



Fertility Assessment of Sexed Semen with Synchronized and Spontaneous Estrus in Crossbred Cows

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ABSTRACT

Background: With the commercial introduction of sexed semen, its use has been recommended for insemination at observed estrus, avoiding the use of a fixed-time insemination due to inherent variation in success of timed programmes. The use of the Ovsynch protocol with sexed semen at defined conditions such as normal clinical and reproductive status of lactating cows might result in a pregnancy rate comparable to that achievable at insemination upon detection of spontaneous estrus with sexed semen. Considering this rationale, present research was implemented with the effect of fertility on synchronized and spontaneous estrus with sexed semen.

Methods: Post-partum multiparous crossbred cows (n=28) were categorized as a group I (Spontaneous; n=14) and group II (Ovsynch; n=14). The fourteen cows from group I were inseminated with sexed semen at observed estrus after initial treatment with vitamin AD₃E and H, urimin and supplemented with chelated mixture and the fourteen cows from the group II were synchronized with Ovsynch synchronization protocol (Inj. Buserline acetate 10 µg I/M) on day 0, (Inj. Cloprostenol sodium 500 µg) on day 7 and (Inj. Buserline acetate 10 µg) I/M on day 9. Thereafter, timed artificial insemination (TAI) was performed 16-20 hours after 2nd dose of GnRH with sexed semen.

Result: The percent intense type of intensity recorded numerically higher in induced estrus. The first service conception rate was higher in spontaneous estrus with the overall conception rate 39.28%. Cows with 60-100 days in milk was showed high conception rate with sexed semen, as the parity increases the conception rate decreases with sexed semen The overall proportion of female calves born was 90.90%.

Key words: Conception rate, Crossbred cows, Sexed semen, Spontaneous, Synchronization.

INTRODUCTION

With the commercial introduction of sexed semen, its use has been recommended for insemination at observed estrus, avoiding the use of a fixed-time insemination due to inherent variation in success of timed programmes (DeJarnette *et al.*, 2010). However, a delay in the resumption of cycling in cows after calving, weak estrous expression and the low detection rates of estrus are primary factors limiting reproductive performance on dairy farms. For timed insemination of lactating cows, (Pursley *et al.*, 1995) elaborated the programme, providing the control of follicular growth, luteolysis and ovulation through a GnRH and PGF_{2a} combination (Ovsynch protocol). After the introduction of the Ovsynch, numerous modifications of the protocol were elaborated, providing a similar or higher pregnancy rate than at natural estrus (Rabiee *et al.*, 2005; Wiltbank and Pursley 2014). Our hypothesis is that the use of the Ovsynch protocol with sexed semen at defined conditions such as normal clinical and reproductive status of lactating cows might result in a pregnancy rate comparable to that achievable at insemination upon detection of spontaneous estrus.

Sexed semen can be used to generate herd replacements and additional heifers for herd expansion at a faster rate from within the herd, thereby minimizing biosecurity risks associated with bringing in animals from different herds. Furthermore, the use of sexed semen can increase herd genetic gain compared with use of non-

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sorted semen (Holden and Butler, 2018). A high fertility sexed semen product has the potential to accelerate herd expansion, minimize waste production, improve animal welfare and increase profitability compared with non-sorted conventional semen. Numerous fertility-related factors, other than methods of synchronization, including stage of lactation, insemination number, parity and milk production, may exert effect on success of insemination in lactating cows (Kurykin *et al.*, 2017). Utilizing sexed semen, the observed conception rates were 20.7% in cows and 35.3% in heifers when compared to Y-sorted semen, it was discovered that X-sorted semen had a better purity (Biswas *et al.*, 2013). Currently, sexed semen in various breeds of cows and buffaloes is offered by ABS India, Prime Bovine Genetics

and Sexing Technologies. Thus, the information on the effect of the fertility related factors on the efficiency of sexed semen use at spontaneous estrus and synchronization of estrus is of interest in the perspective of the facilitation of the insemination of lactating cows.

MATERIALS AND METHODS

The study was conducted on crossbred cows that were selected from Adarsh Goshala farm near Mhaispur district Akola, Maharashtra India and farmers doorstep in nearby villages in Akola district during the period of February 2022 to May 2022. Lactating crossbred cows (n=28) weighed between 300-350 kg, that have completed post-partum period of 60-150 days with normal reproductive genitalia and without any clinical infection with 1st to 3rd parity were selected randomly and divided into two groups. *viz.* spontaneous group (group I; n=14) and Ovsynch group (group II; n=14) and. In group I, cows initial treatment with vitamin AD₃E and H (5 ml l/m single dose), urimin (10 ml l/m for 3 days) and supplemented with chelated mixture 50 gm/day for 20 days) were given and inseminated with sexed semen after observation of heat signs. In group II cows, presynchronization treatment for 7 days consist of vitamin AD₃E and H (5 ml l/m single dose), urimin (10 ml l/m for 3 days) and supplemented with chelated mixture 50 gm/day for 20 days). Seven days after presynchronization treatment cows were synchronized with Ovsynch synchronization protocol (Inj. Buserline acetate 10 µg l/M) on day 0, (Inj. Cloprostenol sodium 500 µg) on day 7 and (Inj. Buserline acetate 10 µg) l/M on day 9. Thereafter, timed artificial insemination (TAI) was performed 16-20 hours after 2nd dose

of GnRH with sexed semen. The pregnancy diagnosis was carried out on day 60 post insemination.

The examination of external genitalia and per rectal examination of internal genital organ of responded cows from two groups were carried out to ascertain their status. The intensity of the estrus was decided by the expression of behavioral symptoms of the estrus and total score method as detailed below (Table 1).

Total score for each estrus was prepared as described by Singh and Kharche, (1985) and the heat was classified accordingly into intense, medium and week (Table 2).

The data was analyzed by using ICAR GOA WASP 2.0 online software. The data regarding intensity of estrus, effect of intensity of estrus on conception rate, first service conception rate, effect of days in milk on conception rate, effect of parity on conception rate were analyzed by using Chi-square test.

RESULTS AND DISCUSSION

The comparative intensity of estrus in spontaneous and synchronized estrus is depicted in Table 3. The intense type of intensity of estrus was higher in induced estrus cows as compared to spontaneous estrus cows. The results are non-significant (p<0.05) between the groups. The intense type of intensity of estrus was non significantly higher in induced estrus cows as compared to spontaneous estrus cows are in agreement with Shahid *et al.* (2019), Alves *et al.* (2009) and Landaeta *et al.* (2002) reported higher intensity of estrus in induced cows as compared with spontaneous cows in Non-Descript, Guzera and Angus breed of cows, respectively. In contrast, Singh *et al.* (2020) reported higher

Table 1: Score card device on status of reproductive organ.

Status of reproductive organs	Weightage	Score
Tumification of vulval lips	10	Highly tummified 10 Moderately tummified
	5	Slightly tummified-3
Vaginal mucous membrane	5	pink 5
i) Color		Pale 0
	5	Moist 5
ii) Condition		Dry 0
Cervical status	5	Complete 5
i) Relaxation		Partial 3, No relaxation 0,
ii) Condition	5	Soft 5, Hard 0,
Uterine tonicity	10	Tonic 10 Moderately tonic 5, Atonic 0
Mucous discharge	10	Copious 10, Moderate 6, Flocculent and watery 4, Scanty or apparently absent 2
Total weightage	50	

estrus intensity in spontaneous cows as compared to induced cows, which is not in agreement with present findings.

A slightly better intensity of estrus observed in present study with induced estrus in cows might be due to the reason that GnRH injection at day 0 might have resulted in synchronous follicular emergence and timely follicular development at the start of treatment. Thus, amore finely delineated endocrine regulation might have induced a wave of follicular development. Low intensity of estrus is a limiting factor in the use of AI and other reproductive management techniques depends upon visualization of estrus. Therefore, the higher estrus intensity score achieved by estrus induction may be helpful in application of AI (Shahid *et al.*, 2019). Social factors could have also contributed to differences observed in the intensity of estrus between synchronized and spontaneous estruses. Synchronization of estrus increases the number of cows in estrus at particular time. Consequently, the coincidence of several cows in estrus leads to formation of sexually active groups, which promote increased mounting activity in females (Lemaster *et al.*,1999).

The overall conception rate in intense and moderate + weak estrus inseminated with sexed semen were depicted in Table 4. The overall conception rate was observed higher (15.90%) with non-significant difference (p<0.05) in intense type of intensity as compared to moderate + weak type of intensity which might be due to unlike strong estrus, weak estrus has been associated with elevated progesterone levels (Schopper *et al.*, 1993) that exert a suppressive effect on the amplitude and the amplitude and the frequency of LH pulses in the preovulatory period (Savio *et al.*, 1993). A shortened duration of estrus in cows with weak signs in connection with improper timing of insemination can be related to fertilization failures (Yoshida and Nakao 2005). The overall difference in first service conception rate observed in intense and Moderate + weak estrus is in accordance with Mallory *et al.* (2013) reported pregnancy rate with sexed semen in heifers that displayed estrus after

synchronization by 20% points higher compared to those that did not express apparent signs.

In present study, the higher first service conception rate was observed in spontaneous estrus cows compared to the synchronized cows with overall first service conception rate 39.28%. The overall proportion of female calves born was 90.90% (Table 5). The first service conception rate observed after inseminated with sexed semen in spontaneous estrus was higher (7.14%) as compared with induced estrus with non-significant (p<0.05) difference. The non significant (p<0.05) higher first service conception rate observed in spontaneous estrus compared to induced estrus might be due to the larger number of ovulations that occurred within the time appropriate for sexed sperm. The tendency towards interaction between the bull and the type of estrus is in accordance with the decreased conception rate related to the use of sexed semen for synchronized services compare to non-synchronized services (Abdel-Azim 2010).

The non-significant difference observed in conception rate in induced and spontaneous estrus cows inseminated with sexed semen is in harmony with Kurykin *et al.* (2017) reported 37.2 and 40.1% conception rate in spontaneous and induced estrus, respectively with non-significant difference. The lower conception rate observed in induced estrus as compared to spontaneous estrus is in agreement with Kurykin *et al.* (2016) observed 41.9 and 53.4 per cent conception rates in induced and spontaneous estrus, respectively. The overall conception rate observed in present findings are in concurrence with Kurykin *et al.* (2017) reported 38.6% conception rate in lactating Holstein cows. Sharma *et al.* (2018) observed 40% conception rate in dairy cows inseminated with sex sorted semen. Joshi *et al.* (2021)

Table 2: Type of oestrus based on score from reproductive status of organ.

Score range	Type of estrus
40 and above	Intense
30 and above	Medium
29 and below	Weak

Table 3: The intensity of estrus (%) in spontaneous and induced estrus groups.

Groups	Intensity of estrus (%)			Chi-square statistic value
	Intense	Intermediate	Weak	
Spontaneous estrus	07/14 (50.00) ^a	05/14 (35.71) ^a	02/14 (14.28) ^a	0.178
Induced estrus	08/14 (57.14) ^a	04/14 (28.57) ^a	02/14 (14.28) ^a	

Percent bearing different superscript in a column different significantly.

Table 4: The overall conception rate in intense and moderate + weak estrus inseminated with sexed semen.

Particulars	Intensity of estrus (%)		Chi-square Statistic value
	Intense	Intermediate+Weak	
First service conception rate (%)	7/1546.66 ^a	4/1330.76 ^a	0.325
The overall difference in first service conception rate (%)	15.90		

Percent bearing different superscript in a column different significantly.

observed the overall conception rate of sex sorted semen was 39.92±0.5 percent in Indian breeds of cattle. Norman *et al.* (2010) observed 39% conception rate with sexed semen in Holstein heifers. Mallory *et al.* (2013) reported 38% overall conception rate in Holstein heifers inseminated with sex sorted semen. Patel and Jethva (2019) observed 39.53% pregnancy rate in crossbred (HF × Gir) heifers. DeJarnette *et al.* (2011) observed 38% conception rates in Holstein heifers using 2.1×10^6 concentration of sex sorted semen and Rhinehart *et al.* (2011) observed 38% conception rates in heifers inseminated with sexed semen. Shinde *et al.* (2021) reported 40% conception rate with sex sorted semen in Gir cows. The overall female calves born observed in present study are in concurrence with Shekalgorabi *et al.* (2017) reported 91.1% female calves in Holstein heifers using sexed semen. Joshi *et al.* (2021) observed 90.9% female calve by using sex sorted semen in Indian cattle breeds. Seidel *et al.* (2008) observed 92% female calves using sexed sperms in Angus cows. Frijters *et al.* (2009) observed 91.6% female calves using sexed semen. Guner *et al.* (2021) observed 89.8% female calves using sex sorted semen in Holstein heifers. Abdalla *et al.* (2014) observed 89.6% female calves inseminated with sexed semen in Holstein heifers.

The effect of days in milk on pregnancy rate in spontaneous and induced estrus was depicted in Table 6. The conception rate is higher in 60-100 days in milk as compared to 101-150 days in milk with non-significant ($p < 0.05$) difference. The higher conception rate observed

in above 60 days in milk are in concurrence with DeJarnette *et al.* (2010) and Maicas *et al.* (2020) stated that conception rate of cows inseminated at above 60 days in milk has greater conception rate compared to below 60 days in milk.

The present finding regarding conception rate observed in 101-150 days in induced estrus is similar with Kurykin *et al.* (2017) reported 37.4% conception rate in induced estrus cows. Thus targeting the use of sexed semen to elite cows based on days in milk at insemination highlighted as suitable criteria for identifying lactating cows suitable for insemination with sex sorted sperm. (Maicas *et al.*, 2020).

The parity wise pregnancy rate in spontaneous and induced estrus was depicted in Table 7. From the present study it was observed that as the parity increases the conception rate decreases with non-significant difference. Difference in fertility between primiparous and multiparous cows may depend on differences in milk yield, energy balance and metabolic changes. Greater energy demands for milk synthesis may increase the risk of fertility influencing problems due to the higher susceptibility of high producing cows to metabolic and endocrine disorders (Chebel *et al.*, 2004). The decrease in conception rate as parity increases of sexed semen in present study are in accordance with DeJarnette *et al.* (2008), Kurykin *et al.* (2017), Maicas *et al.* (2019), Maicas *et al.* (2020), reported decrease in conception rate as parity increases.

The conception rate observed in second parity cows inseminated with sexed semen in spontaneous estrus is in harmony with the findings reported by Kurykin *et al.* (2017)

Table 5: Conception rate, calving rates and gender ratio after AI with sexed semen in spontaneous and induced estrus groups.

Particulars	Spontaneous estrus	Induced estrus	Overall observations	Chi-square statistic value
Pregnancy rate				
No. of Als.	14	14	28	-
No. of cows became pregnant	06	05	11	-
First service pregnancy rate (%)	42.85 ^a	35.71 ^a	39.28	0.150
Calving rate				
No. of calved	06	05	11	-
%calved	100	100	100	-
Gender of calved				
No. of calves	06	05	11	-
No. of female calves	05/06	05/05	10	-
% female calves	83.33	100	90.90	-

Percent bearing different superscript in a row different significantly.

Table 6: The effect of days in milk on pregnancy rate in spontaneous and induced estrus.

Particular	Pregnant cows with days in milk (days)		Conception rate %		Chi-square table value
	60-100	101-150	60-100	101-150	
Spontaneous estrus (n=14)	04/08	02/06	50.00 ^a	33.33 ^a	0.389
Induced estrus(n=14)	03/08	02/06	37.50 ^a	33.33 ^a	0.026
Overall conception rate (n=28)	07/16	04/12	43.75 ^a	33.33 ^a	0.312

Percent bearing different superscript in a row different significantly.

Table 7: The parity wise pregnancy rate in spontaneous and induced estrus.

Particular	Parity wise conception rate (%)			Chi-Square statistic value
	First parity	Second parity	Third parity	
Spontaneous estrus (n=14)	04/07= 57.14 ^a	01/03=33.33 ^b	01/04=25.00 ^b	20.824
Induced estrus (n=14)	04/08=50.00 ^a	01/03=33.33 ^a	00/03=00.00 ^b	
Overall conception rate (n=28)	08/15=53.33 ^a	02/06=33.33 ^{ab}	01/07=14.28 ^{bc}	

Percent bearing different superscript in a row different significantly.

reported 35.6 percent conception rate in second parity lactating HF cows inseminated with sexed semen. The present findings regarding conception rate observed in first parity in induced estrus cows is in accordance with Maicas *et al.* (2019) observed 45.2% conception rate in first parity with sex sorted sperm in induced estrus. The present findings regarding conception rate observed in second parity in induced estrus cows is in accordance with DeJarnette *et al.* (2008) and Karakaya *et al.* (2014) reported 31.3 and 33.3% conception rate in second parity with sexed and sex sorted semen, respectively in cows.

CONCLUSION

The results acquired supported the hypothesis that synchronized estrus cows showed at par conception rate with sexed semen in summer season and can be used in synchronized cows with optimum pregnancy rate and should used at the field level.

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Conflict of interest

All authors declare that they have no conflict of interest.

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