

*Original Research***Impact of Different Ovulation Synchronization Protocols on Plasma Profile of Progesterone, Protein and Cholesterol in Cyclic Repeat Breeder Crossbred Cows**

A. R. Prajapati, A. J. Dhami\*, K. K. Hadiya and J. A. Patel

Department of Animal Reproduction, Gynaecology &amp; Obstetrics, College of Veterinary Science &amp; Animal Husbandry, AAU, Anand-388 001, Gujarat, INDIA

\*Corresponding author: [ajdhami@aau.in](mailto:ajdhami@aau.in)

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**Abstract**

A study was undertaken in field conditions on 50 repeat breeder HF crossbred cows to evaluate the efficacy of four estrus/ovulation synchronization protocols, viz., Ovsynch, Ovsynch Plus, Doublesynch, and Estradoublesynch (10 cows in each protocol, and in control) in terms of conception rate at induced estrus with fixed time AI (FTAI) and monitoring plasma progesterone, protein and cholesterol profile at different time intervals. All the cows received pre-synchronization treatment, i.e., Inj. 100 mg ivermectin s/c, Inj. tono-vita 10 ml each, single shot Inj. enrofloxacin 40 ml i/m, and bolus Minotas 1 daily for 7 days. The conception rates at induced cycle/FTAI were 30, 30, 40 and 20 %, respectively, with Ovsynch, Ovsynch Plus, Doublesynch, and Estradoublesynch protocols, and 10% in control group. The rests were taken as non-conceived cows. The overall mean plasma progesterone concentrations (ng/ml) compared on day 0 (start of treatment), 7/9/10 (PGF<sub>2</sub> $\alpha$  injection), 9/12/13(FTAI) and on day 12 post-AI in cows under different protocols revealed significant ( $P<0.01$ ) effect of sampling days in all 4 protocols with higher values on day of PGF<sub>2</sub> $\alpha$  injection and on day 12 post-AI compared to day 0 and day of FTAI. Moreover, the mean plasma progesterone concentrations were found to be significantly ( $P<0.05$ ) higher in conceived than non-conceived cows on day 12 post-AI under all 4 protocols. No significant influence of sampling days and conceived-nonconceived status of cows was observed on plasma protein profile in any of the groups studied. The overall mean plasma cholesterol concentration (mg/dl) for cows under Ovsynch and Ovsynch Plus protocols were  $140.74\pm 3.29$  and  $151.72\pm 3.78$ , respectively, which did not vary significantly between sampling days. Under Estradoublesynch protocol, the period effect was significant with higher cholesterol during luteal phase of cycle. The non-conceived cows had consistently higher mean cholesterol than conceived cows on all sampling days in all groups, but differed significantly ( $P<0.05$ ) in Ovsynch protocol with pooled means of  $148.85\pm 3.33$  vs.  $121.80\pm 4.23$  mg/dl, and in Doublesynch protocol  $174.20\pm 5.61$  vs.  $150.50\pm 7.90$  mg/dl. The results showed that Doublesynch protocol is better with respect to modulation of plasma progesterone and cholesterol profile and conception rate in repeat breeding cows.

**Key words:** Crossbred Cows, Cholesterol, Conception Rate, Ovulation Synchronization, Plasma Progesterone, Repeat Breeding,

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## Introduction

In the current era, different estrus induction and ovulation synchronization protocols are in use to treat postpartum anestrus and repeat breeding problems in dairy animals, but with inconsistent results (Bhoraniya *et al.*, 2012, Ahmed *et al.*, 2016). The causes of repeat breeding are many and the nutritional inadequacy, endocrine imbalance and asynchrony between artificial insemination (AI) and ovulation are considered the major ones. Plasma progesterone levels denote either the presence or absence of CL and its functional competency which is directly related with fertility of the female. The progesterone hormone is responsible for stimulation of cyclicity, follicular development and also for continuation of pregnancy. During timed AI protocol, elevation in plasma progesterone concentration is generally achieved by ovulation resulting in an accessory CL or by supplementation with exogenous progesterone that decreases double ovulation and increases fertility to the timed AI. After AI, circulating progesterone is critical for embryo growth, and for establishment and maintenance of pregnancy (Wiltbank *et al.*, 2012). The plasma protein and cholesterol profile denote nutritional status of animal and are related with its fertility, but were not influenced significantly by estrus synchronization treatment (Savalia *et al.*, 2014, Nakrani *et al.*, 2015). The cholesterol being precursor of steroid hormones play an important role in steroidogenesis. In view of above facts, this study was aimed to evaluate whether different ovulation synchronization protocols modulate plasma progesterone and biochemical profile and enhance fertility in repeat breeding crossbred cows under field conditions.

## Material and Methods

### Selection of Animals and Pre-Synchronization Treatment

The study was carried out in villages under the milk shed areas of Amul, Anand and Panchamrut Dairy, Godhara, Gujarat from August 2016 to February 2017. Fifty repeat breeding (RB) crossbred cows (>120 days postpartum) were confirmed on the basis of failure of conception with regular cyclicity and quality AI for 3 or more times without visible or palpable genital abnormality. All these cows were initially injected once with 100 mg ivermectin s/c (Inj. Ivectin, 10 ml, Indian Immunologicals Ltd), injection organic phosphorus (Inj. Tonophosphan, MSD Animal Health) and multivitamins AD<sub>3</sub>E (Inj. Intavita-H, Intas Pharma) 10 ml each, and single shot i/m injection of enrofloxacin (Inj. Flobac SA, 40 ml, Intas Pharma), and were supplemented with bolus Minotas (Intas Pharma) @ 1 bolus daily for 7 days. They

were then randomly divided into 5 equal groups (n=10 each) and were subjected to the following four estrus induction/ovulation synchronization protocols, keeping one group as untreated control.

### **Ovsynch and Ovsynch plus Protocols**

Under Ovsynch protocol, the cows were administered with i/m Inj. of GnRH analogue, i.e. Buserelin acetate 20 µg (Receptal, 5 ml, MSD) on day 0, Inj. of PGF<sub>2</sub>α analogue, i.e. Cloprostenol sodium 500 µg (Estrumate, 2 ml, MSD) on day 7, and second Inj. of 10 µg GnRH on day 9, followed by fixed time AI (FTAI) twice at 16 and 24 hrs later. In Ovsynch Plus protocol, the cows received an additional i/m Inj. of PMSG 500 IU (Folligon, MSD) 2 days before the actual Ovsynch protocol.

### **Doublesynch and Estradoublesynch Protocols**

Under Doublesynch protocol, the RB cows received i/m Inj. of 500 µg Cloprostenol sodium on day 0, Inj. of 20 µg Buserelin acetate on day 2, 500 µg Cloprostenol sodium on day 9 and second Inj. of 10 µg GnRH analogue on day 11, followed by FTAI twice at 16 and 24 hrs later, while in Estradoublesynch protocol, the cows received an Inj. of estradiol benzoate 1 mg (Sigma, USA) on day 10, in place of second GnRH injection on day 11 in Doublesynch, with FTAI twice at 48 and 60 hrs post-estradiol injection.

### **Control Group**

Ten repeat breeder cows given pre-synchronization treatment as above, but without any hormonal intervention, and followed for spontaneous estrus and insemination, served as control. Animals inseminated at induced/spontaneous estrus, if not returned to next estrus, were followed for pregnancy per-rectum 60 days post-AI.

### **Blood Sampling and Assay Profile**

Blood samples were collected from jugular vein in heparinized vacutainers on day 0 - just before treatment, at the time of PGF<sub>2</sub>α administration (day 7/9/10 as per protocol), induced estrus/FTAI (day 9/12/13) and on day 12 post-AI. The blood samples were centrifuged at 3000 rpm for 15 minutes. The plasma separated out was stored in deep freezer at -20°C with a drop of sodium merthiolate (0.1%). The plasma progesterone concentration was determined by employing standard Radio-Immuno-Assay (RIA) technique of Kubasic *et al.* (1984). Labeled antigen (I<sup>125</sup>), antibody coated tubes and standards were procured from Immunotech-SAS, France. The plasma total protein and total cholesterol concentrations were estimated by Biuret and CHOD/PAP method, respectively, using standard procedure and assay kits procured from Crest Bio-system, Goa with the help of chemistry analyzer (Mindray, BS 120).

## Statistical Analysis

The cows found pregnant with FTAI and those failed were grouped as conceived and non-conceived subgroups. The data on plasma profiles were analyzed statistically using standard ANOVA (CRD), NMRT and t-test on IBM SPSS 20.00 statistics to know the variations between sampling days, groups and conceived/non-conceived status (Snedecor and Cochran, 1986).

## Results and Discussion

### Conception Rates at Induced Estrus/FTAI

The conception rates obtained at induced estrus with FTAI in RB cows under Ovsynch, Ovsynch Plus, Doublesynch and Estradoublesynch protocols and in untreated control group were found to be 30, 30, 40, 20 and 10 %, respectively. Patel *et al.* (2013) and Prajapati *et al.* (2015) documented the first service conception rate of 40 and 30 %, respectively, with Ovsynch protocol in RB crossbred cows, while Chaudhary *et al.* (2012) and Parmar *et al.* (2015) found higher conception rate at FTAI (50 %). Vijayrajan *et al.* (2009) found first service conception rate of 50 % in RB crossbred cows treated with Ovsynch versus 20% in untreated control group, while Kalra *et al.* (2011) reported the corresponding figures as 50 and 00 %. The present first service conception rate of 40 % found in RB cows under Doublesynch protocol is comparable with 40 and 48 % reported during summer and winter seasons in buffaloes by Kumar *et al.* (2016), but was slightly lower than that reported by Sahoo *et al.* (2017) in cyclic crossbred cows (55%) and by Mirmahmoudi and Prakash (2012) and Mirmahmoudi *et al.* (2014) in cycling buffaloes using Doublesynch (60 %) and Estradoublesynch (62 %) protocols, respectively, which are much higher than the present findings in RB crossbred cows.

### Plasma Progesterone Profile

The mean plasma progesterone concentration in cows put under all four protocols were found to be more than 1 ng/ml on day 0 (initiation of treatment) which suggested that the animals selected were all cyclic repeat breeder with presence of functional CL on the ovary on that day. In all these groups, the plasma progesterone values on day 7/9/10 (i.e. just before PGF<sub>2</sub> $\alpha$  injection) increased significantly. The levels on both above days were statistically similar among all four protocols. Further, within next 2-3 days, i.e., on the day of FTAI (i.e. on day 9/12/13 of concerned protocol), the mean plasma progesterone levels dropped significantly ( $P < 0.05$ ) to basal values of  $< 0.7$  ng/ml in all the groups, yet the value of Ovsynch Plus protocol was significantly higher than all other protocols. The mean values of plasma progesterone were found to be again increased on day 12 post-AI to the levels of  $3.01 \pm 0.96$ ,  $3.18 \pm 0.86$ ,  $2.18 \pm 0.84$  and  $1.79 \pm 0.77$  ng/ml in Ovsynch, Ovsynch-plus, Doublesynch and Estradoublesynch protocols, respectively, due to formation of CL and establishment of pregnancy in some of these animals following induction of ovulatory estrus and FTAI (Table 1). The present findings closely corroborated with Patel *et al.* (2013)

and Nakrani *et al.* (2015) in repeat breeding cows and buffaloes administered mid-cycle PGF<sub>2</sub>α injection. The mean plasma progesterone concentrations were found to be significantly (P<0.01) higher on day 12 post-AI in conceived than non-conceived cows under Ovsynch, Doublesynch and Estradoublesynch (5.83±0.80 vs. 1.81±1.04, 5.02±0.89 vs. 0.29±0.12 and 5.65±1.35 vs. 0.83±0.47 ng/ml, P<0.01), but in Ovsynch Plus protocol the difference was not significant (5.83±0.80 vs 2.24±0.90 ng/ml).

**Table 1:** Plasma progesterone concentrations in cyclic/repeat breeder crossbred cows on different days of various estrus synchronization protocols and on day 12 post-AI

Estrus synchronization protocol	Status	No	Progesterone (ng/ml) on days from treatment/AI				Overall
			D-0	D-PGF <sub>2</sub> α Inj	Day FTAI	D-12 post-AI	
Ovsynch	Concd	3	2.20±1.06	2.55±1.21	0.14±0.04	5.83±0.80 <sup>y</sup>	2.71±0.72
	Non-cd	7	1.99±0.87	2.58±0.76	0.27±0.16	1.81±1.04 <sup>x</sup>	1.63±0.41
	<b>Overall</b>	<b>10</b>	<b>2.05±0.65<sup>pq</sup></b>	<b>2.57±0.61<sup>q</sup></b>	<b>0.18±0.05<sup>ap</sup></b>	<b>3.01±0.96<sup>q</sup></b>	<b>1.96±0.36</b>
Ovsynch plus	Concd	3	1.46±0.58	4.72±1.30	0.36±0.22	5.39±1.40	2.98±0.77
	Non-cd	7	1.06±0.54	2.81±0.79	0.81±0.24	2.24±0.90	1.73±0.35
	<b>Overall</b>	<b>10</b>	<b>1.18±0.40<sup>p</sup></b>	<b>3.38±0.70<sup>q</sup></b>	<b>0.67±0.18<sup>bp</sup></b>	<b>3.18±0.86<sup>q</sup></b>	<b>2.10±0.34</b>
Doublesynch	Concd	4	0.90±0.64	4.57±1.82	0.10±0.00	5.02±0.89 <sup>y</sup>	2.65±0.73 <sup>y</sup>
	Non-cd	6	0.98±0.58	1.23±0.92	0.11±0.01	0.29±0.12 <sup>x</sup>	0.65±0.27 <sup>x</sup>
	<b>Overall</b>	<b>10</b>	<b>0.95±0.40<sup>pq</sup></b>	<b>2.57±1.01<sup>q</sup></b>	<b>0.11±0.01<sup>ap</sup></b>	<b>2.18±0.84<sup>pq</sup></b>	<b>1.45±0.36</b>
Estra-doublesynch	Concd	2	1.65±1.47	5.45±3.55	0.10±0.00	5.65±1.35 <sup>y</sup>	3.21±1.19
	Non-cd	8	1.19±0.43	3.66±1.02	0.24±0.10	0.83±0.47 <sup>x</sup>	1.48±0.37
	<b>Overall</b>	<b>10</b>	<b>1.28±0.41<sup>p</sup></b>	<b>4.01±0.99<sup>q</sup></b>	<b>0.21±0.08<sup>ap</sup></b>	<b>1.79±0.77<sup>p</sup></b>	<b>1.82±0.38</b>

D-0 = Day of starting the treatment, Concd = Conceived, Non-cd = Non-conceived; Means bearing uncommon superscripts within the column differ significantly (P<0.05) between protocols (a,b) or status (x,y), and those within the row (p,q) differ between days.

This trend of lower plasma progesterone profile in non-conceived repeat breeder cows under all four protocols could be associated with ovulatory failure and/or luteal insufficiency even following hormonally synchronized estrus. However, at all other days of treatment/FTAI, the differences in mean plasma progesterone levels between conceived and non-conceived cows were statistically non-significant. A similar trend with relatively lower mean plasma progesterone levels were reported by Panchal *et al.* (1992) in non-conceiving cyclic buffaloes on day 7 and 14 post-AI, while Gupta *et al.* (1998) observed values of 6.29 vs 1.11 ng/ml on day 18 in conceived and non-conceived RB cows. Oyedipse *et al.* (1986) found non-significantly higher plasma progesterone on day 4, 10 and 16 of AI post-synchronization in conceived than non-conceived zebu cows. Chander *et al.* (2002) also reported lower concentration of plasma progesterone on day 10 of cycle in RB cows than the normal cyclic cows, and suggested luteal insufficiency and/or anovulation as a probable cause of RB. In repeat breeder cows under Ovsynch protocol, the overall mean plasma progesterone on day 0 and day 9/FTAI, and the values on day 0, day of PGF<sub>2</sub>α treatment and day 12 post-AI were statistically similar. For repeat breeder cows under Ovsynch Plus protocol, the overall mean plasma progesterone concentrations on day 0 and day 13/FTAI and on day

of PGF<sub>2</sub>α injection and on day 12 post-AI were also similar, however the values on day 0 and day of FTAI were significantly lower than those found on day of PGF<sub>2</sub>α injection and day 12 post-AI (Table 1). The present trend of mean plasma progesterone concentrations observed in RB cattle under Ovsynch protocol concurred well with Savalia *et al.* (2014) and Parmar *et al.* (2015) in RB crossbred cows and buffaloes. Under Doublesynch and Estradoublesynch protocols also, the trend of plasma progesterone concentration was similar to Ovsynch protocol with lowest/basal values on the day of FTAI and the highest (P<0.05) on day 9, whereas the values on day 0, 12/FTAI and day 12 post-AI were statistically similar (Table 1). The present overall mean plasma progesterone concentration on day 9 was similar to Mirmahmoudi and Prakash (2012) and Mirmahmoudi *et al.* (2014) in cyclic buffaloes. Higher plasma progesterone concentrations during the early pregnancy are related to embryonic development and pregnancy rates. In normal cyclic animals, progesterone level is expected to be high during diestrus stage and gradually reduce during estrus stage. Repeat breeder cases carry irregular levels of progesterone during different stages of the estrous cycle, which prevents the conception and development of embryo.

### Plasma Total Protein Profile

The overall mean plasma total protein profile, irrespective of days, for Doublesynch and Estradoublesynch protocols (7.31±0.08 and 7.17±0.13 g/dl) were similar, yet significantly (P<0.05) lower than the values of Ovsynch and Ovsynch Plus protocols (7.60±0.10 and 7.88±0.11 g/dl), and the values of Ovsynch and Doublesynch protocols were statistically similar and intermediate of other two protocols. Moreover, the levels between conceived and non-conceived cows did not vary significantly in any of the protocol, overall or at any of the sampling days, except in Ovsynch protocol on day 0 where the conceived cows had significantly higher protein than non-conceived cows (7.99±0.04 vs. 7.44±0.23 g/dl). Further, the values did not vary significantly between different days or intervals studied in any of the protocols (Table 2).

The present overall mean plasma protein concentration recorded on day 0 was the highest in cows under Ovsynch Plus protocol and the lowest in Estradoublesynch protocol (7.78± 0.35 vs. 6.86±0.29 g/dl, P<0.05), but the values of Ovsynch, Doublesynch and Estradoublesynch protocols were statistically similar, and so also was the status for values of Ovsynch, Ovsynch Plus and Doublesynch protocols (Table 2). Almost similar trend of protein profile was noted on day just before PGF<sub>2</sub>α injection with significantly higher value in cows under Ovsynch Plus than in Doublesynch protocol (7.81±0.15 vs. 7.15±0.12, P<0.05). The overall mean plasma protein levels on day of FTAI were statistically similar in RB cows under Ovsynch, Doublesynch and Estradoublesynch protocols, and significantly lower than in Ovsynch Plus protocol (8.07±0.22 g/dl). However, no significant variation was noted in the plasma total protein contents on day 12 post-AI in cows under four protocols (Table 2).

**Table 2:** Plasma total protein concentrations in cyclic/repeat breeder crossbred cows on different days of various estrus synchronization protocols and on day 12 post-AI

Estrus synchronization protocol	Status	No	Plasma protein (g/dl) on days from treatment/AI				Overall
			D-0	D-PGF <sub>2</sub> α Inj	Day FTAI	D-12 post-AI	
Ovsynch	Concd	3	7.99±0.04 <sup>y</sup>	7.65±0.25	7.54±0.28	7.54±0.29	7.68±0.11
	Non-cd	7	7.44±0.23 <sup>x</sup>	7.47±0.22	7.33±0.19	8.00±0.41	7.56±0.14
	<b>Overall</b>	<b>10</b>	<b>7.61±0.18<sup>ab</sup></b>	<b>7.52±0.16<sup>ab</sup></b>	<b>7.39±0.15<sup>a</sup></b>	<b>7.86±0.30</b>	<b>7.60±0.10<sup>bc</sup></b>
Ovsynch plus	Concd	3	7.93±0.69	7.57±0.21	7.94±0.47	7.62±0.12	7.76±0.19
	Non-cd	7	7.71±0.43	7.91±0.19	8.13±0.26	7.96±0.25	7.93±0.14
	<b>Overall</b>	<b>10</b>	<b>7.78±0.35<sup>b</sup></b>	<b>7.81±0.15<sup>b</sup></b>	<b>8.07±0.22<sup>b</sup></b>	<b>7.86±0.18</b>	<b>7.88±0.11<sup>c</sup></b>
Doublesynch	Concd	4	6.94±0.17	7.15±0.25	7.61±0.39	7.48±0.57	7.29±0.18
	Non-cd	6	7.26±0.15	7.15±0.13	7.15±0.17	7.73±0.09	7.32±0.08
	<b>Overall</b>	<b>10</b>	<b>7.13±0.12<sup>ab</sup></b>	<b>7.15±0.12<sup>a</sup></b>	<b>7.33±0.19<sup>a</sup></b>	<b>7.63±0.21</b>	<b>7.31±0.08<sup>ab</sup></b>
Estra- doublesynch	Concd	2	6.61±0.51	7.20±1.20	6.67±0.64	6.98±0.77	6.86±0.32
	Non-cd	8	6.92±0.36	7.35±0.27	7.31±0.30	7.41±0.16	7.25±0.14
	<b>Overall</b>	<b>10</b>	<b>6.86±0.29<sup>a</sup></b>	<b>7.32±0.28<sup>ab</sup></b>	<b>7.18±0.27<sup>a</sup></b>	<b>7.32±0.18</b>	<b>7.17±0.13<sup>a</sup></b>

D-0 = Day of starting the treatment, Concd = Conceived, Non-cd = Non-conceived; Means bearing uncommon superscripts within the column differ significantly ( $P < 0.05$ ) between protocols (a,b,c) or status (x,y).

Our findings on plasma protein profile on different days of Ovsynch protocol corroborated well with the observations of Savalia *et al.* (2014) and Parmar *et al.* (2015) in RB cows and buffaloes. Sahoo *et al.* (2017) also observed similar findings on plasma total protein with non-significant differences before and after treatment in cyclic cows treated with Doublesynch and Estradoublesynch protocols. Further, Khan *et al.* (2010) found highest and lowest values of plasma protein on day 5 and day 20 of cycle in RB cows, and the levels were significantly lower than those in normal cyclic cows. Patel *et al.* (2013) however, did not find significant variation in total protein profile of RB cows treated with mid-cycle PGF<sub>2</sub>α and in conceived-non conceived groups.

### Plasma Total Cholesterol Profile

The overall mean plasma cholesterol concentrations were found to be non-significantly different in Ovsynch vs Ovsynch plus, and Doublesynch vs Estradoublesynch (140.74 ±3.29 vs. 151.72±3.78 and 164.20±10.29 vs. 174.15±3.76 mg/dl) protocols, but were significantly higher in latter groups as compared to former groups. The mean plasma cholesterol concentrations were almost identical on day 0 and day of PGF<sub>2</sub>α injection in cows under Ovsynch and Ovsynch Plus protocols and also in Doublesynch and Estradoublesynch protocols. Further, in Ovsynch and Doublesynch protocols the values were statistically similar on day of FTAI and on day 12 post-AI, and all these values were significantly lower than the corresponding values recorded for Ovsynch Plus and Estradoublesynch protocols on day of FTAI and for Doublesynch and Estradoublesynch protocols on day 12 post-AI (Table 3). The effect of different sampling days on mean plasma cholesterol concentrations was significant only in Estradoublesynch

protocol with the lowest values on day 9 and 12 (FTAI) of treatment ( $161.70 \pm 5.75$  and  $167.60 \pm 7.29$  mg/dl) and highest on day 12 post-AI ( $188.80 \pm 7.36$  mg/dl). The mean plasma cholesterol concentrations were found to be significantly ( $P < 0.05$ ) higher in non-conceived than conceived group on all days of treatment including overall means ( $148.85 \pm 3.33$  vs.  $121.80 \pm 4.23$  mg/dl) in repeat breeder cows under Ovsynch protocol.

**Table 3:** Plasma total cholesterol concentrations in cyclic/repeat breeder crossbred cows on different days of various estrus synchronization protocols and on day 12 post-AI

Estrus synchronization protocol	Status	No.	Plasma cholesterol (mg/dl) on days from treatment/AI				Overall
			D-0	D-PGF <sub>2</sub> α inj.	Day FTAI	D-12 post-AI	
Ovsynch	Conceived	3	$123.33 \pm 11.89^x$	$116.33 \pm 11.25^x$	$117.23 \pm 3.21^x$	$130.33 \pm 7.33$	$121.80 \pm 4.23^x$
	Non-Cd	7	$155.42 \pm 6.64^y$	$152.28 \pm 5.59^y$	$143.00 \pm 4.68^y$	$144.71 \pm 9.24$	$148.85 \pm 3.33^y$
	<b>Overall</b>	<b>10</b>	<b><math>145.80 \pm 7.35^a</math></b>	<b><math>141.50 \pm 7.29^a</math></b>	<b><math>135.27 \pm 5.13^a</math></b>	<b><math>140.40 \pm 6.94^a</math></b>	<b><math>140.74 \pm 3.29^a</math></b>
Ovsynch plus	Conceived	3	$134.00 \pm 9.53$	$132.66 \pm 10.49$	$158.33 \pm 4.84$	$153.66 \pm 11.89$	$144.66 \pm 5.34$
	Non-Cd	7	$150.14 \pm 8.02$	$144.42 \pm 9.83$	$164.57 \pm 9.05$	$159.85 \pm 11.65$	$154.75 \pm 4.83$
	<b>Overall</b>	<b>10</b>	<b><math>145.30 \pm 6.49^a</math></b>	<b><math>140.90 \pm 7.46^a</math></b>	<b><math>162.70 \pm 6.38^b</math></b>	<b><math>158.00 \pm 8.58^{ab}</math></b>	<b><math>151.72 \pm 3.78^a</math></b>
Doublesynch	Conceived	4	$143.75 \pm 16.98$	$160.50 \pm 20.70$	$138.25 \pm 14.52$	$159.50 \pm 13.97$	$150.50 \pm 7.90^x$
	Non-Cd	6	$182.00 \pm 14.25$	$175.83 \pm 18.87$	$165.00 \pm 12.16$	$174.00 \pm 14.94$	$174.20 \pm 7.22^y$
	<b>Overall</b>	<b>10</b>	<b><math>166.70 \pm 12.05^{ab}</math></b>	<b><math>169.70 \pm 13.49^b</math></b>	<b><math>154.30 \pm 9.82^{ab}</math></b>	<b><math>168.20 \pm 10.29^{bc}</math></b>	<b><math>164.72 \pm 5.61^b</math></b>
Estradoublesynch	Conceived	2	$163.50 \pm 3.50$	$161.50 \pm 16.50$	$143.00 \pm 12.00$	$182.00 \pm 4.00$	$162.50 \pm 6.56$
	Non-Cd	8	$182.25 \pm 8.97$	$161.75 \pm 6.60$	$173.75 \pm 7.30$	$190.50 \pm 9.19$	$177.06 \pm 4.29$
	<b>Overall</b>	<b>10</b>	<b><math>178.50 \pm 7.52^{bpq}</math></b>	<b><math>161.70 \pm 5.75^{abp}</math></b>	<b><math>167.60 \pm 7.29^{pb}</math></b>	<b><math>188.80 \pm 7.36^{cq}</math></b>	<b><math>174.15 \pm 3.76^b</math></b>

D-0 = Day of starting the treatment. Means bearing uncommon superscripts within the column differ significantly ( $P < 0.05$ ) between protocols (a,b,c) or status (x,y), and those within the row (p,q) differ between days, Non-cd = Non-conceived.

A very similar trend of non-significantly higher plasma cholesterol in non-conceived than conceived cows was also observed for all other protocols on all days, except for overall pooled means in Doublesynch protocol ( $174.20 \pm 7.22$  vs.  $150.50 \pm 7.90$  mg/dl), which differed significantly ( $P < 0.05$ ). Patel *et al.* (2013) reported higher value of cholesterol in non-conceived than conceived ( $230.25 \pm 7.74$  vs  $155.20 \pm 14.40$  mg/dl) RB cows under mid-cycle PGF<sub>2</sub>α protocol. Srivastava and Sahni (2000) observed higher level of cholesterol at estrus/AI in cows turned out to be pregnant than non-pregnant ( $80.29 \pm 3.11$  vs.  $67.34 \pm 1.93$  mg/dl). In contrast to our findings, Sahoo *et al.* (2017) did not observe any variation in plasma total cholesterol content in cyclic cows before and after use of Doublesynch and Estradoublesynch protocols. The present trend of cholesterol on different sampling days however concurred with the earlier report of Savalia *et al.* (2014) in RB cows and buffaloes treated with Ovsynch and CIDR protocols. Rajagopal *et al.* (2011) reported significantly higher value of serum total cholesterol ( $214 \pm 11.94$  vs  $157.5 \pm 14.23$  mg/dl) at synchronized estrus with Ovsynch in RB cows compared to control group. Moreover, Khan *et al.* (2010) found significantly lower value in repeat breeders than in normal cyclic cows, while Guzel and Tanriverdi (2014) noted insignificantly higher values in repeat breeder than normal cyclic cows.

Steroid hormones have a direct relationship with cholesterol metabolism. The higher cholesterol level in the cycling animals is indicative of more secretion of steroids during estrus due to increased ovarian activity. Savalia *et al.* (2014) opined that the low cholesterol level might have resulted in inadequate synthesis of sex steroid hormones leading to anestrus and repeat breeding condition. The differences reported by different workers in cholesterol levels could be attributed to variation in nutritional, reproductive and health status of animals, apart from seasonal and analytical differences.

### Conclusion

The present findings showed that the hormonal protocols of estrus induction and ovulation synchronization used in crossbred cows did not influence the plasma levels of total protein, though there was modulation in plasma progesterone and cholesterol profile in the treated animals, and the Doublesynch was found better in respect of conception rate in repeat breeding crossbred cows.

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