

Original Research Article

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Behavioural Signs and Fertility Response Following Spontaneous and Ovsynch Induced Estrus in Sahiwal Cows

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ABSTRACT

The study was designed to evaluate behavioural estrus signs and fertility response following spontaneous and Ovsynch induced estrus in Sahiwal cows. Group 1: (Spontaneous estrus group; n=20) cows were observed visually and ultrasonographically from a random day till occurrence of spontaneous estrus and ovulation. Group 2 (Ovsynch group; n=10) were administered with standard Ovsynch protocol (two GnRH 20µg on day 0 and 9 and PGF 500µg on day 7, i.m.) started at random day of estrous cycle to synchronize estrus and ovulation. In group 1, the estrus characteristics viz. cervico-vaginal mucus discharge, mounting, vulvar swelling, bellowing and frequent urination were observed in 70, 65, 30, 10 and 20 percent cows, respectively as compared to 70, 40, 20, 30 and 20 per cent in group 2. In ovsynch group, higher size of dominant follicle (7 to 10 mm) at time of prostaglandin injection favoured subsequent ovulation, whereas, cows having smaller dominant follicle (< 7 mm size) failed to undergo ovulation. Similarly, average diameter of DFs on Day 10 was higher in cows showing ovulatory estrus as compared to non ovulatory cows in the Ovsynch group (11.9±0.27 vs. 9.25±1.18, respectively, P<0.05). Results suggested that CVM discharge and mounting activity were the two prominent signs expressed during estrus and efficacy of Ovsynch protocol initiated at random day of estrous cycle was lower in inducing ovulatory estrus and pregnancy in comparison to spontaneous estrus in Sahiwal cows.

Keywords

Estrus, Ovsynch,
Ovulation,
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Introduction

The precise detection of estrus is essential to ensure high herd fertility and economic returns from a dairy farm. Generally, majority of farmers use visual observations to detect estrus in cattle. However, *Bos indicus* do not exhibit pronounced behavioural estrus signs making estrus detection a difficult task.

Sahiwal is considered as the best milch breed of indigenous cattle. Like other breeds of *Bos indicus* cattle, estrus detection is difficult in Sahiwal cows due to less prominent estrus signs and shorter duration of standing estrus (average 10 hours) as compared to *Bos taurus* cows (Galina and Arthur 1990, Pinheiro *et al.*, 1998). Application of estrus detection aids have been found advantageous, however, their

use require extra expenditure which may not be feasible and economical for poor and marginal farmers. Fixed timed artificial insemination (FTAI) protocols could be used to achieve acceptable pregnancy rates without estrus detection in cattle. However, protocols developed in *Bos taurus* sometime did not yield similar success in *Bos indicus* cows (Pinheiro *et al.*, 1998) this could be due to minor differences observed in their endocrinology and physiology. Therefore, present study was planned to compare the behavioural estrous signs and fertility response following spontaneous and Ovsynch induced estrus in Sahiwal cows.

Materials and Methods

Thirty pluriparous Sahiwal cows weighing between 350 to 450 Kg, 60 to 90 days postpartum, having apparently normal genitalia and estrous cycle were included in the study. Cows were maintained under loose housing system and were fed with chaffed green fodder, wheat straw, concentrates, common salt, mineral mixture and ad libitum drinking water.

Cows were divided into following two groups-

Group 1: Spontaneous estrus group; Sahiwal cows (n=20) were followed to detect spontaneous estrus through visual observations and ultrasonography. No treatment was administered in this group.

Group 2: Ovsynch group; Sahiwal cows (n=10) were administered standard Ovsynch protocol at the at random stage of estrous cycle to synchronize estrus. On day 0, GnRH analogue (20 µg Buserelin acetate) was administered followed by PGF₂α (Cloprostenol 500 µg) on day 7 and again GnRH analogue (20 µg Buserelin acetate) on day 9.

All cows were observed visually to record estrual signs like cervico-vaginal mucous (CVM) discharge, mounting activity, bellowing, micturition etc. The ovaries of enrolled animals were scanned daily in Spontaneous estrus group till ovulation and on days 0, 3, 7, 9, 10, 11, 12 in the Ovsynch group to monitor number, size, fate of the follicles and size of corpus luteum.

Statistical analysis

The data was analyzed for means and standard errors (SE) for all variables. After confirming the normality of data and homogeneity of variance, Student's t test (two tailed) was applied to compare average size of follicles in different treatment groups. The ovulation and pregnancy rates were compared using chi-square test. A probability level of P < 0.05 was considered significant.

Results and Discussion

The characteristics of estrus and fertility response in spontaneous and Ovsynch induced estrus are depicted Table 1. In the spontaneous estrus group, 75 percent of the cows (n=20) showed either one or combination of behavioural estrus signs and 25 percent did not show any visual signs of estrus but were found in estrus on per rectal and ultrasonographic examination. Two most prominent behavioural signs of estrus observed were the CVM discharge and mounting activity, which were expressed by 70 and 65 percent of cows, respectively. Similar to the present study, Mattoni *et al.*, (1988) observed vaginal mucus discharge in 64% of estrus periods in Zebu cattle. Unalan *et al.*, (2016) stated that the standing to be mounted was the most observable behaviour estrus sign, followed by mounting, vaginal mucus discharging, and the other estrus signs in Holstein heifers.

Chicoteau *et al.*, (1989) also observed that acceptance of mounting as the characteristic sign of estrus in Baoule cows. Similar to this, Roelofs *et al.*, (2005a) observed mounting activity among herd mates in 90% cases of estrus in HF cows which was higher than recorded in the present study. Roelofs *et al.*, (2005b) further reported that the percentage of cows expressing mounting activity was higher when more than two cows were in estrus simultaneously. Membrive (2000) recorded the number of mounts during estrus and reported 23.0 ± 16.9 mounts per estrus, that were distributed in 10.0 ± 9.7 mounts (43.5%) between 7 AM to 7 PM. and 13.0 ± 12.4 mounts (56.5%) between 7 PM to 7 AM in *Bos indicus* × *Bos taurus* heifers.

The percentage of cows expressing bellowing sign was low in the present study. Contrary to the present study, Singh *et al.*, (1984) observed that segregation and bellowing confirmed estrus in 83.69% and 80.43% cattle, respectively. Srivastava *et al.*, (1977) stated that to detect a cow in proper heat, for successful conception the visual signs of being mounted, chasing and teaser mounting were the reliable indicators. However, Llewelyn *et al.*, (1987) observed that only 27% cows showed mounting behaviour in Boran cattle.

Following the spontaneous estrus, the percentages of other behavioural estrus signs viz. vulvar swelling, bellowing and frequent urination were comparatively lower than that of the CVM discharge and mounting activity. This could be partially due to the fact that cows were observed visually for half an hour in the morning 7 PM and evening 6 PM for expression of estrus signs in the present study. Roelofs *et al.*, (2005b) reported that intensive visual observations of 30 minutes rounds at 3 hours intervals were required to achieve ~80 percent precision in estrus detection rate in cattle. Cows were kept in loose housing

system which could be another reason for differential expression of estrus signs in the present study compared to others. Roelofs *et al.*, (2010) also reported that expression of estrus could be influenced by postpartum days, lactation number, milk production, health, nutrition, season, housing and herd size.

Out of the 10 cows administered Ovsynch protocol, behavioural estrus signs viz. CVM discharge, mounting activity, vulvar swelling, bellowing and micturition were present in 7 cows, whereas, 3 cows did not show any estrus sign. This could be due to the fact that possibly three cows with no visible estrus signs didn't respond to the Ovsynch protocol and were not in estrus which was also indicated by per rectal and ultrasonographic findings. Otherwise, no significance difference in expression of estrus signs was observed between Ovsynch induced estrus and spontaneous estrus in the present study. Udin *et al.*, (2017) evaluated the efficacy of Ovsynch protocol in Simmental cattle and observed that 40 percent of the cows showing behavioural estrus signs had CVM discharge and 100% cows were having intense uterine tone. Ammu *et al.*, (2012) observed that 83.33% Gir cows showed more prominent behavioural estrus signs especially uterine tone and mucus discharge following ovsynch.

Uterine tonicity during estrus is one of the important criteria for evaluation of estrus. In the spontaneous estrus group, 70 percent of the cows had intense uterine tone and remaining 30 percent had moderate uterine tone. None of the cow showing behavioural estrus signs had no or low uterine tone in the spontaneous estrus group (Table 1). In a similar study, Gunasekaran *et al.*, (2008) reported higher incidence of moderate uterine tone compared to mild and intense uterine tone (69.8 vs. 24.3 and 5.8 percent) in crossbred cows. However, Singh and Kharche

(1985) reported that 63.9 percent cows showed intense uterine tone followed by low tone in 26.39 and atonic in 9.72% Crossbred cows.

Results of present study showed that the percentage of cows with Intense, Moderate and Low uterine tone following Ovsynch induced estrus was 40, 30 and 30 percent, respectively (Table 1). Whereas, in case of spontaneous estrus, none of the cow had no or low uterine tone indicating that the three cows of Ovsynch group with low or no uterine tone might have not responded to the treatment.

Ovulation and pregnancy rate

In the spontaneous estrus group, 18 cows expressed ovulation and 2 cows failed to ovulate resulting in 90 percent ovulation rate. In the Ovsynch, 6 cows ovulated and 4 failed to ovulate as confirmed by ultrasonographic examination. Lower ovulation rate observed could be due to failure of three cows to respond to Ovsynch protocol. Hassan *et al.*, (2017) reported 50 percent ovulation rate following Ovsynch protocol which was comparable to the ovulation rate achieved in the present study. Sartori *et al.*, (2001) and Gimenes *et al.*, (2008) stated that full ovulatory capacity was obtained when the DF reaches a diameter of 10 mm in *Bos indicus* and 12 mm in *Bos taurus* cattle. However, Tortorella *et al.*, (2013) obtained a low ovulation rate even in large follicles following eCG treatment.

Out of 20 inseminated cows, 11 got pregnant resulting in 55 percent pregnancy rate in the Spontaneous estrus group. In the Ovsynch group, out of the six cows ovulated, 3 become pregnant following AI resulting in overall pregnancy rate of 30 percent in the Ovsynch group. In similar studies, Ammu *et al.*, (2012) and Udin *et al.*, (2017) obtained 50 and 60 conception rate by Ovsynch in Gir and Simmental cows, respectively which was

higher than achieved in the present study. The pregnancy rates depending upon type of estrus sign expressed following the Ovsynch induced and the spontaneous estrus was also analysed. No significance difference in pregnancy rate based on the type of estrus sign expressed between the Ovsynch and the spontaneous estrus group was observed (Table 2). However, in the Ovsynch group none of the cows having low or moderate uterine tone conceived indicating that cows were not in proper estrus at the time of insemination.

In both the groups, cows expressing behavioural signs had higher pregnancy rate compared to cows with no visible signs of estrus (66.7 vs. 20 and 42.8 vs 0, respectively). Similarly, higher percentage of cows got pregnant in both the groups under study when they had intense uterine tone indicating that intense uterine tone was a reliable indicator of estrus expression. Gunasekaran *et al.*, (2008) found very high association between type of estrus sign exhibited and conception rate in the crossbred cows. Fernandes *et al.*, (2001) suggested that use of Ovsynch protocol was more effective in synchronizing ovulation in cycling than non cycling Nellore cows, and reported higher pregnancy rate after FTAI (45%) in cycling than in non-cycling cows (20%). Lamb *et al.*, (2001) and Larson *et al.*, (2006) reported 56% pregnancy rates in *Bos taurus*, while less than 33.5% was obtained in *Bos indicus* in GnRH based protocols by Saldarriaga *et al.*, (2007) and Vasconcelos *et al.*, (2009).

Effect of ovarian status, follicle size and growth rate on efficacy of Ovsynch protocol

Ovarian status on Day 0

The influence of ovarian status of Sahiwal cows administered Ovsynch protocol at random stage of estrous cycle was also

evaluated in present study. Out of the total 6 ovulations recorded in the Ovsynch group, four occurred in cows having ≥ 8 mm Follicle + CL at the beginning of protocol. The average size of dominant follicle in these four cows was 9.2 ± 0.80 . The results indicated that presence of DF measuring ≥ 8 mm + CL favoured ovulation following administration of Ovsynch protocol.

Mapletoft *et al.*, (2006) reviewed that the first GnRH administration of the Ovsynch protocol induced ovulation in the dominant follicle measuring > 10 mm in diameter which lead to emergence of new follicular wave at 48 hrs of GnRH administration. Thus, the efficacy of GnRH protocol was expected to be better in cows having a follicle measuring > 10 mm diameter at the time of first GnRH administration. Similar trend was observed in the Sahiwal cows having ≥ 8 mm Follicle + CL at the time of first GnRH injection which showed higher ovulation rate than the rest of

the animals (4/5 vs. 2/5). However, the results needed to be verified on larger population to generate statistically significant data.

Size and growth rate of dominant follicles

Effect of largest follicle present on day 7 and its growth rate in inducing ovulatory estrus was also analysed (Table 3). It was observed that the average size of the DF on day 7 was higher in ovulatory cows compared to anovulatory cows. In the Ovsynch group, the size of the DF on day 7 in the cows undergoing ovulation ranged from 7 to 10 mm and none of the cow having follicle measuring < 7 mm ovulated or expressed estrus behaviour. On Day 10, significantly higher average diameter of DF was observed in cows showing ovulatory estrus compared to anovulatory in the Ovsynch induced estrus (11.9 ± 0.27 vs. 9.25 ± 1.18 , respectively, $P < 0.05$).

Table.1 The characteristics of estrus and fertility response following Spontaneous vs. Ovsynch induced estrus in Sahiwal cows

S. No.	Parameters	Spontaneous Group (n=20) % (n)	Ovsynch Group (n=10) % (n)	P Value
1	Behavioural estrus signs	75 (15)	70 (7)	0.770
	i. CVM discharge	70 (14)	40 (4)	0.113
	ii. Vulvar swelling	30 (6)	20 (2)	0.559
	iii. Mounting	65 (13)	30 (3)	0.070
	iv. Bellowing	10 (2)	20 (2)	0.447
	v. Frequent urination	20 (4)	20 (2)	1
2	No visible estrus signs	25 (5)	30 (3)	0.770
3	Uterine tonicity			
	i. No or Low uterine tone	0	30 (3)	0
	ii. Moderate uterine tone	30 (6)	30 (3)	1
	iii. Intense uterine tone	70 (14)	40 (4)	0.113
4	Ovulation rate	90 (18)	60 (6)	0.052
5	Pregnancy rate	55 (11)	30 (3)	0.195

Table.2 Pregnancy rate in relation to expression of estrus signs following Spontaneous vs. Ovsynch induced estrus in Sahiwal cows

SN	Parameters	Pregnancy rate (n)	
		Spontaneous Group	Ovsynch Group
1	Behavioural estrus sign	66.7 (10/15)	42.8 (3/7)
	i. CVM discharge	57 (8/14)	50 (2/4)
	ii. Vulvar swelling	50 (3/6)	50 (1/2)
	iii. Mounting	54 (7/13)	66.7 (2/3)
	iv. Bellowing	100 (2/2)	100 (2/2)
	v. Frequent urination	75 (3/4)	100 (2/2)
2	No visible estrus signs	20 (1/5)	0 (0/3)
3	Uterine tonicity		
	i. No or Low uterine tone	0	0 (0/3)
	ii. Moderate uterine tone	37.5 (3/8)	0 (0/3)
	iii. Intense uterine tone	75 (9/12)	75 (3/4)
4	Pregnancy rate in ovulated cows	61.1 (11/18)	50 (3/6)

Table.3 Dominant follicle size and its growth rate (Ovulatory vs. Anovulatory estrus) following Ovsynch protocol

SN	Parameter	Ovulatory Estrus	Anovulatory Estrus	Overall
1	No. of cows	6	4	10
2	Av. Dominant Follicle Size Day 7 (mm)	8.3±0.55 (7-10)	6.5±1.55 (4-11)	7.6±0.71 (4-11)
3	Av. Dominant Follicle Size on Day 10 (mm)	11.9±0.27 ^a (11 - 13)	9.25±1.18 ^b (6 - 11)	10.85±0.63 (6 to 13)
4	Follicle Growth Rate from day 7 to 10 (mm)	1.19±0.15	0.91±0.41	1.08±0.18

Values marked with different superscript within the same row differed significantly P<0.05

The results of the study indicated that the growth rate of dominant follicle from day 7 to 10 was higher in cows having ovulatory estrus in comparison to the anovulatory estrus (1.19±0.15vs. 0.91±0.41 mm per day, respectively). This indicated that the actively growing follicles had more competence to undergo ovulation. Similar to our results, Udin *et al.*, (2017) also observed that diameter of ovulatory follicle was more in

ovsynch protocol than co-synch protocol ((10.53±2.66 and 9.18±1.65, p>0.05) in Simmental cows. Quezada *et al.*, (2015) observed that the growth rate of preovulatory DF from deviation to ovulation was higher after synchronized estrus than after spontaneous estrus (0.9 ± 0.2 and 0.8 ± 0.2 mm/d, respectively; p<0.05). Hassan *et al.*, (2017) recorded 1.18±0.26 mm per day follicle growth rate from day 7 to day 10,

12.30±0.92 mm average size of dominant follicle at the time of ovulation, and 27 ± 5.56 hours interval from the second GnRH to ovulation, 50% ovulation and 43% pregnancy rate following Ovsynch protocol in Sahiwal cows.

Interval from PG to estrual discharge and ovulation

In seven cows showing behavioural estrus signs, average interval from PG to estrus discharge was 68.7±1.11 hrs (range 66 to 72 hrs), whereas, the interval from PG to ovulation was 86.0±2.00 hrs (range 84 to 96 hours). Mattoni *et al.*, (2000) observed that mean estrus interval from PGF₂α analogue treatment to the onset of estrus was shorter in the Zebu cows compared to Baoule cattle. Zebu showed estrus ranging from 48 hrs to 60 hrs after treatment. The highest proportions of animals were observed in estrus over a period of 60 hrs and 72 hrs after treatment in Baoule cows. Pinheiro *et al.*, (1998) reported that ovulation occurred 26.6 h after the onset of estrus in Nelore (*Bos indicus*) cows.

Kastelic and Ginther (1991) reported that the time from the administration of PGF₂ α to ovulation was 3 days, if there was an emergent DF and reached static phase; in that case the time from PGF₂ α to ovulation was 3 days. However, the time from PGF₂ α to ovulation was 4.5 days if a new DF emerged at the time of luteolysis.

In conclusions, cervicovaginal discharge and mounting activity were the two major behavioural signs of estrus in Sahiwal cows. In addition, the efficacy of Ovsynch protocol at random stage of estrous cycle in inducing ovulatory estrus and pregnancy was lower as compared to spontaneous estrus in Sahiwal cows. Further, large scale studies are required in Sahiwal cows to draw concrete results on efficacy of Ovsynch in this breed.

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Compliance with ethical standards

The presented research was conducted in accordance with the ethical standards of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India.

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