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Original Research

Effect of Estrus and Diestrus Urine on Libido of Buffalo (*Bubalus bubalis*) Bulls

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Abstract

The present experiment was designed to study the effect of estrus and diestrus urine on libido, reaction time, and occurrence of flehmen in buffalo bulls. Six buffalo bulls (4 to 5 years of age) and one vasectomised teaser bull maintained at the Germ Plasm Centre (Division of Animal Reproduction, Indian Veterinary Research Institute, Izatnagar) were used for this study. Urine samples from six buffalo cows at estrus and during the diestrus stage of the cycle (10 to 14 days post estrus) were used for assaying the bull's sexual behavior. A male dummy was restrained in a service bail and urine was sprayed at the root of the tail and perineal area of the dummy till it was sufficiently wet and started dripping off the body. Distilled water was used similarly as control. The bull was allowed to investigate the dummy and libido score, reaction time, and frequency of flehmen response, and their duration was recorded over a 10 minutes period. The libido score was significantly higher for the bulls exposed to estrus urine (4.44±0.11) compared to diestrus urine (2.61±0.26) or distilled water (3.60±0.18). Reaction time was minimum with estrus urine (67.08±8.52 sec) which was significantly ($p<0.05$) less than with diestrus urine (189.32±30.00 sec) or control (160.91±21.73 sec). The frequency of flehmen behavior increased significantly ($p<0.05$) with estrus urine (3.94 ±0.28) compared to diestrus urine (1.94 ±0.15) or control (1.66 ± 0.13). We conclude that estrus urine is a strong stimulator of sexual activity in buffalo bulls. Furthermore, specific pheromonal compounds present in estrus urine may be responsible for a higher frequency of flehmen behavior in bulls.

Keywords: Buffalo , Diestrus, Estrus, Libido, Urine

Introduction

Bull fertility is influenced by a complex interaction of factors including seminal quality and quantity, sex drive, mating ability and social interactions among animals in the breeding pasture (Chenoweth, 1984). Expression of fertility is affected not only by the phenotype of the sire but also by that of the female and the resulting progeny (Koger, 1980). Consequently, a great difficulty is encountered in attempting to predict bull fertility from single or even multiple characteristics (Chenoweth, 1984). Various scoring systems employing a number of seminal and physical characteristics have been used to predict bull fertility.

High libido in bulls is advantageous to herd fertility and has probable beneficial effects on the fertility of subsequent female progeny. Bulls that score highly in libido/service capacity tests achieve more pregnancies than bulls that have low scores (Parkinson, 2004). Although the expression of libido is a response to endogenous or exogenous stimuli mediated through hormonal events, relationships between blood levels of LH or testosterone and bull libido are not well established (Henney *et al.,* 1990). It has been reported however that, high libido bulls do have high levels of androgens (Elsaesser, 1980). Osman and his coworkers (1984) have observed that the levels of testosterone in buffalo bulls were nearly half of those reported in cattle, which may be the cause of sluggish libido and shorter breeding life of buffalo bulls.

Large variations in libido have been associated with factors such as genetics (Blockey, 1978; Chenoweth *et al.*, 1979; Williams, 1988), age and experience (Boyd *et al.*, 1991; Chenoweth *et al.*, 1996), nutrition, (Chenoweth, 1983), social environment (Rodriquez *et al.*, 1993), inadequate stimuli (Chenoweth, 1983), temperament (Price and Wallach, 1991), rearing and handling (Borg *et al.,* 1993), housing conditions (Forrest, 1989), type of test (Chenoweth, 1981), pathologies or traumatic causes (Fraser and Broom, 1990) and genotype-environment interactions (Chenoweth, 1996).

Paleologou (1977) demonstrated that a volatile odor in the cervicovaginal mucus of estrous cows is a source of sexual attraction for bulls. Vaginal secretions have been shown to have pheromonal properties in many species. The production of vaginal pheromones in primates appears to depend on interactions between vaginal bacteria and the vaginal secretions controlled by ovarian hormones (Keverne, 1974). The concentrations of volatile fatty acids in vaginal secretions of cows remain elevated on the day before estrus and fall sharply thereafter (Hradecky *et al.*, 1983). These changes in the concentrations of volatile fatty acids show signs of hormonal changes in the cow mediated by the vaginal microflora.

Undoubtedly, a female present a combination of olfactory, visual, tactile, and auditory cues, all of which a male has at its disposal to use in determining whether the female is in estrus. Improving libido in males by having semen collected for A.I. might be one means by which signaling pheromones that stimulate sexual activity could be practically applied. These pheromones might also be useful in facilitating the training of young males to mount dummies, which are often used in semen collection procedures (Izard, 1983). The present investigation was therefore designed with the objectives to study the effect of estrus and diestrus urine on libido, reaction time, and occurrence of flehmen in buffalo bulls.

Materials and Methods

Experimental Animals

The present study was carried out on Murrah buffalo bulls stationed at the Germ Plasm Centre and buffalo cows at Livestock Production and Management Section (Cattle and Buffalo Farm), Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India. Six buffalo bulls and one vasectomised teaser buffalo bull (4 to 5 years of age), maintained at the Germ Plasm Centre were used for this study. Out of the six buffalo bulls, three bulls were regularly used for semen collection. The remaining three bulls were not used for regular semen collection but had normal reproductive behavior and semen picture. The study was conducted from early February to early March. Urine from six buffalo cows (5 to 8 years of age), during their first to the fifth lactation in estrus and diestrus stage (10 to 15 days after estrus) was collected in pre-sterilized glass beakers. The Germ Plasm Centre and the Cattle and Buffalo Farm are situated at 28°22’ (N) latitude and 79°24’ (E) longitude, at an altitude of 169.2 meters above mean sea level. Agro-ecologically, the experimental site is situated in the upper Gangetic plain region (Sastry, 1995) with extreme conditions, where the temperature varies from 37.5°C to 39.5°C from the month of May to June (summer)

and 5°C to 10°C from December to February (winter).

Feeding and Management

The bulls at the Germ Plasm Centre were maintained in semi-open sheds. All the animals received seasonal green fodder at the rate of 20 to 25 kg along with 4 to 4.5 kg of concentrate mixture and 6 kg of chaffed wheat straw per animal per day. The animals had free access to clean drinking water. The animals of the Livestock Production and Management Section were maintained under stall-feeding with free access to clean drinking water. About 30 kg of seasonal green fodder (maize/berseem/oats) was provided along with 3.5 kg of concentrate mixture to each animal per day.

Detection of Estrus in Buffaloes

The phases of the estrous cycle were carefully determined for three naturally occurring consecutive cycles and confirmed by examination of genitalia per rectum. The stages of the estrous cycle were identified according to several criteria; including vaginal swelling, frequent urination, the bellowing of females, and male flehmen behavior. The onset of estrus was checked twice daily (7:00 and 17:00 hours) by teaser bull parading. The time when any animal stood for mounting by the teaser bull was considered as the time of onset of estrus and as evidence that the animal was in standing heat. The length of the normal estrous cycle based on the onset of behavioral estrus ranged from 18 to 22 days.

Sample Collection

Collection of urine samples from estrus animals was done approximately two to four hours from the onset of estrus. Approximately, 250-300ml of urine sample from each of the animals was collected during midstream urination (Rajanarayan and Archunan, 2011). Collection of urine samples from diestrus animals was done 10 to 15 days after estrus. Immediately after collection, the urine samples were filtered using Millipore (0.22µm) membrane filter, aliquoted into 30 ml vials, and stored at – 20ºC till assayed for bull's sexual behavior.

Preparation of Sample for Bioassay

The exposure of the particular urine sample, i.e., estrus urine, diestrus urine, or distilled water (control) was randomized among the bulls. Fifteen minutes prior to the assay, 3 vials (30 ml capacity) containing urine samples were thawed at room temperature and then filled into a glass sprinkler (Borosil; 100 ml capacity), which was fitted with a rubber bulb.

Behavioral Parameters Studied

A male dummy was restrained in a service bail and urine was sprayed at the root of the tail and perineal area of the dummy till it was sufficiently wet and started dripping off the body. Distilled water was used similarly as control. Behavioral parameters which included libido score test, reaction time, and frequency of flehmen and their duration were recorded over 10 min. period.

Libido was quantified according to the method of Hultnas (1959) as described below:

0 = No interest in dummy, although bull was led up and invited to mount
1 = Little interest in mounting, despite sniffing at the rear end of the dummy and vague mounting attempts.
2 = Mounting after obvious repeated hesitation with weak clasping and seeking.
3 = Comparatively quick mounting without obvious eagerness. Satisfactory holding and seeking.
4 = Quick mounting with bull's attention focused upon the dummy with very good holding and seeking.
5 = Eager mounting with very good holding and seeking.
6 = Uncontrolled eager mounting with very good holding and intensive seeking.

Reaction time was considered as the time that elapsed after seeing the dummy up to the first mount and expressed in seconds.

Flehmen behavior was induced when the bull smelled the perineal region of the dummy which was a characteristic

raised posture of the head and an elevation of the upper lip. The frequency and the duration of flehmen responses were recorded for each bull.

Results

Behavioral Parameters

When the bull was released to investigate the dummy, it first sniffed the perineal area of the dummy sprayed with urine or distilled water. Short physical contact of the bull's muzzle with the urine elicited immediate flehmen, regardless of the type of urine sample. A single flehmen lasted for about five to fifteen seconds, during which the bull lifted its head, straightened its neck, curled its upper lip, and breathed through narrowed nostrils.

Libido Score

Table 1 depicts the mean libido score with distilled water, diestrus urine, and estrus urine. Libido score of the bulls ranged from 0 to 5 when exposed to distilled water and diestrus urine, while with estrus urine, it ranged from 3 to 5. One bull on a single occasion with distilled water and two bulls on 3 occasions with diestrus urine failed to express libido. The majority of the bulls (52.78%) had scores of 5 with estrus urine followed by scores, of 4 (38.89%) and 3 (8.33%). The mean libido score of bulls with estrus urine (4.44±0.11) was significantly higher ($p<0.05$) than those exposed to diestrus urine (2.61±0.26) or to distilled water (3.60±0.18).

Table 1: Effect of estrus and diestrus urine on libido, reaction time, and flehmen response in buffalo bulls (mean ± S.E.)

Type of urine	Libido (0-6 scale)	Reaction time (sec)	No. of Flehmen
Distilled water (control)	3.60 ± 0.18^{a} (n=36)	160.91 ± 21.73^{a} (n=33)	1.66 ± 0.13^{a} (n=30)
Diestrus urine	2.61 ± 0.26^{b} (n=36)	189.32 ± 30.00^{a} (n=22)	1.94 ± 0.15^{a} (n=36)
Estrus urine	4.44 ± 0.11^{c} (n=36)	67.08 ± 8.52^{b} (n=36)	3.94 ± 0.28^{b} (n=36)

Means bearing different superscripts within a column differ significantly ($p<0.05$), tested by one-way analysis of variance.

Reaction Time

The reaction time of the bulls ranged from 16 to 492 seconds with distilled water, 18 to 530 seconds with diestrus urine, and 16 to 246 seconds with estrus urine. No reaction time was recorded for three bulls on 3 occasions (8.33%) when exposed to distilled water, while with diestrus urine, four bulls on 14 occasions (38.89%) did not exhibit any reaction time. However, reaction time was recorded in all the bulls when exposed to estrus urine. The mean reaction time was minimum with estrus urine (67.08±8.52 seconds) which was significantly ($p<0.05$) less than with diestrus urine (189.32±30.00 seconds) or distilled water (160.91±21.73 seconds).

Flehmen Response

The number of flehmens varied from 1 to 4 when bulls were exposed to distilled water, 1 to 5 with diestrus urine, and from 1 to 9 following exposure to estrus urine. Four bulls on 6 occasions did not show any flehmen with distilled water. The estrus urine induced significantly ($p<0.05$) a greater number of flehmen responses (3.94±0.28) as compared to exposure of bulls to diestrus urine (1.94±0.15) or distilled water (1.66±0.13).

Duration of Flehmen

The duration of flehmen response of bulls exposed to estrus urine, diestrus urine, and distilled water is presented in Table 2. The comparison of the mean duration of flehmen with estrus urine, diestrus urine, and distilled water revealed no significant ($p>0.05$) difference, however, it tended to be lower with estrus urine.

Table 2: Duration of flehmen response (in seconds) of buffalo bulls to estrus and diestrus urine (mean ± S.E.)

Type of urine	Flehmen response I	Flehmen response II	Flehmen response III	Flehmen response IV	Flehmen response V
Distilled water (control)	11.20±0.89 (n=30)	8.93±1.09 (n=16)			
Diestrus urine	11.28±0.71 (n=36)	11.47±0.75 (n=23)	11.50±1.83 (n=6)		
Estrus urine	9.58±0.85 (n=36)	9.06±0.73 (n=35)	7.90±0.66 (n=30)	7.22±0.71 (n=28)	7.00±0.74 (n=12)

Discussion

A significantly greater libido score with minimum reaction time for the bulls exposed to estrus urine, compared to diestrus urine or control clearly indicated the magnitude of their attraction towards estrus urine and its properties in stimulating the bulls sexually. It has been demonstrated that males can discriminate between estrus and diestrus urine based on chemical cues (Rajanarayanan and Archunan, 2004). A wide array of urinary compounds has been identified in several species in recent years. For instance, Kumar *et al.* (2000) identified undecane from cow urine during the postovulatory stage of the estrous cycle. Patra and his coworkers (2009) identified decane, 6 ethyl, 2 methyl, that was specific to diestrus phase in cow urine. As estrus-specific pheromonal compounds such as 1-iodoundecane, di-n-propyl phthalate, octane and 3-hexanol, 2,2-dimethyl in bovine urine (Kumar *et al.* 2000; Patra *et al*. 2009), (Z)-7-dodecenyl acetate in elephant urine (Rasmussen *et al.,* 1996), hydroperoxide, 1-nitropentane and 4-azidoheptane in rat urine (Selvaraj and Archunan, 2002) and 1-iodo-2 methylundecane in mouse urine (Achiraman and Archunan, 2006) have been identified, similar chemical cues of estrus urine seem to have provoked the manifestation of higher libido and flehmen response in our experimental bulls as has been demonstrated by Sankar and Archunan (2002) and Kumar and Archunan (2002).

Pheromones are perceived by the sense of smell, which is mediated by the main olfactory system and the accessory olfactory system the vomeronasal organ (Hradecky *et al*., 1983). While the main olfactory system serves as a general detecting system, the vomeronasal organ is capable of more precise or specialized evaluation of chemical information contained in urine. In males, the function of the vomeronasal organ is associated with flehmen. Bulls receive the estrus-specific chemosignal present in estrus urine and then exhibit flehmen behavior. Flehmen is preceded by fast tongue strokes over the rostral and medial part of the palate, which has separate innervations and yield strokes that actually trigger the flehmen response (Jacobs *et al*., 1980). Comparatively less frequent flehmen response observed with diestrus urine in the bulls of the present study suggests the appearance of sex pheromones in connection with some other events such as interovulatory follicle growth (Kiddy and Mitchell, 1981). This is in consonance with the reports of Hradecky *et al.* (1983), who studied the distribution of flehmen reaction of the bulls throughout the bovine estrous cycle and found that flehmen behavior was not limited to the estrus phase only as a non-estrual phase also provoked the similar response.

An interesting feature in the present investigation was an expression of flehmen by the bulls exposed to distilled water, which in turn can be attributed to the fact that the bulls used for the bioassay perhaps became habituated of showing some flehmen due to their repeated semen collection using a male dummy. The presence of pheromonal compounds such as nonacosane, 1, 2, benzenedicarboxylic acid, dihexyl ester, and heptacosane in bull urine has been recently demonstrated (Patra *et al*., 2009). Similarly, other excretory substances such as feces (Sankar and Archunan, 2008), saliva (Sankar *et al*., 2007), preputial gland secretions (Kanan, 1998), and cheek gland secretions (Kanan and Archunan, 1999) have been shown to contain an array of pheromonal compounds. Therefore, the other possibility that the pheromonal cues from anal gland secretions, urine, and/or feces adhering to the perineal area of the dummy, elicited the flehmen behavior in our experimental bulls, cannot be ignored. These findings are, however, contrary to the report of Sankar and Archunan (2004) who recorded no flehmen response in bulls exposed to dummy cows sprayed with water to the perineal area.

In the present investigation, the mean duration of flehmen with estrus urine, diestrus urine, or distilled water did not differ significantly ($p>0.05$), though its duration tended to be lower with estrus urine. In contrast, however, Sankar and Archunan (2004) observed flehmen behavior of maximum duration in bulls exposed to dummy cows smeared with vaginal mucus, saliva, feces, or milk of estrus stage.

The use of bulls with greater libido has been shown to benefit pregnancy rates, time of conception, length of calving season, homogeneity of calves at weaning, and more efficient use of personnel (Blockey, 1978; Godfrey and Lunstra, 1989; Chenoweth, 1997). As assessment of libido has been found to be a better predictor of bull fertility than seminal parameters (Smith *et al*., 1981), the improved libido and least reaction time in bulls exposed to estrual urine in the present investigation seems to indicate its potential use for enhanced reproductive function of buffalo bulls at semen collection centres and to facilitate the training of young males to mount dummies.

Conclusion

From this study, it can be concluded that estrus urine is a strong stimulator of sexual activity in buffalo bulls.

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Conflict of Interests

There is no conflict of interest.

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References

1. Achiraman, S. and Archunan, G. (2006). 1-iodo-2methylundecane, a putative estrus-specific urinary chemo-signal of female mouse (*Mus musculus*). *Theriogenology*, 66, 1913-1920.
2. Blockey, M.A. (1978). The influence of serving capacity of bulls on herd fertility. *Journal of Animal Science*, 46, 589-595.
3. Borg, K.E., Esbenshade, K.L. and Johnson, B.H. (1993). Effects of the peripubertal rearing environment on endocrine and behavioral response to estrus female exposure in the mature bull. *Applied Animal Behavior Science*, 35, 245-253.
4. Boyd, G.W., Healy, V.M., Mortimer, R.G. and Piotrowski, J.R. (1991). Serving capacity tests are unable to predict the fertility of yearling bulls. *Theriogenology*, 36, 1015-1025.
5. Chenoweth, P.J. (1981). Boars and rams: a review. *Theriogenology*, 16, 155-1577.
6. Chenoweth, P.J. (1984). Examination of bulls for libido and breeding ability. *Veterinary Clinics of North America Large Animal Practice*, 5, 59-74.
7. Chenoweth, P.J. (1997). Bull libido/serving capacity. *Veterinary Clinics of North America Food Animal Practice*, 13, 331-344.
8. Chenoweth, P.J., Chase, C.C., Larsen, R. Thatcher, M.J., Bivens, J.F. and Wilcox, C.J. (1996). The assessment of sexual performance in young *Bos taurus* and *Bos indicus* bulls. *Applied Animal Behavioral Science*, 48, 225-236.
9. Chenoweth, P.J., Brinks, J.S. and Nett, T.M. (1979). A comparison of three methods of assessing sex-drive in yearling beef bulls and relationships with testosterone and LH levels. *Theriogenology*, 12, 223-233.
10. Elsaesser, F. (1980). Effects of active immunization against estradiol 17–β, testosterone or progesterone on receptivity in the female rabbit and evaluation of specificity. *Journal of Reproduction and Fertility*, 58, 213-218.
11. Forrest, D.E. (1989). Serving capacity and reproductive performance in Brahman influenced bulls. In: Pope LS, editor. Beef Cattle Science Handbook. International Stockmen's School, TX, USA, p. 132-38.
12. Fraser A.F. and Broom D.M. (1990). Male Sexual Behavior. In: Fraser AF, Broom DM. (editors). Farm Animal Behavior and Welfare, 3rd edition, Baillere-Tindale;, p.185-91.
13. Godfrey, R.W. and Lunstra, D.D. (1989). Influence of single or multiple-sire and serving capacity on mating behavior of beef bulls. *Journal of Animal Science*, 67, 2897-2903.
14. Henney, S.R., Killian, G.J. and Deaver, D.R. (1990). Libido, hormone concentrations in Holstein bulls. *Journal of Animal Science*, 68, 2772-2784.

15. Hradecky, P., Sis, R.F. and Klemm, W.R. (1983). Distribution of flehmen reactions of the bull throughout the bovine estrous cycle. Theriogenology, 20, 197-204.
16. Hultnas, C.A. (1959). Studies on variation in mating behavior and semen picture in young bulls of the Swedish Red and White breed and on causes of this variation. *Acta Agriculturae Scandinavica Supplement,* 6, 1-82.
17. Izard, M.K. (1983). Pheromones and Reproduction in Domestic Animals. In: Izard MK. (editor). Pheromones and Reproduction in Mammals, J.G. Vandenbergh ed. Academic Press, New York., p. 253.
18. Jacobs, V.L., Sis, R.F. and Cappock, C.E. (1980). Tongue manipulation of the palate assists estrous detection in the bovine. *Theriogenology,* 13, 353-356.
19. Kannan, S. (1998). Histomorphology, biochemistry and chemical identification of rat scent glands in view of rodent pest management. A new light in IPM programme. Ph.D Thesis, Bharathidasan University, Tiruchirappalli, India.
20. Kannan, S. and Archunan, G. (1999). Identification of volatile compounds from cheek glands of lesser bandicoot rats and assessment of behavioral response for identified compounds. *Indian Journal of Experimental Biology*, 37, 798-802.
21. Keverne, E.B. (1974). Sex attractants in primates. *New Scientist,* 63, 22-24.
22. Kiddy, C.A. and Mitchell, D.S. (1981). Estrus-related odors in cows: Time of occurrence. *Journal of Dairy Science,* 64, 267-271.
23. Koger, M. (1980). Effective crossbreeding systems utilizing Zebu cattle. *Journal of Animal Science*, 50, 1215-1220.
24. Kumar, R.K. and Archunan, G. (2002). 1-iodoundecane: An effective sex attractant from bovine estrus urine. Proceedings of XX Symposium on Reproductive Biology and Comparative Endocrinology, 2002, 7-9 at Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.
25. Kumar, R.K., Archunan, G., Jeyaraman R. and Narasimhan, S. (2000). Chemical characterization of bovine urine with special reference to estrus. *Veterinary Research Communications*, 24, 445-454.
26. Osman, A.M., Ghaniema, S.A. and Abdelkalik, M.O. (1984). Testosterone level in male buffaloes as related to age and season. *Buffalo Bulletin*, 3, 9-14.
27. Paleologou, A.M. (1977). Detecting estrus in cows by a method based on bovine sex pheromones. *Veterinary Record*, 100, 319-320.
28. Parkinson, T.J. (2004). Evaluation of fertility and infertility in natural service bulls. *Veterinary Journal,* 168, 215-229.
29. Patra, M., Kumar, H., Yadav, M.C., Meur S. K. and Mahmood S. (2009). Volatile chemical compounds in bovine urine. *Indian Veterinary Journal,* 86, 912-914.
30. Price, E.O. and Wallach, S.J. (1991). Sexual and aggressive behavior of Hereford bulls: inter relationship of variables and problems with the use of composite scores to describe overall sexual performance. *Journal of Animal Science,* 69, 1028-1033.
31. Rajanarayan, S. and Archunan, G. (2011). Identification of urinary sex pheromones in female buffaloes and their influence on bull reproductive behavior. *Research in Veterinary Science,* 91, 301-305.
32. Rajanarayanan, S. and Archunan, G. (2004). Occurrence of flehmen in male buffaloes [*Bubalus bubalis*] with special reference to estrus. *Theriogenology,* 61, 861– 866.
33. Rasmussen, L.E.L., Lee, T.D., Roelofs, W.L., Zhang, A. and Daves, G.D. (1996). Insect pheromone in elephants. *Nature*, 379, 684.
34. Rodriquez, C., Galina, C.S., Gutierrez, C., Navarro, R. and Piccinalli, R. (1993). Evaluation of sexual activity in Zebu bulls used for multiple matings with $PGF_2\alpha$ synchronised females. *Ciencias Veterinarias Journal*, 15, 41-49.
35. Sankar, R. and Archunan, G. (2002). Occurrence of flehmen reaction in bull towards bovine (*Bos taurus*) estrus urine. Proceedings of XX Symposium on Reproductive Biology and Comparative Endocrinology, 2002, 7-9 at Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.
36. Sankar, R. and Archunan, G. (2004). Flehmen response in bull: role of vaginal mucus and other body fluids of bovine with special reference to estrus. *Behavioral Processes*, 67, 81-86.
37. Sankar, R. and Archunan, G. (2008). Identification of putative pheromones in bovine (*Bos taurus*) faeces in relation to estrus detection. *Animal Reproduction Science*, 103, 149-153.
38. Sankar, R., Archunan, G. and Habara Y. (2007). Detection of estrus-related odour in bovine (*Bos taurus*) saliva: bioassay of identified compounds. *International Journal of Animal Science,* 9, 1237-1400.
39. Sastry, N.S.R. (1995). Livestock sector of India: Regional Aspects. New Delhi: International Book Distributing Company.
40. Selvaraj, R. and Archunan, G. (2002). Chemical identification and bioactivity of rat (*Rattus rattus*) urinary

compounds. *Zoological Studies*, 41, 127-135.
41. Smith, M.F., Morris, D.L., Amos, M.S., Parish, N.R., Williams, J.D. and Wiltbank, J.N. (1981). Relationship among fertility, scro tal circumference, seminal quality and libido in Santa Gertrudis bulls. *Theriogenology*, 16, 379-397.
42. Williams, G.L. (1988). Breeding capacity, behavior and fertility of bulls with Brahman genetic influence during synchronised breeding of beef females. *Theriogenology*, 30, 35-44.

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