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Effect of estrus on vaginal and vulvar temperature following induction of estrus during winter and summer in anestrus Murrah buffalo and heifer

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Abstract
Fixed-time artificial insemination method (FTAI) has been used in buffalo to improve reproductive efficiency and rate. Estrus intensity and duration vary significantly among individuals and are affected by many factor. Therefore, estrus identification technology plays an important role in reproduction management of dairy herds. Vaginal and vulvar temperature measurement are more reliable and realistic method to use for the purposes of regulating reproduction and managing production. Hence present study was designed to investigate the change in vaginal and vulvar temperature during estrus induction protocol on 193 buffaloes and buffalo heifers during summer and winter under farm and field conditions. Study concluded that average vaginal temperature on the day of estrus increased in both winter and summer seasons in both buffalo and buffalo heifers.

Keywords: Vaginal temperature, vulvar temperature, buffalo, heifer, farm, field

Introduction
Buffalo is hardy animal having better adaptability for harsh environmental conditions and important livestock resource for the rural economy due to the fundamental role played by them in many climatically disadvantaged agricultural systems and having good feed conversion efficiency with low maintenance requirements (Paul *et al*., 2002; Gasparrini, 2013) [24, 11]. However, despite of these merits, buffaloes are blamed for slow reproduction, owing to long calving interval, delayed puberty, poor estrus expression and seasonality in breeding (El- Wishy, 2007) [9]. The breeding season of buffalo starts in rainy season, and winter is the most favourable period, while summer is most unfavourable time for buffalo breeding (Sule *et al*., 2001) [33]. The low pregnancy rate of buffaloes has been a major issue in India.

To achieve efficient reproduction, the buffaloes should have an inter-calving interval of 12-13 months for which ovarian activity should commence between 30-45 days of calving and conceive within 90 days of parturition (Abdalla, 2003) [1]. Shy reproductive behavior of buffalo may be due to the lower circulating concentration of hypophyseal and gonadal hormones and suboptimal functioning of hypothalamo-hypophyseal (HPS) and gonadal axis (Madan *et al*., 1983) [17]. Heat stress has a direct adverse effect on breeding efficiency of female buffaloes and reduces the intensity and duration of estrus (Raut and Kadu, 1988; Lopez *et al*., 2004 and Singh *et al*., 2013) [26, 16, 33]. poor estrus detection (30–40%; Barkawi *et al*., 1993) [2], poor expression of estrus signs (Perera, 2011) [25], a variable duration of estrus length (4–64 h; Baruselli *et al*., 2001) [3] and the difficulty in predicting the exact time of ovulation, are responsible for the limited use of artificial insemination (AI) in this species.

Fixed-time artificial insemination method (FTAI) has been used in buffalo to improve reproductive efficiency and rate (Baruselli *et al*., 2013) [4]. Ancillary methods are studied in search of an enhanced efficiency of the FTAI in cattle, and markers can be used to observe which females were produced by the other animals through homosexual behaviour typical of estrous expression (Sá Filho *et al*., 2011) [28]. In buffalo, homosexual behavior is not expressed, and it is necessary to develop practical methods to identify estrus (Singh *et al*., 2000; Hockey *et al*., 2010) [31, 13].

Estrus intensity and duration vary significantly among individuals and are affected by many factors (Reith and Hoy 2018) [27]. Therefore, estrus identification technology plays an important role in reproduction management of dairy herds (Miller *et al*. 2007; Fricke *et al*. 2014) [20, 10]. It was reported that estrus (Suthar *et al*. 2011) [34], pregnancy (Gil *et al.* 2001) [12], parturition (Wright *et al*. 2014) [37], and the postpartum period (Burfeind *et al.* 2014) [5] in cows are all accompanied by changes in body temperature. Compared with surface temperature measurement, vaginal and vulvar temperature are strongly correlated with core body temperature (EI-Sheikh *et al*., 2013; Miura *et al*., 2017) [8, 21] and are not significantly affected by external factors. Vaginal and vulvar temperature measurement are more reliable and realistic method to use for the purposes of regulating reproduction and managing production (Holman *et al*., 2013) [14]. Studies also showed that the estrous detection based on temperature measurement was superior to using ultrasound and hormone detection (Sakatani *et al*., 2016; Miura *et al*., 2017) [30, 21]. Hence present study was done to investigate change in vaginal and vulvar temperature from anoestrous to estrus phase during estrus induction in buffaloes by using digital and infrared thermometer.

Materials and Methods

The present study was carried out on 193 animals including pluriparous Murrah buffaloes (n=141) and buffalo heifers (n=52) maintained at 1) Central Institute for Research on Buffalo, Hisar (n=88), and 2) in rural areas, nearby Hisar district (n=105) during summer (May to August) and winter (November to February). The selected animals had a history of anestrous without showing any proper signs of heat such as bellowing, micturition, restlessness, vaginal discharge for the past more than 60 days following calving in post-parturient buffaloes, and after attaining the age of sexual maturity in heifers. Under field conditions, all animals were subjected to repeated rectal examination at 12 days interval for confirmation of anoestrous condition and to confirm absence of any cyclic structure on either of the ovary. In farm animals, additionally transrectal sonography was also done to confirm acyclicity without any ovarian structure before the start of estrus induction protocols in farm condition. All animals included in the field study were reared on stall-feeding in their respective villages, and milked and suckled twice a day. The stall feeding practices were as per the availability of seasonal green fodder and wheat straw, with concentrates as per recommended the production potential of individual animals. The study was conducted in two experiments as per parity of animal and which was further categorized into different groups:

Experiment 1: The study was conducted on 143 anoestrous healthy pluriparous buffalo's belonged (between 2nd and 5th parity) and categorized in different groups according to the days post-partum and location of buffalo as given below:

Group 1 (n= 16): Anoestrous buffaloes of 60 to 90 days post-partum reared under farm conditions during winter.

Group 2 (n= 17): Anoestrous buffaloes of 60 to 90 days post-partum reared under farm conditions during summer.

Group 3 (n= 17): Anoestrous buffaloes of>90 days post-partum reared under farm conditions during winter.

Group 4 (n= 17): Anoestrous buffaloes of >90 days post-partum reared under farm conditions during summer.

Group 5 (n= 10): Anoestrous buffaloes of 60 to 90 days post-partum reared under field conditions during winter.

Group 6 (n= 23): Anoestrous buffaloes of 60 to 90 days post-partum reared under field conditions during summer.

Group 7 (n= 14): Anoestrous buffaloes of >90 days post-partum reared under field conditions during winter.

Group 8 (n= 27): Anoestrous buffaloes of >90 days post-partum reared under field conditions during summer.

Experiment 2: The study was conducted under farm and field conditions involving 52 healthy anoestrous buffalo heifers who had attained age of sexual maturity and had not shown signs of estrus. The buffalo heifers were free from any infectious or anatomical reproductive problem and had normal genitalia. The criteria to confirm anoestrous was the same as followed for buffalo in experiment number 1. Their anoestrous condition was confirmed on the basis of history obtained from farmers and subsequently confirmed through rectal examination at 12 days interval. The study was conducted during summer (n=31) and winter (n=21) months to check the efficacy of estrus induction protocol described above in experiment 1. The buffalo heifers were divided in different group as mentioned below:

Group 9 (n= 11): Anestrous buffalo heifers reared under farm conditions during winter.

Group 10 (n= 10): Anestrous buffalo heifers reared under farm conditions during summer.

Group 11 (n= 10): Anestrous buffalo heifers reared under field conditions during winter.

Group 12 (n= 21): Anestrous buffalo heifers reared under field conditions during summer.

All the buffalo and buffalo heifers were subjected to measure the vaginal temperature and vulvar surface temperature on days 7 and 9 post-insertion of CIDR. The vaginal temperature was recorded by a clinical digital thermometer in degree Fahrenheit (°F), and thereafter the value recorded was converted into degree Celsius (°C). The vulvar surface temperature was recorded with the help of an infrared digital thermometer (Fisher Scientific™ Traceable™ Noncontact Infrared Thermometer) by keeping it 5 to 7 cm away from the desired vulvar surface site.

Results and Discussion

The average vaginal and vulvar surface temperature data for the buffalo and heifers on days 7 and 9 post-insertion of CIDR during winter and summer season are depicted in the table1, Figure 1 and 2, respectively. The measured vaginal temperature was significantly higher ($p<0.05$) on day 9 i.e. corresponding to the day of estrus than day 7 post-insertion of CIDR in both winter and summer season. Further analysis of data revealed that the average vaginal temperature was increased by 0.3 °C in both buffalo and heifer. However, no significant ($p<0.05$) vulvar temperature variations were recorded between days 7 and 9 post-insertion of CIDR in both seasons for buffalo and heifer (Table 1; Fig. 2). In summer, most of the buffalo group showed increased (p<0.05) vulvar temperature than winter season, however, trend of increased (p<0.05) vaginal temperature during former than later season was recorded for only few of buffalo groups.

It was observed that the body temperature of bovine changes during different stages of the estrus cycle (Suther *et al*., 2011; Wang *et al*., 2020) [34, 36] with greatest increase on the day of estrus (Wang *et al*., 2020) [36]. The present findings of higher average vaginal temperature by 0.3 °C on the day of oestrus are in agreement with earlier study in dairy cow (Wang *et al*., 2020) [36] and vulvar surface temperature in buffalo (De Ruediger *et al*., 2018) [6]. Additionally, increased body temperature on the day of estrus was reported by others (Dolecheck *et al*., 2015; Miura *et al*., 2017) [7, 21]. In the present study, the similar pattern of variation of the temperature on the day of estrus was not recorded for vulvar surface temperature. In contrast, study in cow (Osawa *et al*., 2004) [23] and sow (Sykes *et al*., 2012) [22] reported increased vulvar surface temperature in estrus compared to diestrus periods. The discrepancy in the present and earlier studies might be due to species difference. The current results are in agreement with the findings reported by Nabenishi *et al*. (2011) [22] in cattle, and suggested that environmental effects could not be avoided (Kendall and Webster, 2009; Miura *et al*., 2017) [15, 21]. Contrary, Sakatani *et al*. (2012) [29] and Sakatani *et al*. (2016) [30] reported no variation in the vaginal temperature in Japanese Black cows in response to change in weather. Darker body coat and relatively presence of smaller number of sweat glands makes the buffalo more susceptible to weather change and heat stress (Marai *et al*., 2009; Marai and Haeeb, 2010) [19, 18]. Changes in the vulvar surface temperature in response to the weather variation may be explained by heat dissipation to the environment due to vasodilation and increased blood flow to the peripheral tissues of the body (Marai and Haeeb, 2010) [18]. In support of thiscurrent study also indicated that vaginal temperature was not much affected with variation of weather, however, vulvar temperature varied with change of weather to acclimatize the body under heat stress condition. Additionally, within summer season, heifer reared under farm condition exhibited higher vaginal and vulvar temperature than other category of buffalo group. Moreover, the buffalo belonging to 60-90 days post-partum and >90 days post- partum in the farm, and buffaloes of 60-90 days post-partum reared under field condition showed greater vulvar surface temperature than any other category of buffalo group. The exact reason behind the changes in temperature in above mentioned category is not clear, however, greater number of different category animal with smaller number of population size in each group would not be enough to suggest any valid reason. A study is warranted in larger population size to shed the light on this important issue related to variation in vaginal and vulvar surface in buffalo.

Table 1: Vulva and vaginal temperature (°C, Mean±SE) on days 7 and 9 post-insertion of CIDR in summer and winter season of different group of buffaloes and heifers

Group	Season	Vaginal temperature		Vulvar temperature	
		Day 7	Day 9	Day 7	Day 9
1 (n=16)	Winter	38.04±0.05^{a}	38.24±0.07^{a}	32.94±0.56$^{b, i}$	32.49±0.43^{b}
2 (n=17)	Summer	38.24±0.08^{A}	38.42±0.08^{A}	30.48±0.64$^{A, j}$	30.85±0.61^{A}
3 (n=17)	Winter	38.18±0.08^{ab}	38.39±0.06^{a*}	32.43±0.54^{b}	32.46±0.46^{b}
4 (n=17)	Summer	38.04±0.09^{A}	38.35±0.09^{*A}	29.99±0.69^{A}	30.47±0.72^{A}
5 (n=10)	Winter	38.21±0.13^{ab}	38.37±0.14^{a}	29.57±1.84^{ab}	29.81±1.82^{ab}
6 (n=23)	Summer	38.05±0.05^{A}	38.63±0.04^{*A}	29.92±0.19^{A}	30.43±0.22^{A}
7 (n=14)	Winter	38.37±0.06^{b}	38.37±0.09^{a}	30.21±1.48^{ab}	30.50±1.46^{ab}
8 (n=27)	Summer	38.13±0.04^{A}	38.63±0.04^{*A}	30.53±0.29^{A}	30.88±0.28^{A}
9 (n=11)	Winter	37.99±0.05$^{a, c}$	38.27±0.06$^{a*, e}$	28.42±1.08$^{a, k}$	28.58±0.88$^{a, o}$
10 (n=10)	Summer	38.46±0.07$^{d, B}$	38.7±0.03$^{f, A}$	34.55±0.16$^{B, l}$	33.84±0.14$^{B, p}$
11 (n=10)	Winter	38.02±0.07^{a}	38.32±0.09$^{a*, g}$	26.96±1.32$^{a, m}$	27.29±1.29$^{a, q}$
12 (n=21)	Summer	38.10±0.05^{A}	38.65±0.09$^{*, h, A}$	29.71±0.27$^{A, n}$	30.17±0.23$^{A, r}$

A, B different superscript differ significantly within a column in winter season (p<0.05). $^{A, B}$ different superscript differ significantly within a column in summer season (p<0.05). * differ significantly vaginal temperature between day 7 and 9 in a row (p<0.05). ^{c} *vs* $^{d, e}$ *vs* $^{f, g}$ *vs* $^{h, i}$ *vs* $^{j, k}$ *vs* $^{l, m}$ *vs* $^{n, o}$ *vs* $^{p, q}$ *vs* ^{r} differ significantly (p<0.05)

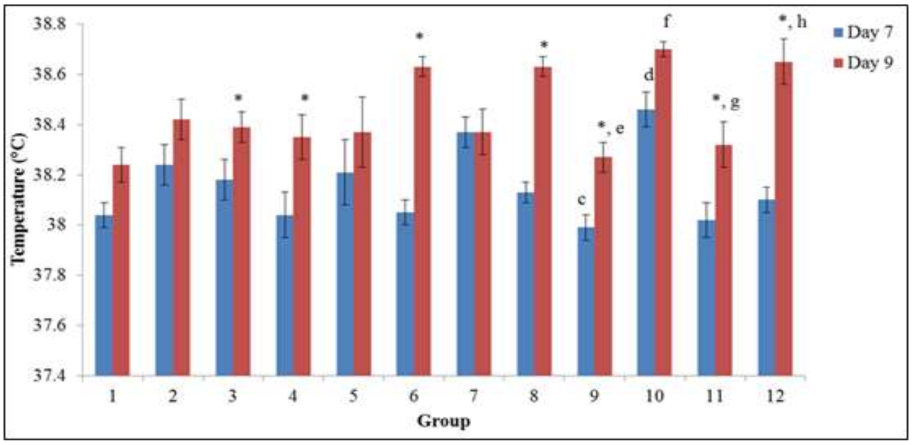

Fig 1: Vaginal temperature (°C, Mean±SE) on days 7 and 9 post-insertion of CIDR during summer and winter season of different group of buffaloes and buffalo heifers. 'differ significantly vaginal temperature between day 7 and 9 within a group (p<0.05). Q vs d' • VS f' g VS h differ significantly (p<0.05)

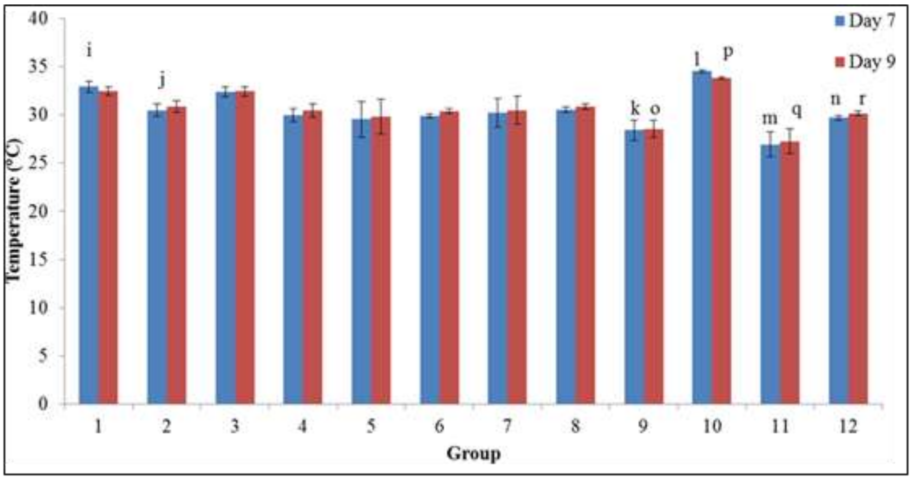

Fig 2: Vulvar surface temperature (°C, Mean±SE) on days 7 and 9 post-insertion of CIDR during summer and winter season of different group of buffaloes and buffalo heifers. i is j, k vs 1, m vs n,VSP,9 VS 1. differ significantly (p<0.05).

Conclusion

The average vaginal temperature on the day of estrus increased in both winter and summer seasons in both buffalo and buffalo heifers.

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