RESEARCH ARTICLE

Biochemical Alterations and Fertility Response to Ovsynch Protocol in Repeat Breeding Crossbred Cows with Prolonged Estrus

Dhaval N. Patel[1]*, Haresh C. Nakhashi[1], Tarun V. Sutaria[1], Ravji K. Chaudhari[1], Prakash M. Chauhan[2]

Abstract

The present study was conducted to determine the biochemical profile and fertility response in repeat breeder crossbred cows having normal and prolonged estrus by using 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 ovsynch protocol starting from 5th day of estrous cycle using GnRH-PG-GnRH (20, 500 and 20 µg) on day 0, 7 and 9 with FTAI on day 10. The blood was collected on day 0, 7th, 9^{th,} and 25th (15th day post-FTAI) of the treatment to determine plasma glucose, total protein and total cholesterol. Pre-treatment mean duration of estrus in Group-I and Group-II cows was 84.60 ± 5.35 and 25.60 ± 2.79 hrs, respectively, and it declined significantly ($p < 0.05$) following ovsynch treatment (39.80 ± 5.56 hrs) in Group-I, although estrous cycle lengths were identical in both the groups. The conception rates in group I and II were 60 and 70%, respectively. The cows with prolonged estrus had significantly lower mean blood glucose concentration than those with normal estrus, and the level was also significantly lower on day 9th (induced estrus) in both the groups. The plasma total protein concentration was significantly lower on 0, 9^{th,} and 25th day in Group-I than in Group-II. A non-significant difference existed for protein levels within the conceived crossbred cows, whereas the difference was significant within non-conceived crossbred cows. The overall plasma cholesterol concentration was non-significantly higher in Group-I than the Group-II. Blood glucose, plasma protein and plasma total cholesterol concentrations differed non-significantly between the conceived and non-conceived crossbred cows at all periods in both the groups.

Keywords: Biochemical profile, Crossbred cow, Fertility, Prolonged estrus, Ovsynch protocol.

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Introduction

Repeat breeding, a major reproductive disorder in dairy cattle, is associated either with failure of fertilization or early embryonic death. Among the major functional causes of repeat breeding, hormonal insufficiency and dysfunction contribute about 40.1% cases. There are altered hormone estrogen, progesterone, and LH levels in repeat breeders associated with prolonged estrus and extended estrus-to-ovulation interval (Bloch *et al.,* 2006). Among the repeat breeding cattle, approximately 30-40 % shows prolonged estrus (37-60 vs. 24-36 hrs) and 70 % recorded marginally elevated plasma progesterone levels (0.30 to 0.35 ng/mL) during or around estrus (Singh *et al*., 2012). Clinically, prolonged estrus can be easily diagnosed and has been treated successfully by synchronized timed insemination protocols (Lopez-Gatius *et al.,* 2001, Patel *et al.,* 2020). The literature on blood biochemical profile in repeat breeding cattle without and with synchronization treatment is meagerly reported and is unclear (Rao, 2008; Kumar, 2014; Nath *et al.,* 2019). Conception rate with ovsynch protocol usually is improved when initiated during mid-diestrus, *i.e*., days 5-12 of the estrous cycle (Tiwari *et al.,* 2019), but its use in repeat breeding cattle with prolonged estrus is meager. Therefore, the present study was planned to evaluate the efficacy of ovsynch protocol in terms of fertility response and biochemical profile in repeat breeding crossbred cattle with normal and prolonged estrus.

[1]Dept of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, India

[2]Dept of Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, India

Corresponding Author: Dhaval N. Patel, Dept of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, e-mail: dhavalpatel134@gmail.com

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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 based on estrus duration; the cows showing estrus duration > 28 hrs were included in the prolonged estrus group (n=10; Group-I), whereas cows with estrus duration of18-28 hrs were included in normal estrus group (n=10; Group-II). Animals of both the groups were treated with ovsynch protocol by using GnRH analogue (Busereline acetate, 20 µg, i/m), on day 5 of the estrous cycle (as day 0). Seven days later $PGF_2\alpha$ injection (Cloprostenol sodium, 500 µg, i/m) was given. Second GnRH Injection was given 48 hrs after the $PGF_2\alpha$ injection, followed by fixed-time artificial insemination (FTAI) twice at 12 and 24 hours later.

The blood samples were collected in K_3EDTA vials on day 0, 7th, 9th and 25th (15th day post-FTAI) of the protocol. The blood glucose was assayed directly from fresh blood samples using Dr. Morepen Gluco One BG-03 meter (M/S Tianjin Empecs Medical Devise Co. Ltd). The blood plasma separated out by centrifugation was stored at -20°C until analyzed for biochemical profile. The plasma total protein and total cholesterol levels were determined using commercial diagnostic kits of Trans-Asia Bio-Medical Ltd., Malpur, Baddi, and Dist. Solan (HP), India, on Biochemistry Analyzer RX-50V (Micro Lab, India). The cows which did not show signs of estrus following FTAI were examined per rectum 60-day post-insemination to confirm the pregnancy. The cows which did not conceive at FTAI were re-inseminated at subsequent estrous cycles. The data on biochemical parameters were analyzed using a general linear model repeated measure ANOVA for group, period, and interaction effects at a 5% level of significance using SPSS software (IBM® SPSS® statistics, version 20.0).

Results and Discussion

Duration of Estrus, Estrous Cycle, and Fertility

In the present study, estrus duration pre-treatment in Group-I (prolonged estrus) and Group-II (normal estrus) cows was recorded as 84.60 ± 5.35 and 25.60 ± 2.79 hrs, respectively. This was in close agreement with the findings of Dadarwal *et al.* (2005) and Singh *et al.* (2012). The prolonged duration of estrus in repeat breeding cows observed might be due to the failure of or delayed release of LH from the anterior pituitary and increased follicular estrogen level leading to intense estrus signs over an extended period.

In the present study, the duration of estrus declined significantly ($p < 0.05$) following ovsynch treatment as compared to pre-treatment value (39.80 ± 5.56 vs. 84.60 ± 5.35 hrs) in Group-I cows (Table 1), which corroborated well with Honparkhe *et al.* (2010), who reported pre-and post-treatment duration of estrus as 74.40 ± 3.90 and 33.60 ± 2.40 hrs. Significant reduction in estrus duration following ovsynch protocol might be associated with timely release of LH from the pituitary leading to ovulation and cessation of estrus (Dadarwal *et al.,* 2005). The slow ovulatory follicle growth and CL regression could be associated with estrus characteristics' persistence, optimized following PGF2α administration during the early-luteal phase of cows (Ghuman *et al.*, 2014).

The estrous cycle length was 19.40 ± 0.76 and 18.30 ± 0.65 days in Group-I and II, respectively, which did not differ significantly. The overall conception rates following administration of ovsynch protocol in repeat breeding crossbred cows with prolonged estrus (group-I), and normal estrus (group-II) with follow up of four cycles were 60 and 70%, respectively (Table 1). These findings concurred well with Honparkhe *et al.* (2010).

Blood Glucose

The concentrations of blood biochemical parameters estimated in two groups of cows at various periods are presented in Table 2 and those of conceived and non-conceived cows in Table 3.

Statistical analysis revealed a significant ($p < 0.05$) difference in glucose concentration between and within the groups. The overall blood glucose concentration was significantly lower in Group-I than the Group-II (56.85 ± 1.68 vs. 72.05 ± 1.68 mg/dl). These observations agreed well with various workers (Nath *et al.*, 2019; Kumar, 2014 and Ahmed *et al.*, 2017), who observed significantly low serum glucose levels during prolonged estrus (2-4 days duration) in repeat breeder crossbred cattle as compared to control group. The low level of blood glucose in prolonged estrus

Table 1: Effect of ovsynch protocol on the duration of estrus, estrous cycle and fertility in repeat breeding crossbred cows with normal and prolonged estrus (Mean ± SE)

Parameters	*Group-I (n = 10)*	*Group-II (n = 10)*
Duration of estrus pre-treatment (hrs)	$84.60 \pm 5.35^{a}{}_{A}$	$25.60 \pm 2.79^{a}{}_{B}$
Duration of estrus post-treatment (hrs)	$39.80 \pm 5.56^{b}{}_{A}$	$24.00 \pm 1.89^{a}{}_{B}$
Length of estrous cycle (days)	19.40 ± 0.76	18.30 ± 0.65
Overall post-treatment fertility (%)	60.00 (6/10)	70.00 (7/10)
Days at fertile estrus post-treatment	45.50	22.14

Means bearing different superscripts (a, b) in column and subscripts (A, B) in raw differ significantly ($p < 0.05$)

Table 2: Plasma glucose, total protein and total cholesterol concentrations in repeat breeding crossbred cows with prolonged (Gr-I) and normal (Gr-II) estrus treated with ovsynch protocol

Days of Ovsynch treatment	*Glucose (mg/dL)*		*Total protein (g/dl)*		*Total Cholesterol (mg/dL)*	
	Group-I (n = 10)	*Group – II (n = 10)*	*Group-I (n = 10)*	*Group – II (n = 10)*	*Group-I (n = 10)*	*Group – II (n = 10)*
0	$60.20 \pm 2.59^{a}_{A}$	$73.40 \pm 2.26^{ab}_{B}$	$5.62 \pm 0.23_{A}$	$7.12 \pm 0.46^{ab}_{B}$	192.56 ± 14.10	188.68 ± 19.45
7th	$57.50 \pm 2.24^{a}_{A}$	$73.50 \pm 2.89^{a}_{B}$	5.27 ± 0.61	6.84 ± 0.59^{a}	176.93 ± 25.47	162.42 ± 19.99
9th	$52.30 \pm 1.86^{b}_{A}$	$69.00 \pm 2.95^{b}_{B}$	$5.21 \pm 0.59_{A}$	$8.11 \pm 0.44^{b}_{B}$	205.21 ± 13.11	178.75 ± 19.68
25th	$57.40 \pm 1.86^{ab}_{A}$	$72.30 \pm 2.22^{ab}_{B}$	$5.12 \pm 0.59_{A}$	$7.46 \pm 0.47^{ab}_{B}$	202.13 ± 12.32	164.46 ± 18.80
Overall	56.85 ± 1.68	72.05 ± 1.68	5.31 ± 0.29	7.38 ± 0.29	194.20 ± 14.09	173.57 ± 14.09

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 3: Plasma glucose, total protein and total cholesterol concentration in ovsynch treated conceived and non-conceived cows of prolonged (Gr-I) and normal (Gr-II) estrus (Mean ± SE)

	Group-I (Prolonged estrus)		*Group-II (Normal estrus)*	
Day of ovsynch treatment	*Conceived (n = 6)*	*Non-conceived (n = 4)*	*Conceived (n = 7)*	*Non-conceived (n = 3)*
Blood glucose (mg/dl)				
0	63.00 ± 3.17^{a}	56.00 ± 3.89	72.14 ± 2.74	76.33 ± 4.19
7th	60.00 ± 2.73^{a}	53.75 ± 3.35	70.57 ± 3.91	80.33 ± 5.97
9th	53.33 ± 2.48^{b}	50.75 ± 3.04	66.71 ± 3.43	74.33 ± 5.25
25th	57.67 ± 2.54^{ab}	57.00 ± 3.11	69.88 ± 2.33	78.00 ± 3.56
Overall mean	58.50 ± 2.39	54.38 ± 3.23	69.82 ± 3.11	77.25 ± 4.89
Total protein (g/dl)				
0	5.35 ± 0.28	6.02 ± 0.34^{ab}	7.27 ± 0.57	6.78 ± 0.87^{ab}
7th	5.52 ± 0.82	4.89 ± 1.00^{a}	6.66 ± 0.73	7.27 ± 01.11^{a}
9th	4.32 ± 0.63	6.56 ± 0.77^{b}	7.49 ± 0.38	9.56 ± 0.59^{b}
25th	5.39 ± 0.79	4.72 ± 0.96^{ab}	7.22 ± 0.58	8.00 ± 0.89^{ab}
Overall mean	5.15 ± 0.58	5.54 ± 0.87	7.16 ± 0.52	7.90 ± 0.79
Total cholesterol (mg/dl)				
0	183.94 ± 18.66	205.49 ± 22.86	202.93 ± 22.88	155.43 ± 34.94^{a}
7th	177.87 ± 34.87	175.51 ± 42.71	160.80 ± 25.32	166.19 ± 38.68^{ab}
9th	210.85 ± 17.67	196.74 ± 21.65	170.84 ± 24.43	197.20 ± 37.31^{a}
25th	206.51 ± 16.69	195.56 ± 20.44	167.44 ± 23.76	157.50 ± 36.29^{ab}
Overall mean	194.78 ± 12.92	193.32 ± 13.37	175.50 ± 12.81	169.08 ± 21.36

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

may be attributed to increased peripheral glucose uptake, failure of gluconeogenesis or glycogenolysis or endogenous hyperinsulinemia (Mukherjee *et al.,* 2011), which may reduce luteal function in repeat breeding cows.

The initial blood glucose level (60.20 ± 2.59 mg/dl) declined significantly on day 9 (52.30 ± 2.24 mg/dl) and thereafter non-significantly rose on 25th day (57.40 ± 1.86 mg/dl) of treatment in Group-I cows. The trend was similar in Group-II with significant difference between 7th and 9th days. Nath *et al.* (2019) observed the higher glucose level on day 3rd and day 5th of estrus in both prolonged and normal estrus duration cows. This variation in the blood glucose level during the estrous cycle might also be associated with hyperpituitarism and increased production of adrenocorticotrophic hormones (Kumar, 2014).

The blood glucose concentrations did not differ significantly between conceived and non-conceived subgroups at any of the periods or overall in Group-I and II cows (Table 3). The non-significantly higher blood glucose level in conceived than non-conceived cows of Group-I (prolonged estrus) agreed with Chandrakar *et al.* (2003). However, in group II (normal estrus), the trend was reversed in our study.

Plasma Total Proteins

The overall mean plasma total protein concentration was

significantly ($p < 0.05$) lower in Group-I than the Group-II (5.31 ± 0.29 vs. 7.38 ± 0.29 g/dL). Further statistical analysis revealed a significant difference ($p < 0.05$) between the groups at various periods of treatment, except on 7th day, in Group-II, but not in group-I (Table 2). Group-II cows showed a significantly ($p < 0.05$) higher total protein concentration on 9th day as compared to 7th day, while the levels on day 0 and 25th were at par. This might be due to induced estrus following $PGF_2\alpha$ administration leading to protein metabolism to avail the essential amino acids needed for synthesis of hormones and ovarian/follicular activity (Dadarwal *et al*., 2005). Nath *et al*. (2019) found a non-significant difference in the level of serum total protein between prolonged estrus (2-4 days) and normal estrus groups, while Rao (2008) recorded higher total protein level in repeat breeder crossbred cattle treated with ovsynch protocol.

Low plasma protein level might cause reproductive hormonal disturbances in animals leading to inactive ovaries (Amle *et al*., 2014). The levels were significantly lower at all the periods in Group-I than those of Group-II, except on 7th day or mid-luteal phase of estrous cycle following first injection of GnRH, which agreed well with the significantly low levels of protein at estrus in repeat breeder cows and non-significantly differing levels at early and mid-luteal phases observed by Manjunatha *et al.* (2001).

Non-significant difference was observed between conceived and non-conceived crossbred cattle for protein level in both the groups (Table 3), which corroborated with findings of Kumar *et al*.(2009). However, a significantly higher total protein level in fertile estrus compared to non-fertile estrus in crossbreds (Srivastava and Sahni, 2000; Chandrakar *et al,* 2003; Pandey *et al.,* 2009) has been reported. The optimum protein level is necessary for the development of endocrine and sex organs.

Plasma Total Cholesterol

The plasma total cholesterol concentration was non-significantly higher in Group-I than in Group-II cattle without period effect with an overall value of 194.20 ± 14.09 vs. 173.57 ± 14.09 mg/dl, respectively. These findings corroborated with Nath *et al*. (2019). Though the difference was non-significant within group, an increased level on the day of induced estrus (9th day) corroborated with the findings of Santhiamoorthy *et al*. (2005), Rao (2008), and Ammu *et al.* (2012) following ovsynch protocol. An increase in the cholesterol level at induced estrus might be due to increased adrenal cholesterol when the repeat breeder female is under the prolonged estrogen-dominant phase or increased steroidogenesis in the active luteal cells midluteal phase of the estrous cycle (Khan *et al*., 2010). The variation in serum cholesterol level might be attributed to several factors like fertility status of the animal, stage of the estrous cycle and genotype (Nath *et al*., 2019). Further, lower cholesterol level in repeat breeding cows indicates positive association of cholesterol with reproductive performance (Kumar, 2014).

The plasma total cholesterol concentration of conceived and non-conceived cows among two groups showed non-significant difference at all the periods and also between periods within the subgroup, except non-conceived cows of Group-II, which showed significantly ($p < 0.05$) higher level on 9th day as compared to 0 day of treatment, while values on 7th and 25th day were intermediate (Table 3). The overall mean concentration of cholesterol in conceived and non-conceived repeat breeder cattle was almost identical in both groups**.** The significantly higher cholesterol levels in non-conceived than conceived groups of repeat breeding cows (Patel *et al*., 2014), and higher cholesterol at estrus and at 20-22 days post-insemination in non-conceived than the conceived crossbred cows (Kumar *et al*., 2009) have been reported earlier. However, a significantly higher level of total cholesterol in fertile estrus as compared to non-fertile estrus was reported in crossbred cows (Srivastava and Sahni, 2000).

The fluctuation of the cholesterol level observed in the present study seems to have a strong relationship with dominant follicle and peak CL activity of estrous cycle that secrete the sex steroidal hormones (Jain and Pandita, 1995). Cholesterol is a known precursor for the biosynthesis of steroid hormones either from gonad or adrenal cortex (Hu *et al.,* 2010). The total cholesterol content at estrus was relatively higher in $PGF_2\alpha$ treated cows (Nath *et al*., 2019).

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