r=0.676, p≤0.01) with FCR (Table 1). Fresh semen attributes like LND, HOST and ACRM had positive and significant association with FCR (r=0.466, p≤0.01; r=0.403, p≤0.05 and r=0.456, p≤0.01, respectively) (Table 2). On the other hand, ABN showed negative and significant correlation with FCR (r=-0.354, p≤0.05). Moreover, post-thaw seminal attributes particularly PTM had positive and significant correlation with FCR (r=0.475, p≤0.01).

In Jaffarabadi buffalo bulls there was significant positive correlation of sexual behaviour parameters with first AI

Table 2: Correlation of seminal attributes (fresh and post-thaw) with fertility (n=192).

Seminal attributes	Volume	Concen-tration	Mass activity	Initial motility	Live sperm	Abnormal sperm	HOST	Acrosomal integrity	First AI conception rate (FCR)
Post-thaw motility (PTM)	0.249**	0.409**	0.470**	0.508**	0.167*	-0.032	-0.119	0.368*	0.475**
Post-thaw live sperm (PLND)	0.014	-0.038	0.062	0.031	0.527**	-0.141	-0.023	-0.018	0.258
Post-thaw abnormal sperm (PABN)	-0.046	0.151	0.052	0.053	-0.098	0.512**	-0.068	-0.114	-0.263
Post-thaw host reactive sperm (PHOST)	-0.079	-0.013	-0.033	-0.041	0.383*	-0.008	0.468**	0.119	0.239
Post-thaw acrosomal integrity (PACRM	-0.052	-0.033	0.080	0.017	-0.021	-0.076	-0.062	0.369**	0.089
First AI conception rate (FCR)	-0.050	0.095	0.060	-0.137	0.466**	-0.354*	0.403*	0.456**	-

**Significant at p<0.01 level; * Significant at p≤0.05 level.

conception rate (FCR) which is in agreement with the observations of Anzar *et al.* (1993) who reported a correlation coefficient of *r*=0.44 between sexual behaviour and fertility in buffalo bulls. In the present study, the correlation coefficient between different sexual behaviour parameters with FCR was comparable with the findings of Anzar *et al.* (1993). Similar results have been reported in crossbred and Nellore bulls (Pal *et al.,* 2012). While in Kankrej bulls, Rathod (2018) found that penile erection was non-significantly correlated with overall conception rate. The PTM had a highly significant positive correlation with FCR in Jaffarabadi buffalo bulls, which indicated that higher the number of progressively motile sperms in post-thaw semen resulted in better conception rate. However, Rathod (2018) found non-significant negative correlation of PTM with overall conception rate in Kankrej bulls. The present investigation showed highly significant positive correlation LND with FCR. The present findings are well supported by the earlier reports in buffalo bulls (Kataria and Tuli, 1992).

CONCLUSION

Taken together it may be concluded that in Jaffarabadi buffalo bulls certain sexual behaviour parameters are correlated with fresh and frozen semen attributes as well as fertility. Similarly sperm quality traits, *viz*., sperm motility, viability, membrane integrity and acrosomal integrity of fresh semen were significantly and positively correlated with their respective post-thaw values and conception rate in Jaffarabadi buffalo bulls.

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