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US Khinda
MVSc Scholar, Department of Veterinary Gynaecology and Obstetrics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

SS Dhindsa
Scientist, Department of Veterinary Gynaecology and Obstetrics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

M Honparkhe
Principal Scientist, Department of Veterinary Gynaecology and Obstetrics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

VS Malik
Senior Scientist, Department of Veterinary Gynaecology and Obstetrics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Prahlad Singh
Professor, Department of Veterinary Gynaecology and Obstetrics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Corresponding Author
US Khinda
MVSc Scholar, Department of Veterinary Gynaecology and Obstetrics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Relationship between Estrus expression and fertility in Sahiwal cattle following modified timed artificial insemination protocols

US Khinda, SS Dhindsa, M Honparkhe, VS Malik and Prahlad Singh

Abstract
Sahiwal is considered as best indigenous milch breeding the country. The present study was conducted on non-pregnant, clinically healthy Sahiwal cows (n=40, >45 days in milk) weighing between 350 to 500 kg having apparently normal estrous cycle and genitalia. All cows were divided into 3 groups on the basis of treatment used or not viz. Group I (Modified Presynch-Ovsynch, n=15), II (Modified Presynch-Heatsynch, n=15) and III (Spontaneous estrus, n=10). In treatment groups, considerable number of cows (66.67-93.33%) showed main signs of behavioural estrus at the time of insemination. On the other hand, only 50-70 percent animals that were inseminated at spontaneous estrus exhibited major estrus signs. Further, all cows that became pregnant later following hormonal treatment showed bellowing, frequent urination, vulvar swelling and CVM. In treatment groups, moderate and high uterine tones were observed in 33.33 to 37.50 and 62.50 to 66.66 percent subsequently pregnant animals, respectively. In conclusion, a positive relationship between estrus expression and subsequent pregnancy rate was observed in the study. Hence, positive conception may be projected if an animal displays main heat signs at the time of insemination like swelling of vulva, bellowing, tonicity of genitalia and vaginal discharge.

Keywords: Estrus signs, synchronization, Presynch-Heatsynch, Presynch-Ovsynch, Sahiwal

Introduction
Sahiwal is one of the best indigenous milch breeds in India. It is quite resistant to ticks (Wambura *et al.*, 1998) [8], environmental heat-tolerant (Jian *et al.*, 2015) [6] due to large number of sweat glands (Jian *et al.*, 2014) [5] and has high resistance to internal and external parasites. Sahiwal remains calm while milking and due to high productivity it has been exported to other countries.

Sahiwal like other *Bos indicus* cows, is not reproductively efficient. It shows incidence of delayed puberty, shows tendency of seasonal breeding, postpartum anestrus is shown to be long and shows small length of heat period along with lesser expression of signs (silent estrus) (Bó *et al.*, 2003) [2] during evening and night hours hence estrus detection is challenging in Sahiwal cows.

Generally visual methods are used by farmers for the detection of estrus in cattle. Due to few overt signs of Sahiwal estrus detection is difficult. Owing to the said explanations, use of artificial insemination (AI) in Sahiwal has not been popular as in cross bred cows. In large herds this is even more challenging leading to heavy economic losses to the farmers. Hence use of estrus detection aids like pedometer, heat watch system, teaser bull, per-rectal examination, fern pattern, record keeping, heat mount detector, chin ball marker and progesterone analysis etc. are of great importance and can lead to increased conception rate. However, these methods require extra time, money expenditure and we have to wait for the animal to come in heat naturally which further lead to increase in days open and decrease in economic returns.

This necessitates the development of fixed timed artificial insemination (FTAI) synchronization protocols (Bello *et al.*, 2006) [1]. Ovsynch and Heatsynch are effective FTAI methods for controlling the timing of insemination in dairy cattle (Pursley *et al.*, 1995; Cirit *et al.*, 2007) [7, 3]. The Estradiol benzoate (EB) used in Heatsynch protocol has several advantages compared to GnRH like cheaper cost, prominent estrus signs, practical scheduling of treatments before TAI, greater uterine tonicity and relatively easy insemination since most injections and AI are at 24 and 48 h interval (Cirit *et al.*, 2007) [3].

Furthermore, a presynchronization strategy before Ovsynch/Heatsynch may be implicated to improve reproductive efficiency of Sahiwal breed (Gumen *et al.*, 2012) [4]. Thus, present study was planned to evaluate a modified Presynch-Ovsynch and Presynch-Heatsynch protocols with the aim of comparing estrus behavior and subsequent fertility to enhance pregnancy rates in Sahiwal cattle.

Materials and Methods

Sahiwal cows (n=40) >45 days postpartum, non-pregnant, clinically healthy weighing between 350 to 500 kg having apparently normal estrous cycle and genitalia were used for the study. All the efforts were done to equally divide the cows with similar body condition score, milk production and days in milk into all groups. All the cattle were kept in a loose housing system and were offered green and dry fodder, concentrate feed, mineral lick, mineral mixture and clean fresh water. Sahiwal cows (n=40), were divided into three groups; Group I (Modified Presynch-Ovsynch, n=15), Group II (Modified Presynch-Heatsynch, n=15) and Group III (Spontaneous estrus, n=10). The postpartum Sahiwal cattle (n=10), having corpus luteum (CL) upon ultrasonographic examination were selected as control group. In group I (Modified Presynch-Ovsynch, n=15), on day 0 (beginning of the experiment) and 12, each cow was administered prostaglandin analogue (Cloprostenol sodium 500 µg, intramuscular). On 14th day, gonadotropin releasing hormone analogue (Buserelin acetate 10 µg, intramuscular) was given. On 21st day, all animals were administered prostaglandin analogue (Cloprostenol sodium 500 µg, intramuscular) followed by gonadotropin releasing hormone analogue (Buserelin acetate 10 µg, intramuscular) on 23rd day. FTAI was performed twice (AM, PM) by using good quality frozen semen of Sahiwal bulls on day 24. In group II (Modified Presynch-Heatsynch, n=15), all the injections were administered similar to group I except day 23 injection that was replaced by estradiol (Estradiol benzoate, 1 mg, intramuscular) on 22nd day. The FTAI was performed twice (AM, PM) on day 24. In group III (Spontaneous estrus, n=10), all cows were visually monitored to detect occurrence of spontaneous estrus for 30 days. No treatment was administered and AI was done twice (AM, PM) on observation of estrus signs using good quality frozen semen of Sahiwal bulls. Pregnancy diagnosis was performed at 30 and 60 days post AI in all groups by transrectal ultrasonography using ultrasound scanner (Easi scan curved, BCF Technology, India) equipped with B-mode linear array trans-rectal probe of 5 MHz frequency. The incidence of various behavioural estrus signs viz. bellowing, frequent urination, swollen vulva, cervico-vaginal mucus (CVM) and uterine tone at induced and spontaneous estrus were recorded in all groups. Various estrus signs have been enlisted in Table 1.

Table 1: Estrus signs observed during spontaneous and induced estrus in Sahiwal cows

Parameters	Grading	Observations
1. Cervical mucus discharge	Present	CVM discharge visible either hanging from vulva or sticking to any other body parts like tail, rump, hindquarter
	Absent	CVM discharge not externally visible
2. Vulvar swelling	Present	• Edema of vulvar lips • Disappearance of vulvar wrinkles
	Absent	• No vulvar edema • Vulva wrinkled
3. Bellowing	Present	Typical loud roar
	Absent	No typical loud roar
4. Frequent urination	Present	More than 2 times urination during the observation period of 30 min
	Absent	No frequent urination during observation period
5. Uterine tonicity	No or Low uterine tone	Flaccid uterine horns that got toned following massage through rectal examination
	Moderate uterine tone	Toned uterine horns that were easy to lift and straighten up
	Intense uterine tone	Toned uterine horns which were hard to pull up and uncurl

In the present study a comparison of estrus signs between pregnant and non-pregnant animals of both the groups was done. To compare the heat signs shown by pregnant and non-pregnant cows of both treatment groups, "χ^2 test" and "F-test" were used (Statistical Package for the Social Sciences).

Results and Discussion

The present study findings showed that the constraint of silent heat, inconstant heat length and lower heat expressibility in Sahiwal cow may be fixed via adopting estradiol and GnRH based synchronization protocols as in the present study, appreciable number of animals (66.67-93.33%) showed major estrus signs viz. Bellowing, vulvar swelling, frequent urination and CVM at the time of AI. On the other hand only 50-70 percent animals that were inseminated at spontaneous estrus (control group) exhibited major estrus signs. Behavioural estrus signs exhibited by Sahiwal cows of different groups are shown in Fig. 1.

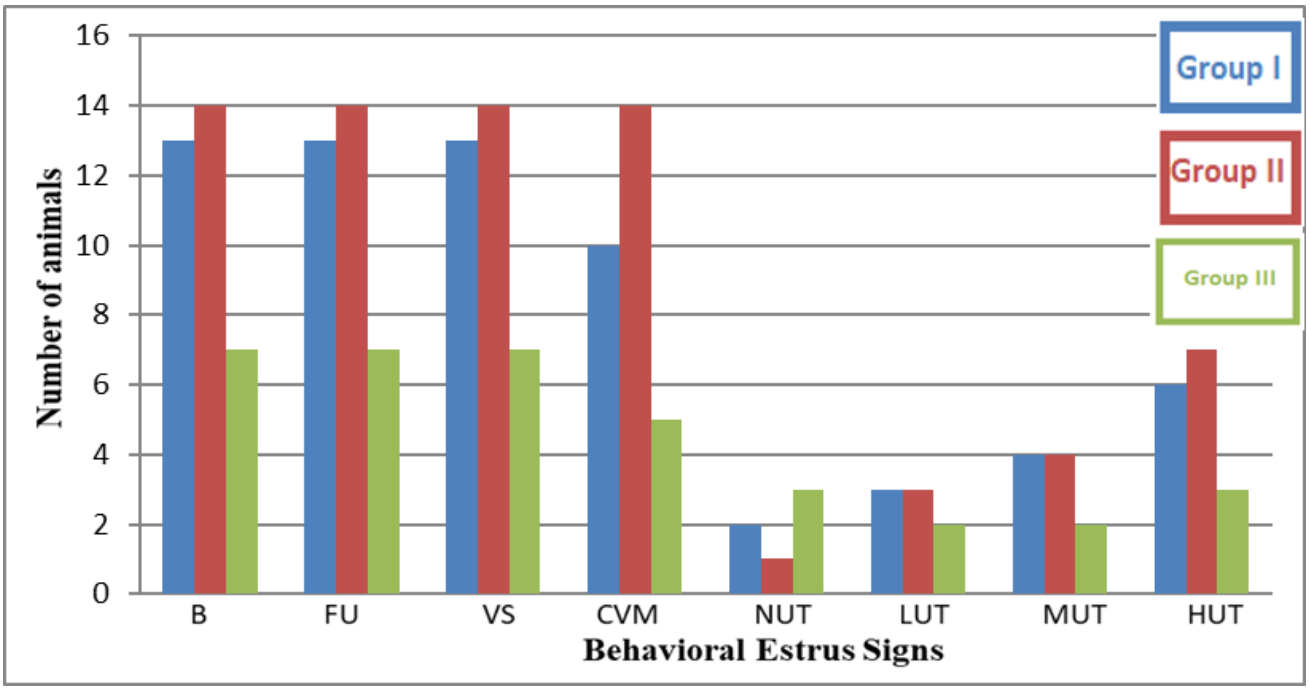

Fig 1: Behavioural heat signs shown by animals of Presynch-Ovsynch, Presynch-Heatsynch treatments and control group at the time of AI

B- Bellowing, FU- Frequent urination, VS- Vulvar swelling, CVM- Cervico vaginal mucus, NUT- No uterine tonicity, LUT- Low uterine tonicity, MUT- Moderate uterine tonicity, HUT- High uterine tonicity

In group I, 86.66 percent (13/15) cows showed bellowing, frequent urination and vulvar swelling and only 66.67 percent (10/15) cows showed CVM. In group II, 93.33 percent (14/15) Sahiwal cows showed estrus signs. In group III, 70.00 (7/10) percent cows showed bellowing, frequent urination and vulvar swelling while only 50.00 (5/10) percent cows showed CVM. Another important estrus sign was uterine tonicity which was assessed through rectal examination. No uterine tonicity was observed in 13.33, 6.67 and 30.00 percent cows in group I, II and III, respectively. Low uterine tonicity was observed only in 20.00 percent cows in all groups. Moderate uterine tonicity was observed in 26.67, 26.67 and 20.00 percent cows in group I, II and III, respectively. High uterine tonicity was observed in 40.00, 46.67 and 30.00 percent cows in group I, II and III respectively. This was clearly observed that in Presynch-Heatsynch protocol estrus signs were more prominent than other groups. In the present study, overall pregnancy rates were 40.00, 53.33 and 30.00 percent in group I, II and III, respectively.

Table 2: Comparison between behavioural estrus signs expressed by pregnant and non-pregnant Sahiwal cows of Presynch-Ovsynch and Presynch-Heatsynch treatments at the time of insemination

Parameter		Pregnancy status	Presynch-Ovsynch (Group I) Percentage of cows (Number of cows/ Total number of cows)	F value	Presynch-Heatsynch (Group II) Percentage of cows (Number of cows/ Total number of cows)	F value
Bellowing		Pregnant	100.00 (6/6)	NS	100.00 (8/8)	NS
		Non pregnant	77.77 (7/9)	0.48	85.71 (6/7)	0.47
Frequent urination		Pregnant	100.00 (6/6)	NS	100.00 (8/8)	NS
		Non pregnant	77.77 (7/9)	0.48	85.71 (6/7)	0.47
Vulvar Swelling		Pregnant	100.00 (6/6)	NS	100.00 (8/8)	NS
		Non pregnant	77.77 (7/9)	0.48	85.71 (6/7)	0.47
CVM (Cervico vaginal mucus)		Pregnant	66.66 (4/6)	NS	100.00 (8/8)	NS
		Non pregnant	66.66 (6/9)	1	85.71 (6/7)	0.47
Uterine tonicity	No	Pregnant	00.00 (0/6)	NS	00.00 (0/8)	NS
		Non pregnant	22.22 (2/9)	0.48	14.28 (1/7)	0.47
	Low	Pregnant	00.00 (0/6)	NS	00.00 (0/8)	NS
		Non pregnant	33.33 (3/9)	0.23	42.85 (3/7)	0.08
	Moderate	Pregnant	33.33 (2/6)	NS	37.50 (3/8)	NS
		Non pregnant	22.22 (2/9)	1	14.28 (1/7)	0.57
	High	Pregnant	66.66 (4/6)	NS	62.50 (5/8)	NS
		Non pregnant	22.22 (2/9)	0.13	28.57 (2/7)	0.31

Figures in parenthesis indicate number of animals, F value – Fischer's Exact Test, NS- Non-significant

Conclusion

Poor estrus expression and detection in Sahiwal cow is a major limit to its reproductive performance. From the present study, it was observed that Sahiwal cows subjected to Presynch-Heatsynch showed better estrus signs at the time of insemination and pregnancy rates than other two groups. A

positive relationship between the estrus expression and subsequent fertility was observed. Introduction of presynchronization based FTAI protocols can lead to major leap in reproductive efficiency of Sahiwal cows.

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