

## RESEARCH ARTICLE

# Hormonal Profile and Fertility Response to Modified Ovsynch Protocol in Repeat Breeding Crossbred Cows with Prolonged Estrus

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## ABSTRACT

The present study was conducted to determine the hormonal profile and fertility response in prolonged estrus affected repeat breeder crossbred cows by using modified ovsynch protocol. Total 20 crossbred cows, not conceiving even after more than four services were selected from the semi-arid area of Banaskantha district in Gujarat. They were divided into two equal groups: Group-I (n=10) - cows with prolonged estrus period (> 28 hrs) and Group-II (n = 10) - cows with normal estrus period (18 to 28 hrs). The animals of both groups were treated with modified ovsynch protocol using double dose of GnRH (@ 20 µg) starting from 5<sup>th</sup> day of estrous cycle. The blood was collected on day 0, 7<sup>th</sup>, 9<sup>th</sup> and 25<sup>th</sup> (15<sup>th</sup> day post-FTAI) of the treatment for determination of plasma progesterone and estradiol hormones. The conception rates in group I and II were 60 and 70%, respectively. The cows with prolonged estrus had non-significantly higher mean plasma progesterone ( $3.51 \pm 0.45$  vs  $2.97 \pm 0.38$  ng/ml) and lower estradiol ( $44.36 \pm 1.74$  vs  $54.56 \pm 2.49$  pg/ml) as compared to normal estrus cows on day 0 of protocol. The progesterone levels were significantly ( $p < 0.05$ ) higher on day 7<sup>th</sup> (day of PG injection) and 9<sup>th</sup> (day of second GnRH injection) of protocol in prolonged estrus cows than normal estrus group. The plasma estradiol concentration was significantly ( $p < 0.05$ ) lower at all the periods in prolonged estrus cows than those of normal estrus group, except on 25<sup>th</sup> day. In repeat breeding cows with prolonged estrus, the plasma progesterone concentration was significantly higher on day 25 (day 15 post-AI) of treatment in conceived as compared to non-conceived cows, whereas in normal estrus cows, there was no significant difference. While, estradiol concentration was significantly lower in conceived than non-conceived cows in both the groups.

**Keywords:** Crossbred cow, Prolonged estrus, Modified ovsynch, Fertility, Progesterone, Estradiol.

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## INTRODUCTION

Repeat breeding is a major reproductive disorder in dairy cattle that leads to major economic losses to the dairy farmers (Modi *et al.*, 2011). Repeat breeding may be associated with failure of fertilization or early embryonic death. Among the major functional causes, hormonal insufficiency and dysfunction contribute about 40.1% cases of repeat breeding. There is altered levels of hormones such as estrogen, progesterone, luteinizing hormone (LH) in repeat breeders associated with prolonged estrus and extended estrus-to-ovulation interval (Bage *et al.*, 2002; Bloch *et al.*, 2006). Approximately 30-40% of the total numbers of repeat breeding cattle showed prolonged estrus (37-60 vs. 24-36 hours) and 70% recorded marginally elevated progesterone levels (0.3 to 0.35 ng/ml) during or around estrus (Singh *et al.*, 2012).

In repeat breeder cattle with prolonged estrus, the use of single insemination along with administration of GnRH analogue, is sufficient, however, in the absence of hormonal treatment, delayed insemination (Villarreal and Lane, 2010) or double insemination at 24 h interval (Sharma *et al.*, 2006) also gives optimal results. Clinically, prolonged estrus can be easily diagnosed and has been treated successfully by

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synchronized timed insemination protocols (Lopez-Gatius *et al.*, 2001). Conception rate with ovsynch protocol usually is improved when initiated during mid-diestrus, *i.e.*, days 5-12 of estrous cycle (Tiwari *et al.*, 2019), but its use in repeat breeding cattle with prolonged estrus is meager. Therefore,

this study was conducted to evaluate the efficacy of modified ovsynch protocol in terms of hormonal changes and fertility response in repeat breeding crossbred cattle with prolonged estrus under semi-arid region of Banaskantha district in Gujarat.

## MATERIALS AND METHODS

A total of 20 crossbred cows, aged between 3-10 years, which had not conceived even after more than four consecutive quality services with variable duration of estrus were selected at doorstep of farmers from the semi-arid region of Banaskantha district in North Gujarat (India). The animals were divided into two equal groups on the basis of estrus duration; the cows showing estrus duration > 28 hrs were included in prolonged estrus group (n=10; Group-I), whereas cows with normal estrus duration (18-28 hrs) were included in normal estrus group (n=10; Group-II). Animals of both the groups were treated with modified ovsynch protocol by using double dose of GnRH analogue (Busereline acetate, 20 µg, i/m; Inj. Ashored, Carus laboratories, Haryana), which was initiated on day 5 of estrous cycle as day 0. Seven days later PGF<sub>2</sub>α injection (Cloprostenol sodium, 500 µg, i/m; Inj. Zolcol, Carus laboratories) was given. Second GnRH Injection was given 48 hrs after the PGF<sub>2</sub>α injection, followed by fixed time artificial insemination (FTAI) twice at 12 and 24 hrs later.

The blood samples were collected on day 0, 7<sup>th</sup>, 9<sup>th</sup> and 25<sup>th</sup> (15<sup>th</sup> day post-FTAI) of the protocol. The blood plasma separated by centrifugation was stored at -20°C till hormonal analysis. The plasma progesterone (ng/ml) and estradiol (pg/ml) hormones were quantified by using commercially available ELISA kits, manufactured by Calbiotech. The cows which did not show signs of estrus following FTAI were examined per rectum 60 day post-insemination to confirm the pregnancy, and the cows which did not conceive at first insemination were re-inseminated at subsequent estrous cycles. The data on hormonal parameters were analyzed using general linear model repeated measure ANOVA for group, time period and their interaction effects (Snedecor and Cochran, 1994) at 5% level of significance using SPSS software (IBM® SPSS® statistics, version 20.0).

## RESULTS AND DISCUSSION

The overall conception rates following administration of modified ovsynch protocol in repeat breeding crossbred cows with prolonged estrus (group-I) and normal estrus (group-II) were 60 and 70%, respectively. The concentration of progesterone on 5<sup>th</sup> day of cycle (i.e. day 0 of ovsynch protocol) was non-significantly higher in prolonged estrus as compared to normal estrus group of repeat breeding cows. Further, the P<sub>4</sub> concentration was significantly (p < 0.05) higher on day 7<sup>th</sup> and 9<sup>th</sup> in repeat breeding cows with prolonged estrus than that of normal estrus group. The progesterone concentration declined significantly on day 9<sup>th</sup> following injection of PGF<sub>2</sub>α on day 7<sup>th</sup>, thereafter, significantly rose on day 25<sup>th</sup> (day 15<sup>th</sup> post-AI) in both the groups. In the present study, plasma 17-β estradiol concentration was significantly (p < 0.05) lower in prolonged estrus as compared to normal estrus group of repeat breeding cows at all the periods. In both the groups, the initial 17-β estradiol concentration declined significantly (p < 0.05) on day 7, and rose significantly (p < 0.05) on day 9 of ovsynch protocol (Table 1).

Similarly elevated P<sub>4</sub> concentration in animals that had extensive prolonged estrus (69-96 hrs) as compared to prolonged estrus (36-60 hrs) was reported by Singh *et al.* (2012). Dadarwal *et al.* (2007) and Bedi *et al.* (2007) also reported suprabasal progesterone levels at estrus and delayed post-ovulatory rise in progesterone associated with prolonged estrus. The prolonged duration of estrus might be due to failure of timely release of LH from the anterior pituitary and increase in the level of follicular estrogen leading to intense estrus signs over a period in repeat breeder cows. Rao (2008) observed higher P<sub>4</sub> concentration (5.77 ± 0.36 ng/ml) on day 10 of estrous cycle following treatment with ovsynch protocol. The response of ovsynch protocol depends on the day of its initiation during estrous cycle (Vasconcelos *et al.*, 1999). The higher estradiol concentration in repeat breeder cows synchronized with ovsynch was also reported by Ahammed *et al.* (2018). Significantly higher estradiol concentration observed might be due to increased follicular activity post-treatment of double dose of GnRH and PGF<sub>2</sub>α (Noseir, 2003). Significantly higher P<sub>4</sub> and lower E<sub>2</sub> on day 7<sup>th</sup> and 9<sup>th</sup> of the treatment observed in prolonged estrus

**Table 1:** Plasma progesterone and 17-β estradiol concentrations in repeat breeding crossbred cows with prolonged (Gr-I) and normal (Gr-II) estrus treated with ovsynch protocol

Days of Ovsynch treatment	Progesterone (ng/ml)		Estradiol (pg/ml)	
	Group-I (n=10)	Group-II (n=10)	Group-I (n=10)	Group-II (n=10)
0	3.51 ± 0.45 <sup>a</sup>	2.97 ± 0.38 <sup>a</sup>	44.36 ± 1.74 <sup>a</sup> <sub>A</sub>	54.56 ± 2.49 <sup>a</sup> <sub>B</sub>
7 <sup>th</sup>	2.83 ± 0.23 <sup>a</sup> <sub>A</sub>	2.08 ± 0.19 <sup>a</sup> <sub>B</sub>	36.17 ± 2.67 <sup>b</sup> <sub>A</sub>	45.35 ± 2.79 <sup>b</sup> <sub>B</sub>
9 <sup>th</sup>	1.99 ± 0.13 <sup>b</sup> <sub>A</sub>	1.18 ± 0.16 <sup>b</sup> <sub>B</sub>	51.10 ± 2.24 <sup>c</sup> <sub>A</sub>	66.27 ± 1.78 <sup>c</sup> <sub>B</sub>
25 <sup>th</sup>	5.33 ± 0.41 <sup>c</sup>	4.45 ± 0.38 <sup>c</sup>	31.35 ± 1.56 <sup>b</sup> <sub>A</sub>	46.79 ± 2.23 <sup>b</sup> <sub>B</sub>

Means bearing different superscripts (a, b, c) within the column and different subscripts (A, B) within the row for a parameter differ significantly (p < 0.05).

**Table 2:** Plasma progesterone and estradiol concentration in ovsynch treated conceived and non-conceived cows of prolonged (Gr-I) and normal (Gr-II) estrus (Mean  $\pm$  SE)

Day of ovsynch treatment	Group-I (Prolonged estrus)		Group-II (Normal estrus)	
	Conceived (n=6)	Non-conceived (n=4)	Conceived (n=7)	Non-conceived (n=3)
<i>Progesterone (ng/ml)</i>				
0	3.67 $\pm$ 0.61 <sup>a</sup>	3.28 $\pm$ 0.75 <sup>ab</sup>	3.09 $\pm$ 0.47 <sup>ab</sup>	2.69 $\pm$ 0.72 <sup>ab</sup>
7 <sup>th</sup>	2.87 $\pm$ 0.31 <sup>a</sup>	2.75 $\pm$ 0.38 <sup>ab</sup>	2.14 $\pm$ 0.24 <sup>a</sup>	1.94 $\pm$ 0.36 <sup>a</sup>
9 <sup>th</sup>	1.99 $\pm$ 0.18 <sup>b</sup>	1.99 $\pm$ 0.22 <sup>a</sup>	1.34 $\pm$ 0.16 <sup>c</sup>	0.78 $\pm$ 0.25 <sup>c</sup>
25 <sup>th</sup>	6.11 $\pm$ 0.34 <sup>c</sup> <sub>A</sub>	4.15 $\pm$ 0.42 <sup>b</sup> <sub>B</sub>	4.72 $\pm$ 0.45 <sup>b</sup>	3.81 $\pm$ 0.69 <sup>b</sup>
<i>Estradiol (pg/ml)</i>				
0	44.49 $\pm$ 2.38 <sup>ab</sup>	44.18 $\pm$ 2.91 <sup>ab</sup>	53.74 $\pm$ 3.11 <sup>a</sup>	56.45 $\pm$ 4.76 <sup>ab</sup>
7 <sup>th</sup>	37.13 $\pm$ 3.61 <sup>a</sup>	34.72 $\pm$ 4.43 <sup>b</sup>	47.34 $\pm$ 3.29 <sup>ab</sup>	40.68 $\pm$ 5.03 <sup>b</sup>
9 <sup>th</sup>	49.74 $\pm$ 2.97 <sup>b</sup>	53.13 $\pm$ 3.64 <sup>a</sup>	66.12 $\pm$ 2.26 <sup>c</sup>	66.63 $\pm$ 3.45 <sup>a</sup>
25 <sup>th</sup>	28.15 $\pm$ 1.16 <sup>c</sup> <sub>A</sub>	36.15 $\pm$ 1.42 <sup>b</sup> <sub>B</sub>	43.35 $\pm$ 1.76 <sup>b</sup> <sub>A</sub>	54.81 $\pm$ 2.68 <sup>b</sup> <sub>B</sub>

Means bearing different superscripts (a, b, c) within the column and different subscripts (A, B) within the row for a parameter differ significantly ( $p < 0.05$ ).

group might have resulted in lower response to ovsynch in terms of ovulation and fertility in the present study.

In prolonged estrus group, the initial plasma progesterone concentration declined gradually on day 7 and 9 of treatment in both conceived and non-conceived cows with statistically significant ( $p < 0.05$ ) difference in conceived subgroup. In contrast, the initial plasma estradiol concentration dropped a little on day 7 and then increased significantly in both the subgroups (Table 2). Similar trend was also observed in both conceived and non-conceived sub-groups of normal estrus cows with little lower values at all periods than the prolonged estrus cows. The plasma progesterone level in conceived cows of prolonged estrus group was significantly ( $p < 0.05$ ) higher on 25<sup>th</sup> day of initiation of protocol (15<sup>th</sup> day post-FTAI) as compared to non-conceived cows (6.11  $\pm$  0.34 vs. 4.15  $\pm$  0.42 ng/ml), whereas, estradiol concentration showed inverse trend (Table 2). Treatment with 2<sup>nd</sup> GnRH injection prior to insemination is responsible for timely ovulation and increase in progesterone that necessarily supports the development of an embryo, hence resulting in better conception in repeat breeder animals (Savalia *et al.*, 2014). Doubling the GnRH dose results in an increase in ovulatory response only in cows with P<sub>4</sub> concentrations exceeding 1 ng/ml at the time of GnRH administration (Giordano *et al.*, 2013). Higher conception rate (80 %) using 2.5 ml GnRH intramuscular at insemination in prolonged estrus cattle has been reported by Prasanth (2016). Response to ovsynch protocol also may rely upon the preovulatory concentrations of estradiol following ovsynch (Lamb *et al.*, 2010) and day of initiation of protocol during estrous cycle (Vasconcelos *et al.*, 1999). The higher conception rate achieved in the present study, might be due to double dose of GnRH that would have increased the magnitude of LH surge, and thereby improving the ovulatory and fertility response in repeat breeding cattle with prolonged as well as normal estrus (Giordano *et al.*, 2013).

In repeat breeder cattle, GnRH administration at estrus or during luteal phase increases plasma progesterone and delays luteolytic response, thus enhancing the embryo survival rate (Jaswal *et al.*, 2016). The ovulation is delayed in the animals showing prolonged estrus than those with normal duration of estrus (Singh, 2003). Repeat breeder cows studied might have delayed rise in progesterone after treatment which could be due to either delay in ovulation leading to delayed CL formation or suboptimal progesterone production by the CL formed after normal ovulation (Honparkhe *et al.*, 2006; Bedi *et al.*, 2007). It is concluded that the modified ovsynch protocol improves the conception/ fertility response in repeat breeder crossbred cows with prolonged estrus by modulating estrogen-progesterone levels.

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