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# Association of estrous behaviour and cervical mucus properties with conception in Gir cows

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**Abstract**
The present study was designed to associate the behavioral signs of estrus and properties of cervical mucus with fertility in Gir cows. Cardinal signs of estrous, cervical mucus properties and gynaecological observations were recorded in forty seven estruses. Estrous was detected by visual observations and rectal palpation, and were inseminated using good quality frozen semen by trained inseminator. Pregnancy diagnosis was performed on 60$^{th}$ day post-insemination. Majority of cows exhibited various cardinal signs of estrous (standing to be mounted, mounting to herdmates, chin resting, sniffing/licking of vulva, tail raising, navel twitching, micturition, ear play and restlessness/ aggressiveness) with moderate intensity (65.91-87.23%). Cervical mucus properties like quantity of mucus discharge was less in majority of estruses (48.94%) and 51.06% estruses had thin consistency of mucus. However, 51.06% estruses had cloudy appearance of mucus and 48.94% had spinnbarkeit value of 8-16cm. About 46.81% estruses had arborisation pattern either typical or atypical. Conception rate increased with increase in intensity of ear play activity ($\chi2$=7.27, df=2, *P*=0.02). Estrous cows with clear appearance of mucus ($\chi2$=3.81, df=1, *P*=0.05), higher spinnbarkeit value ($\chi2$=6.24, df=2, *P*=0.04) and typical arborization pattern ($\chi2$=8.13, df=2, *P*=0.01) had higher conception rate. The results indicated that estrus cows having more ear play activity as well as clear mucus, higher spinnbarkeit value and typical fern pattern has higher conception rate.

**Keywords:** Gir cow, spinnbarkeit value, arborization pattern, conception rate

## Introduction
In a dairy enterprise, reproduction management is considered as a key economic determinant for its success. Estrus detection is one of the crucial activities among the different aspects of reproduction management which contributes significantly towards successful conception (Layek *et al*., 2011). Therefore, proper estrus detection is the key element for efficient reproduction as well as high milk production by utilization of superior germplasm available through Artificial insemination (AI) or natural service (Foote, 1975). Under field condition, estrous in bovines is mostly observed through visual appraisal of its cardinal behavioural signs. However, the cardinal signs exhibited by the dairy bovines show significant individual variation among the crossbred (Madkar *et al*., 2015) as well as indigenous zebu cattle (Layek *et al*., 2011). Thus, to achieve higher conception rate the animal in estrus should be inseminate at proper time of estrus. Additionally, insemination based on properties of cervical mucus particularly fern pattern and spinnbarkeit value improves conception rate (Modi *et al.*, 2011). Cattle and buffalo encounter several reproductive problems during their productive life in which their estrus discharge may be turbid, thick and muco-purulent due to uterine and cervical infection (Kumar *et al.*, 2017). Some studies on association of estrus signs and properties of cervical mucus with conception rate have been reported particularly in crossbred and exotic cattle (Madkar *et al.*, 2015; Mellado *et al.*, 2015*;* Bernardi *et al.*, 2016). However, such informations are lacking in indigenous cow like Gir. The present study was planned to provide baseline information of zebu cattle with the objective to associate cardinal signs of estrus and cervical mucus properties with conception rate in Gir cows.

## Materials and Methods
### Experimental animals
The study was conducted on healthy Gir cows maintained at Cattle Breeding Farm, Junagadh Agricultural University, Junagadh. The experimental animals were more than 60 days postpartum and kept under loose housing system. The cows were free from any anatomical defect or reproductive problems. Forty seven estruses (30 first and 17 second estruses) were included in this study and all the cardinal signs of estrous, cervical mucus properties and

gynaecological observations were recorded. Estrous detection was carried out twice daily using visual observation of cardinal signs of estrus along with heat expectancy chart. Cervical mucus samples were collected on the day of estrus prior to the artificial insemination using sterile blue sheath fitted in a "Universal artificial insemination gun". The mucus was aspirated from the mid cervix by recto-vaginal method and collected in a sterile tube (15 ml) to study the physical characteristics (quantity, consistency, appearance, spinnbarkeit value and arborisation pattern). Estrus was confirmed by per-rectal examination and animals with true estrus were inseminated by recto-vaginal method. All the cows were examined for pregnancy on 60$^{th}$ day post insemination.

**Evaluation of cervical mucus properties**

**Consistency:** A few drops of mucus sample were put on a glass slide held in sloppy direction and the speed of drop on the slide was noted. On the basis of consistency, mucus was classified as thick, moderate or thin (Samad *et al.,* 2002).

**Appearance:** Direct visual observation method was used to judge appearance of cervical mucus. On that basis, mucus samples were categorised into dirty, cloudy and clear.

**Spinnbarkeit Value:** Spinnbarkeit value was measured by putting 2-3 drops of collected mucus sample on a grease free glass slide. Then another grease free glass slide was placed over it and gradually moved away from the first slide to stretch the mucous between the slides. Then the maximum length distance between the two slides just before the breakage of the mucus string was considered as Spinnbarkeit value and measured through a scale (cm scale) mounted on the side wall (Verma *et al.,* 2014).

**Arborization pattern:** Cervical mucus of about 2-3 drops was spread uniformly over a clean, grease free glass slide. The slide was air dried and was examined under microscope using low power objective (10X) for arborisation pattern (Verma *et al.,* 2014).

**Scoring of cardinal signs of estrous**

Different cardinal signs of estrous observed in Gir cattle were assigned with a score depending upon their frequency or intensity of expression and animals were categorized into three groups based on median ± SD of frequencies and assigned scores of 1-3 (Table- I).

**Table I:** Scales for scoring cardinal signs of estrous

| S. No | Cardinal signs | Frequency of expressions | | |
|---|---|---|---|---|
| | | Weak (Score-1) | Moderate (Score-2) | Intense (Score-3) |
| 1. | Standing to be mounted | <4 | 4-6 | >6 |
| 2. | Mounting to herdmates | <4 | 4-6 | >6 |
| 3. | Chin resting | <4 | 4-6 | >6 |
| 4. | Sniffing/licking of vulva | <4 | 4-6 | >6 |
| 5. | Tail raising | <3 | 3-5 | >5 |
| 6. | Navel twitching | <5 | 5-7 | >7 |
| 7. | Micturition | <3 | 3-5 | >5 |
| 8. | Ear play | <4 | 4-6 | >6 |
| 9. | Restlessness/ Aggressiveness | <4 | 4-6 | >6 |
| 10. | Quantity of mucus | Scanty | Moderate | Copious |
| 11. | Consistency of mucus | Thick | Moderate | Thin |
| 12. | Appearance of mucus | Dirty | Cloudy | Clear |
| 13. | Spinnbarkeit value (cm) | 0-8 | 8-16 | >16 |
| 14. | Arborization pattern | Nil | Atypical | Typical |
| 15. | Tumefection of vulva | Weak | Moderate | Intense |
| 16. | Reddening of vulvar membrane | Pale | Moderate | Intense |
| 17. | Uterine tonicity | Mild | Moderate | Intense |

**Statistical Analysis**

Cows were grouped according to degree of expression of different parameters and expressed as percent for better interpretation. Conception rate among groups (weak, moderate and intense) were compared by chi-square test ($\chi 2$) and the difference was considered as significant if $P<0.05$; whereas, it was considered as trend if "P" value remained between 0.05 and 0.1.

**Results and Discussions**

**External signs of estrous**

In the present study, out of 47 estruses, in majority of cases the intensity of cardinal signs of estrus was observed to be moderate. The external signs like standing to be mounted, mounting to herdmates, chin resting, sniffing/licking of vulva, tail raising, navel twitching, micturition, ear play and restlessness/ aggressiveness behaviour were observed to be moderate intensity in 76.60, 74.47, 65.96, 70.21, 87.23, 70.21, 85.11, 78.72 and 76.60% estruses, respectively.

**Table II:** Cardinal signs of estrus in Gir cows

| Cardinal Signs | Weak (%) | Medium (%) | Intense (%) |
|---|---|---|---|
| Standing to be mounted | 12.77 | 76.60 | 10.64 |
| Mounting to herdmates | 12.77 | 74.47 | 12.77 |
| Chin resting | 17.02 | 65.96 | 17.02 |
| Sniffing/licking of vulva | 17.02 | 70.21 | 12.77 |
| Tail raising | 2.13 | 87.23 | 10.64 |
| Navel twitching | 25.53 | 70.21 | 4.26 |
| Micturition | 8.51 | 85.11 | 6.38 |
| Ear play | 19.15 | 78.72 | 2.13 |
| Restlessness/ Aggressiveness | 6.38 | 76.60 | 17.02 |

In this study, most of the estruses exhibited moderately intense behavioural signs of heat which is comparable to Sahiwal (Layek *et al*., 2011) and crossbred cows (Madkar *et al.*, 2015). Layek *et al.* (2011) reported same trend (moderately intense) of behavioural expression, particularly standing to be mounted (40.35%), chin resting (42.31%), tail raising (62.50%) and bellowing (45.45%) behaviour. On the other hand, behavioural signs such as mounting to herdmates, sniffing/licking of vulva, micturition, restlessness and aggression (56.37, 47.17, 90, 50 and 51.72%, respectively) were found to be less intense. Madkar *et al.* (2015) highlighted expression of various behavioural signs like standing to be mounted and mounting with higher intensity (43.75 and 50.00%, respectively), chin resting,

sniffing/licking of vulva, bellowing, frequent micturition, restlessness and aggression with less intensity (41.67, 38.47, 40.00, 50.00, 41.67 and 50.00%, respectively), while tail raising behaviour with moderately intensity (50.00%) in Crossbred cows.

Standing to be mounted and mounting to other herdmates behaviour were expressed for short period of time in majority of Gir cows. Further, in dairy cows the mounting behaviour markedly influenced by number of cows in the sexually active group as well as their social hierarchy (Hurnik *et al.*, 1975). Additionally, the endocrine profile during the periestrual period of an individual animal has also been reported to affect the estrus behaviour expression (Roelofs *et al.*, 2005). Although, sniffing and licking of vulva (70.21%) and chin resting (65.96%) behaviour was displayed by most of the cows during estrus but these behaviours were not exclusive to the estrus period (van Eerdenburg *et al.*, 2002; Roelofs *et al.*, 2005). The differences in expression of cardinal signs of estrus in Gir cows as compared to other breeds might be associated with factors such as the genotype, environment, nutritional status, season and management.

**Cervical mucus properties**

**Table III:** Cervical mucus characteristics in Gir cows

| Cardinal Signs | Weak (%) | Medium (%) | Intense (%) |
|---|---|---|---|
| Quantity of mucus | 48.94 | 34.04 | 17.02 |
| Consistency of mucus | 6.38 | 42.55 | 51.06 |
| Appearance of mucus | 0.00 | 51.06 | 48.94 |
| Spinnbarkeit value | 6.38 | 48.94 | 44.68 |
| Arborization pattern | 6.38 | 46.81 | 46.81 |

Properties of cervical mucus during estrus in Gir cows are presented in Table III. Quantity of mucus discharged was less in majority of estruses (48.94%). Moreover, out of 47 estruses, 51.06% estruses had thin consistency of mucus. But, 51.06% had a cloudy appearance of mucus and 48.94% had spinnbarkeit value of 8-16cm. Typical as well as atypical arborisation pattern of cervical mucus was observed to be 46.81% in both groups (medium and intense groups).

In dairy animas, it has been reported that visual appearance of cervical mucus is a good indicator of reproductive health status (Tsiligianni *et al.*, 2001). In Gir cows, majority of estruses had thin consistency (51.06%) of mucus. Similar findings of mucus consistency have been reported by various researchers (Modi *et al.*, 2011, Sharma *et al.*, 2011). Contrary to Gir cows, non-significant difference in consistency of cervical mucus in normal and repeat breeder crossbred cows was observed by Rangnekar *et al.* (2002). About 46.81% estruses had typical fern pattern which might be due to increased peripheral estrogen concentration at the time of estrus indicating right time of insemination. The Spinnbarkeit value is reduces under the influence of progesterone and increases with high level of estrogen and reaches at peak during ovulatory phase. In Kankrej cows, the average spinnbarkeit value of cervical mucus was observed to be 12-19cm (Modi *et al.*, 2011). In a similar line, in Gir cows, majority of estruses had spinnbarkeit value of 8-16 cm.

**Gynaecological observations**

Gynaecological observations of Gir cows during estrus are presented in Table-IV. In Gir cows, majority of estruses exhibited moderate intensity for tumefaction of vulva (55.32%), reddening of mucus membrane (53.19%) and uterine tonisity (57.45%). Although tumefaction, reddening of vulva and uterine tonisity was moderate intense in majority of estruses in Gir cows, Madkar *et al.* (2015) in Crossbred cows and Layek *et al.* (2011) in Sahiwal cows reported more intense signs of estrous. During the time of estrous, estrogen hormone is found to be at peak which enhances blood supply towards the genital organs in estrous female which causes the swelling of vulva and reddening of vulval mucus membrane.

**Table IV:** Cardinal signs of estrous in experimental animals based on gynecological examinations

| Cardinal Signs | Weak (%) | Medium (%) | Intense (%) |
|---|---|---|---|
| **First & Second estrous** | | | |
| Tumefection of vulva | 4.26 | 55.32 | 40.43 |
| Reddening of vulvar membrane | 0.00 | 53.19 | 46.81 |
| Uterine tonicity | 6.38 | 57.45 | 36.17 |

**Association of cardinal signs of estrous with conception rates**

Conception rate of Gir cows classified under various classes of external signs of estrous are presented in Table V. Among different cardinal signs of estrus, the conception rate increased with increase in intensity of ear play activity ($P$ = 0.026). Moreover, there was increasing trend of conception rate in cows with increase in intensity of navel twitching ($P$ = 0.091).

**Table V:** Conception rate in various classes of external signs of estrous

| Cardinal Signs | Weak | Medium | Intense | Chi-sq., df, 'P' |
|---|---|---|---|---|
| Standing to be mounted | 16.67(1/6) | 33.34(12/36) | 60.00(3/5) | 2.315, 2, 0.314 |
| Mounting to herdmates | 0(0/6) | 37.14(13/35) | 50.00(3/6) | 3.927, 2, 0.140 |
| Chin resting | 12.5(1/8) | 32.26(10/31) | 62.5(5/8) | 4.583, 2, 0.101 |
| Sniffing/licking of vulva | 12.5(1/8) | 33.34(11/33) | 66.67(4/6) | 4.505, 2, 0.105 |
| Tail raising | 0(0/1) | 31.71(13/41) | 60.00(3/5) | 2.116, 2, 0.347 |
| Navel twitching | 8.33(1/12) | 42.42(14/33) | 50.00(1/2) | 4.792, 2, 0.091 |
| Micturition | 0(0/4) | 37.50(15/40) | 33.33(1/3) | 2.278, 2, 0.320 |
| Ear play | 0(0/9) | 40.54(15/37) | 100.00(1/1) | 7.278, 2, 0.026 |
| Restlessness/ Aggressiveness | 0(0/3) | 30.56(11/36) | 62.50(5/8) | 4.629, 2, 0.099 |

**Association of cervical mucus characteristics with conception rates**

Conception rate was observed to be significantly higher in Gir cows those showing higher intensity of appearance of mucus, spinnbarkeit value and arborization pattern (Table VI). Our results are in line with Mellado *et al.* (2015), who stated that acceptable conception rates in high milk yielding Holstein cows can only be obtained with cows showing clear and trans-

lucid mucus at artificial insemination. About 52.38% conception rate was observed in cows with high Spinnbarkeit value (>16cm) might be attributed to proper stage of estrus. Generally, maximum spinnbarkeit value was observed immediately before or during the ovulatory phase owing to the influence of estrogen, but later decreases with increase in level of progesterone during luteal phase in cows. Our findings are supported by Modi *et al.* (2011) and Dodamani and Honnappagol (2004) who observed that typical fern pattern and high spinnbarkeit value favoured better fertility in Kankrej and Crossbred cows, respectively. Contrary to the present study, Crane *et al.* (1960) did not observe any difference in the spinnbarkeit value of mucus between fertile and infertile cows. In normal breeding Kankrej cows, Modi *et al.* (2011) observed that cows with thin and clear discharge and spinnbarkeit value >15cm had typical fern pattern of cervical mucus as compared to repeat breeders. Typical arborisation pattern may enhance conception rate by facilitating movement of spermatozoa as compared to nil or atypical arborisation pattern (Alena *et al.*, 2008). In consonance to present result, other researchers reported higher pregnancy with typical arborisation pattern in dairy cattle (Selvaraj *et al.*, 2002; Shrivastava and Sahni, 2000; Bernardi *et al.* 2016). Higher pregnancy with typical fern pattern might be due to ovulation at proper time as typical fern pattern is an indicative of ovulatory heat (Gandotra *et al.*, 1990).

**Table VI:** Conception rate in various properties of cervical mucus

| Cardinal Signs | Weak | Medium | Intense | Chi-sq., df, 'P' |
|---|---|---|---|---|
| **First & Second estrous** | | | | |
| Quantity of mucus | 26.08(6/23) | 43.75(7/16) | 37.5(3/8) | 1.362, 2, 0.506 |
| Consistency of mucus | 33.33(1/3) | 25.00(5/20) | 41.67(10/24) | 1.350, 2, 0.509 |
| Appearance of mucus | 0(0/0) | 20.83(5/24) | 47.82(11/23) | 3.811, 1, 0.050 |
| Spinnbarkeit value | 0(0/3) | 21.74(5/23) | 52.38(11/21) | 6.244, 2, 0.044 |
| Arborization pattern | 0(0/3) | 18.18(4/22) | 54.54(12/22) | 8.132, 2, 0.017 |

**Association of gynaecological examinations with conception rates**

Higher conception rate was observed in intense condition of tumefection of vulva, red mucous membrane and tonisity of uterus, but there was no significant difference among different categories of cardinal signs observed gynecologically in Gir cows (Table VII).

**Table VII:** Conception rate based on gynecological examinations

| Cardinal Signs | Weak | Medium | Intense | Chi-sq., df, 'P' |
|---|---|---|---|---|
| **First & Second estrous** | | | | |
| Tumefection of Vulva | 0(0/2) | 26.92(7/26) | 47.36(9/19) | 3.12, 2, 0.210 |
| Reddening mucus membrane | 0(0/0) | 24(6/25) | 45.45(10/22) | 2.399, 1, 0.121 |
| Uterine Tonicity | 33.33(1/3) | 29.63(8/27) | 41.17(7/17) | 0.620, 2, 0.733 |

**Summary**

In Gir cows, majority of estrus exhibited cardinal signs of estrous with moderate intensity. Conception rate increases with increase in ear play activity. Estrus cows with clear mucus, higher spinnbarkeit value and typical fern pattern has higher conception rate.

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