

## Original Research Article

# Behavioral and Physical Signs of Estrous Manifested in Hormonally Estrus-Induced Boran and Holstein Friesian Dairy Cattle

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Received: 12.02.2024

Accepted: 19.03.2024

Published: 22.03.2024

**Journal homepage:**<https://www.easpublisher.com>**Quick Response Code**

**Abstract:** One of the major contributors to poor fertility and fail to attain a one-year calving calendar of a dairy herd is inability to know all behavioral oestrus sign and ineffective detection of estrus. As behavioral oestrus sign and physical change is different between breed and even individual variability is there, knowledge of primary and secondary oestrus sign is crucial. Recently, it has become evident that cow factors contribute largely to low detection rates. Until now, standing behavior, a primary oestrus sign has been the only considered symptom and used to determine the right moment for insemination. However, standing behavior is not observed in more than 50% of the cows in estrus. Therefore, the aim of the study was to map out the different behavioural and physical signs of estrus manifested in Boran and Holstein Friesian dairy cow after hormonally oestrus induced. A total of 40 (20 Boran and 20 Holstein Friesian breed) 31 multiparous and 9 primiparous which are apparently healthy cattle were used to study behavioral and physical signs of estrus, diurnal variations in the occurrence of estrus and the effect of estrus intensity on conception rate (CR). Seven (78%) of nine primiparous cows showed standing oestrus in addition to secondary oestrus sign. The other two primiparous cows showed secondary oestrous signs only. Twenty-seven (87%) of thirty-one multiparous cows showed standing oestrous in addition to secondary oestrus sign and the other four cows showed secondary oestrous signs only. Standing to be mounted, the primary oestrus sign was more pronounced in Holstein Friesian 90% (18/20) compared to Boran breed 65% (13/20). Parity wise, standing to be mounted was equally manifested in primiparous and multiparous animals regardless of breed. Regardless of parity, standing to be mounted (65, 90%) Vaginal mucus discharge (55,90%), Bellowing (20,25%), head and side mounting (15,25%), grouping and circling (40,70%), sniffing and liking (60,95%), chain resting (25,15%), swelling and reediness of vulva (50,55%), relaxation of Os-cervix (75,90%) were recorded in Boran and Holstein Friesian respectively. On the other hands Regardless of breed, Vaginal mucus discharge (56,87%), Bellowing (33,16%), head and side mounting (33,22%), grouping and circling (55,39%), sniffing and liking (67,65%), chain resting (78,71), swelling and reediness of vulva (78,65%), Relaxation of Os-cervix (67,90%) were recorded in primiparous and multiparous category of females. No drastic variation was observed on conception rate in primiparous and multiparous but, breed wise, greater conception was noted in Holstein Friesian compared to Boran breed after inseminated with inseminated with sexed semen. There is no difference in conception rate for an animal inseminated with detection of primary oestrous sign with secondary oestrous sign and secondary oestrous sign alone.

**Keywords:** Behavioural and Physical Estrus Signs, Boran, Conception Rate Estrus Intensity, Holstein Friesian.

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## 1. INTRODUCTION

The fertility of dairy cows is of growing concern. Normally, in dairy and beef cattle, there is a well-defined breeding and calving schedule. Accordingly, for an optimal dairy cow breeding and calving schedule, the womb of a cow is open and gives milk for two to three months. After two or three months of post-partum, the cow should get breeding and conceive in addition to giving milk for a consecutive seven month and become dry for the last two month and this cycle is called a one-year calving interval (Figure 1).

Reproductive efficiency is one of the most important factors determining the profitability of the herd of dairy cows. Calving interval (CI) is the deciding parameter used for the assessment of the reproductive efficiency. One-year calving interval is considered as economically advantageous as well as physiologically acceptable (Schmidt, 1989). The failure in dairy cattle to perform one-year calving interval (CI) is a sign of fertility problems that can generate an important economic loss. The CI in dairy cattle is influenced by a time lag between calving and the resumption of ovarian activity in the postpartum (Opsomer *et al.*, 2000).

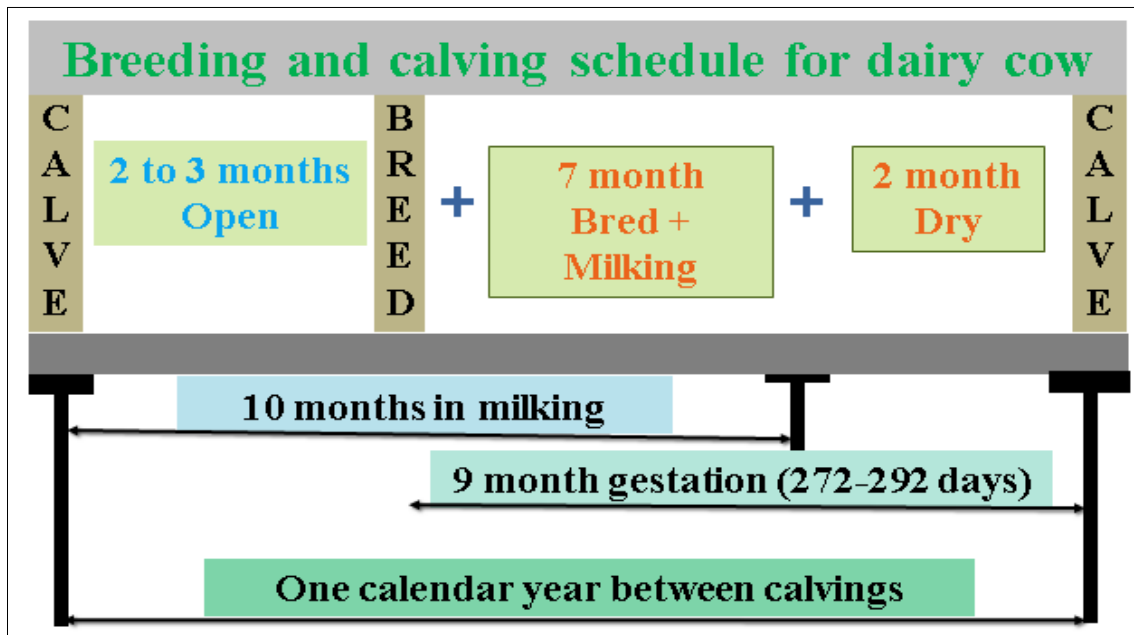


Figure 1: Breeding and calving schedule for dairy cow (Source: personal sketch).

Reproductive physiology is concerned with the mechanisms that animals used to control reproduction. Hence, the knowledge of reproductive physiology, especially the differences between *Bos taurus* and *Bos indicus*, is important for the development and application of different techniques of reproductive management in cattle.

The cow's reproductive cycle consists of a series of events that occurs in a definite order over a period of days. The estrus cycle in the cow averages 21 days (range is 18-24). During this time, the reproductive tract is prepared for estrus or heat (the period of sexual receptivity) and ovulation (egg release) for fertilization. A number of reports indicate that, despite increased knowledge and professional attention; reproductive indices that are used to assess reproductive management show a negative trend during the past decade (Butler, 2000; Opsomer, 2002; Lopez-Gatius, 2003).

The success of application of assisted reproductive technologies like artificial insemination (AI) is greatly dependent on efficient and successful heat detection that reduces extended periods of calving. To

maintain an ideal 12-13 months calving interval, cows must be pregnant by 100 days after calving. As a guideline, 90% of the cows should have been observed in heat by 40-50 days after calving. In certain dairy farm; if the calving interval is 390 days instead of 365 days in a 100-cow herd, this added cost will approximately be \$7500/ year (Jeffrey and Paul, 2007).

Standing to be mounted by herd mates is considered as the primary oestrous sign in cattle. The cattle also show some secondary oestrous signs including mounting other cows, like restlessness, swelling, relaxation and congestion of the vulva, clear mucus discharge, bleeding after oestrus and reduction of appetite and milk yield (Diskin and Sreenan, 2000). Generally, standing oestrus is considered as the true oestrous sign. However, not all the cows in heat show standing activity especially for those kept in tie-stalls (Kerbrat and Disenhaus, 2004). In such animals, oestrus can be detected based upon secondary oestrous signs including friendly approach sign. It is, therefore, very important to fully understand the type, duration and feature of secondary oestrous signs for different type of breed. Kerbrat and Disenhaus (2004) also suggested to

update knowledge on the behavioural aspects of oestrus in the modern dairy cows to define oestrus. It is considered that characteristics of oestrous signs in dairy cattle vary among different breed and regions because of climate, nutrition and the nature of management.

As inadequate estrus detection leads to missed breeding and low conception rate (CR), improved estrus detection practices allow for heifers and cows to be bred artificially with genetically superior sire and also to achieve optimum calving interval. However, no estrus detection aid can ever completely substitute for the keen, conscientious observer. Therefore, close visual observation is very much required in efficient estrus detection (Jeffrey and Paul, 2007). Although there are subjective reports of improving CR of dairy cows, yet the actual outcome of such research has not been undertaken for local and crossbred cows or heifers under field conditions particularly in Ethiopia. Thus, the present study was undertaken to unravel different behavioural and physical signs of estrus manifested in hormonally estrus induced Boran and Holstein Frisian dairy cow.

## 2. MATERIALS AND METHODS

### 2.1. Experimental Anima Management Land Synchronization Procedure

Data were collected from a research herd of Debre Zeit Agricultural Research Center dairy farm. A

total of 40 (20 Boran and 20 Holstein Frisian breed) apparently healthy cattle were used to generate the data. Transrectal palpation for the presence of well-developed and functional CL in either ovary was examined and were injected with 2 ml CICALAR hormone (Synthetic prostaglandin luteolytic agent) intramuscularly. Red/Orange fluorescent colors coated ESTROTECT were applied on halfway between the hip and tail head perpendicular to the spine after brushing the hair thoroughly to create optimal condition for adhesion. Hence with each mount, the surface will gradually turn from silver to its indicator color indicating a true standing heat. As per the manufacturers recommendation approximately 50% of the silver rub off coat removed should indicate standing heat.

After synchronization, responded animals were inseminated with sexed semen following the standard procedure. Pregnancy was checked by following the animal for returning to oestrus 18-24 days post insemination and for non- returning animals transrectal palpation pregnancy diagnosed technique were applied 40 days later for confirmation. Their parity ranged between one and six. The average milk yield per cow per lactation (for Holstein–Friesian cows) was about 3300 kg. They were milked twice a day at 5:30 AM and 4:00 PM hours manually. The free stall floor was concrete.

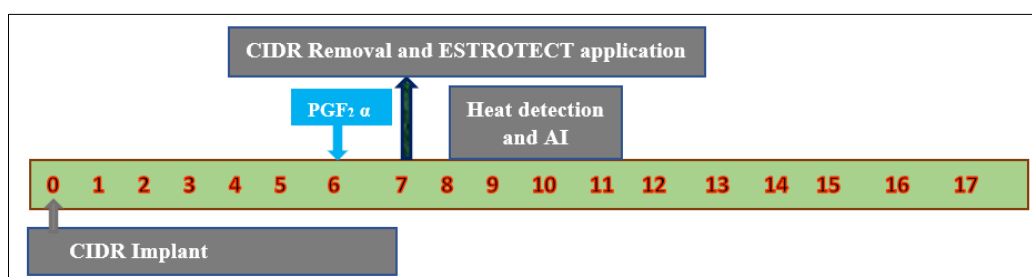


Figure 2: CIDR plus prostaglandin based estrous synchronization protocol.

### 2.2. Oestrus Detection and Insemination Practices

#### • Visual Observation of Oestrous Signs in Free Stall

One researcher, was assigned to oestrous observation during day time in addition to assistants working on animal managements. Experimental animals were visually observed for approximately 30-40 minutes for oestrous sign by the experienced researcher and research assistant staff at three fixed times per day (08:00 morning, 11:00 mid-day and 16:00 afternoon) when the animals get free from tie-stall for movement here and there.

As suggested by Cowen *et al.*, (1989) and previously studied by Stevenson *et al.*, (1996), Apart from standing to be mounted, the rest oestrous signs were considered as secondary oestrous signs and were classified into two sexual activity and external genital change which were also monitored and recorded. A

scratch-off heat mount detector, ESTROTECT was used for the animal showing oestrous during the night especially for mounting. Secondary oestrous signs were classified into two sexual activity and external genital change which were also monitored and recorded. The sexual activity of oestrous signs included secondary oestrous signs such as attempt to mount the other cows, chasing herd mates, restlessness, chin-resting, sniffing of the vagina of herd mates and bellowing. The external genital change type of secondary oestrous signs included typical changes in external genitalia such as Hyperaemia, congestion, relaxation and swelling of the vulva, and mucus discharge from the vulva where recorded and graded qualitatively (Table 1). In addition to the above, for suspected animal for silent heat, per-rectal palpation technique was carried out to confirm the presence or absence of tonicity of uterus or uterine horn, relaxation of os-cervix and palpable follicle.

**Table 1: Qualitative grading of personal observation of differing behaviour oestrous symptoms in Boran and Holstein Friesian**

No	Oestrous symptoms	Breed wise qualitative grading	
		Boran	Holstein Friesian
1	Standing to be mounted	i) Yes ii) No	Yes 2) No
2	Mounting	i) No ii) Few iii) Frequent	No 2) Few 3) Frequent
3	Lowering of the back	i. Yes ii) No	Yes 2) No
4	Head mounting	i) Yes ii) No	Yes 2) No
5	Grouping and circling	i) Yes ii) No	Yes 2) No
6	Chain resting	i) Yes ii) No	Yes 2) No
7	Sniffing and licking	i) Yes ii) No	Yes 2) No
8	Vaginal mucus discharge	i) Yes ii) No	Yes 2) No
9	Swelling of vulva	i) Yes ii) No	Yes 2) No
10	Reddening of vulva	i) Yes ii) No	Yes 2) No
11	Bloody discharge	i) Yes ii) No	Yes 2) No
12	Anxiety	Normal ii) Weak iii) strong	Normal 2) Weak 3) strong

At each oestrus observation, one or more oestrous symptoms were recorded and scored according to a scoring system modified from Van Eerdenburg *et al.*, (1996) and used by the Swedish Dairy Association (Table 2). Data regarding progesterone and estradiol

levels were not available for the herd staff at the time of oestrus detection. The decision to proceed with artificial insemination (AI) was made by the herd staff and AI technician.

**Table 2: Oestrus intensity scoring system as defined by the Swedish Dairy Association**

S/N	Score	Definition
1	Very weak	Very weak uncertain symptoms (e.g., symptoms of dried vulvar discharge)
2	Weak	Weak uncertain symptoms (e.g., discharge, a red and swollen vulva and anxiety)
3	Normal	More evident symptoms (e.g., lowering of the back when touched, clear and stringy discharge, mounting other cows and occasionally standing to be mounted)
4	Strong	Spontaneous lowering of the back, plentiful stringy discharge, several recorded mountings and standing to be mounted
5	Very strong	Very strong sexual activity, spontaneous lowering of the back, very frequent mountings and standing to be mounted

### 3. RESULT AND DISCUSSION

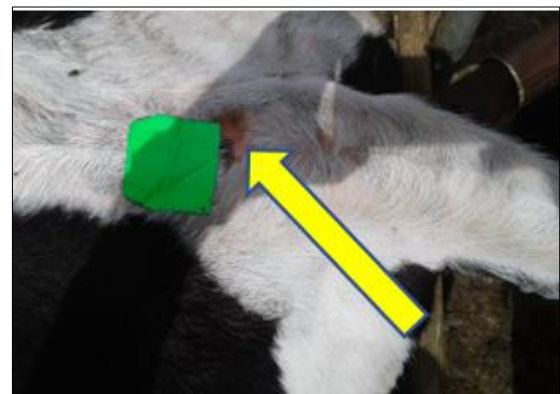
A total number of cows which were observed in oestrus during the experimental period was forty, thirty-one multiparous cows and nine primiparous. Seven (78%) of nine primiparous cows showed standing oestrus. The other two primiparous cows showed secondary oestrous signs only. Twenty-seven (87%) of thirty-one multiparous cows showed standing oestrus and the other four cows showed secondary oestrous signs only.

#### 3.1. Primary Oestrus Sign in Boran and Holstein Friesian

- Oestrus Intensity and Duration of Standing Oestrus in Boran and Holstein Friesian**

Duration of standing oestrus in Holstein Friesian was longer than Boran breed of cattle. In Holstein Friesian cows the duration was on average 7.3 ± 6.1 h (±SD), ranging between 4 and 28 hours. Even though most of the primiparous animals are Boran, again, when duration of oestrus was evaluated in terms of parity, there was a tendency that the duration of oestrus was longer in multiparous than primiparous cows. In Holstein Friesian cows, surprisingly it is beyond allowing to be mounted by others, since, due to excessive

mounting rubbed off on the tail head, which is normal for standing oestrus but until sloughing of the skin on the cow's tail head (figure 1). Furthermore, the mounting animals (if on heat), stay on the back of the mounted animals for one to two minute.



**Figure 3: Sloughing of the skin on the cow's tail head due to excessive mounting Holstein Friesian**

Unlike in Holstein Friesian, in case of Boran breed, there was a tendency that the duration of oestrus sign in general was shorter ranging between 4 and 10













hours and on average the cows' elapses on standing oestrus ranging between 2 to 4 hours. Duration of standing to be mounted in Boran breed is shorter, one to two minutes in average. Again, it is not frequent like that of Holstein Friesian. A video record for five minutes on

behavioral oestrus manifestation in Holstein Friesian cow

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**Table 3: Pictorial representation and description of behavioral estrus symptoms manifested in Boran and Holstein Friesian**

No	Behavioral sign	Description	Breed	
			Boran	Holstein Friesian
1	Standing to be mounted	The primary sign of estrus is a cow standing to be mounted by another cow(s). Standing heat is the most sexually intensive period of the estrous cycle and is applicable only in free stall or pasture.		
2	Mucus discharge	Long viscous, sticky, clear elastic strands of mucus generally hang from the vulva. Sometimes, however, the mucus does not appear externally until the cow is palpated during insemination and the mucus is expelled. Mucus also may be smeared on the tail, thighs, flanks, or perineal region and is just serve as a good secondary sign estrus.		
3	Rubbed off mark	When cows mount each other excessively throughout the day they can leave small rubbing marks on each other's hind quarters. Usually just in front of the pin bones or below the hook bones. The legs and flanks may be smeared with mud or manure. Size, appearance, and freshness of these marks along with the fact that few other events can cause similar signs; these rubbed marks are one of the most reliable secondary indicators of estrus.		
4	Swelling & reddening of the vulva	During heat, the vulva swells and becomes moist and red on the interior. However, these symptoms appear before heat and remain for a short period after, so alone, they are not a precise indicator of estrus.		
5	Head mounting	During heat period, in addition to back or side mounting in some animals there is mounting via head. This behavioral sign noticed in both Boran and Holstein Friesian.		

No	Behavioral sign	Description	Breed	
			Boran	Holstein Friesian
6	Standing besides, sniffing and licking each other	Considerable Sniffing and licking the genitalia or neck of other cows occurs much more frequently with cows before and during estrus. This behavioral sign noticed in both Boran and Holstein Friesian.		
7	Bellowing, Restlessness and trailing	Cows in heat are more restless and alert to their surroundings. When allowed to interact with other cattle, cows coming into heat "proestrus" and cows in heat persistently trail behind to try to mount other cows. Research findings shows that cows in heat spend less time resting than non-estrous herd mates. Prior to and during heat, they remain standing and alert while their herd mates are lying down and resting. Although these are not definitive signs of heat, Cattle may bellow more frequently during estrus. During the study, this behavioral sign is less common in Boran and very well-expressed behavioral sign in Holstein Friesian.		
8	Grouping and Circling	Cows in heat tend to look for willing partners to get involved in estrus-related activities. These sexually active groups are a clear indication that at least one cow inside one of these groups is in estrus. Applies to free stall and pasture. In case greater number of animals are on heat, one follows the other and move in circling manner. During this study, this behavioral sign is more noticed in Holstein Friesian than Boran breed.		
9	Chin resting,	Chin resting is thought to be testing by herd mates to determine if an individual is receptive to being mounted. This testing is performed by first resting a chin on the back of the cow.		
10	Bloody Discharge	Few hours post standing heat; a streak of blood in the mucus usually means that that cow had a high peak of estrogen one to three days ago, therefore indicating that the heat was missed. It is therefore recommended to record that heat and date it two days ago. This only indicates that she has been in heat. It has no relationship with timing of ovulation or whether she conceived. This type of behavioral sign is less observed in Boran and very well-noticed in Holstein Friesian.		

- Secondary oestrus sign in Boran and Holstein Friesian

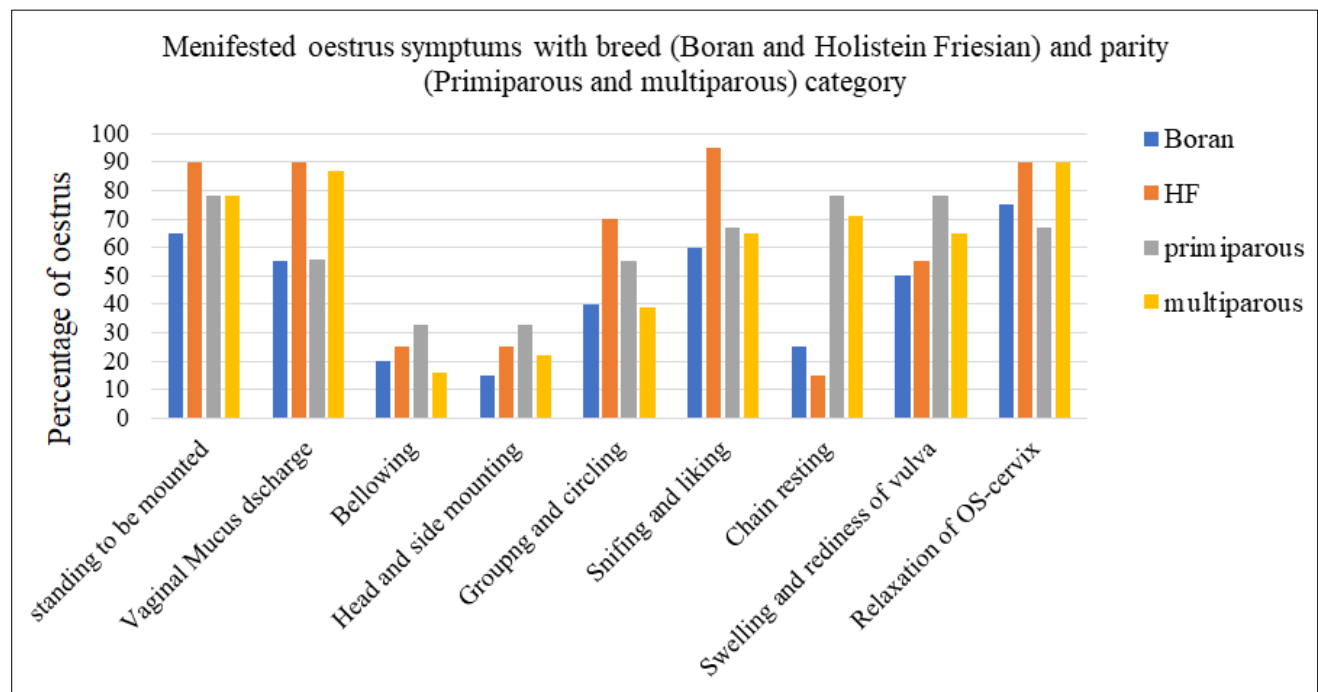


Figure 4: Manifested oestrous symptoms based on visual observations out of total oestruses per breed (Boran and Holstein Friesian) and parity (primiparous and multiparous) category.

#### 4. DISCUSSION AND CONCLUSION

This study shows that the traditional way of detecting cows in oestrus (i.e., observing cows for

standing oestrus only), was not satisfactory and economical. The finding of (Cowen *et al.*, 1989), and others, that the use of a combination of oestrus signs

detection had a positive association with reproductive efficiency, is confirmed here. In both breed of Boran and Holstein Friesian, even though the cow/heifers are on heat, there is a probability of missed or fail to observe standing oestrus.

With few expressions of standing and mounting behaviour together with few oestrus observations per day, behaviour changes can easily be overlooked, and more continuous observation is therefore needed. This has an implication that local oestrus symptoms, that are expressed more frequently and for longer periods, are therefore of high importance for detecting an animal on heat. Hence, our findings imply that local symptoms should not be neglected in oestrus detection, especially when standing and mounting behaviour are decreasing and only found in about 50% of the cows in oestrus (Roelofs *et al.*, 2005; Dobson *et al.*, 2008).

The finding of this study showed that, regardless of breed, of the total 40 cows in oestrus, from a total thirty-one multiparous cow, twenty-seven (87%) of them showed standing oestrus, while from a total of nine primiparous female, nine (78%) showed only secondary oestrous signs without standing oestrus. Rather in USA (Stevenson *et al.*, 1998) and the Netherlands (Van Vliet and Van Eerdenburg 1996), were reported lower percentages of cows 50 and 37%, respectively exhibiting standing oestrus. This indicates that oestrous detection in dairy cows has been becoming more difficult. Standing to be mounted, the primary oestrus sign was more pronounced in Holstein Friesian 90% (18/20) compared to Boran breed 65% (13/20). Parity wise, standing to be mounted was equally

manifested in primiparous and multiparous animals regardless of breed Figure 4.

Based on visual observations at 3-h intervals for three times per day (08:00 morning, 11:00 mid-day and 16:00 afternoon) showed that the average duration of secondary oestrous signs before standing was  $7.6 \pm 8.1$  h and  $15.4 \pm 13.8$  h after standing oestrus. This showed that the duration of secondary oestrous signs before and after the primary sign has not been substantially reduced. It seems that substantial number of cows show weak oestrous expression that lacks standing activity and the cows with weak oestrous signs also show short. In general, Boran breed express weak oestrous sign and also short duration of oestrus.

In this study, when duration of oestrus was evaluated in terms of parity, in multiparous and primiparous cows there was a tendency that the duration of oestrus was longer multiparous female than primiparous but not significant difference. However, earlier study by Stevenson *et al.*, (1998), showed that duration of oestrus in heifers was not as short as in cows. Likewise, Aoyagi *et al.*, (2003) reported the duration of standing oestrus in heifers to be 16.0 h on average.

No drastic variation was observed on conception rate in primiparous and multiparous but, breed wise, greater conception was noted in Holstein Friesian compared to Boran breed (Table 4) after inseminated with inseminated with sexed semen. There is no difference in conception rate for an animal inseminated with detection of primary oestrous sign with secondary oestrous sign and secondary oestrous sign alone.

**Table 4: Conception rate of animas after synchronized and inseminated using sexed semen.**

Animal category		No of animal synchronized	No of animal responded & AI	Conception rate	Female skewness
Breed	Boran	20	13	61.5% (8/13)	87.5% (7/8)
	HF	20	19	94.7 % (18/19)	100% (18/18)
	Total	40	32		
Parity	Primiparous	23	19	78.9 (15/19)	100% (15/15)
	Multiparous	17	13	76.5% (11/13)	91% (10/11)
	Total	40	32		

## 5. RECOMMENDATION

For private company or governmental sectors involving in dairying system, only traditional way of detecting cows in oestrus (i.e., observing cows for standing oestrus only), was not satisfactory and economical. Hence knowledge and understanding of secondary oestrous sign is very important for breeding and calving schedule of dairy cow and attain a one-year calving calendar. For further identification and characterization of the behavioral oestrous in both breed, knowledge level of different reproductive hormonal profile is crucial. Hence, further research and investigation are needed reproductive hormone analysis

especially level of progesterone and estradiol determination, are highly encouraged.

**Conflict of Interest:** The authors declared no potential conflicts of interest relative to the research, authorship, and/or publication of this article.

### Acknowledgment

The authors gratefully acknowledged the financial and research inputs support of this study by the Ethiopian Institute of Agricultural Research (EIAR), Debre Zeit agricultural research center.

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**Cite this Article:** Sayid Ali, Mosissa Dirre, Asnaku Funga, Tamrat Degefa, Ayda Mohammed, Asmarech Yeshaneh (2024). Behavioral and Physical Signs of Estrous Manifested in Hormonally Estrus-Induced Boran and Holstein Friesian Dairy Cattle. *EAS J Vet Med Sci*, *6*(1), 1-8.

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