# EFFECT OF PUERPERIUM, ESTRUS SYNCHRONIZATION AND POSTBREEDING PERIODS ON PLASMA PROFILE OF TOTAL PROTEIN, CHOLESTEROL AND TRIGLYCERIDES IN GIR COWS

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## ABSTRACT

This study was carried out on 24 Gir cows of 2nd to 4th parity from calving till 140 days postpartum on an organized farm. The objective was to monitor the plasma total protein, cholesterol and triglyceride profile at 10 days interval during first 90 days postpartum and then during various estrus synchronization (CIDR, Ovsynch and Cosynch) protocols till 40 days post-AI. The behavioural estrus induction response was observed in 83.33, 83.33 and 100.00 % of cows under CIDR, Ovsynch and Co-synch protocols, respectively. The conception rates at induced estrus (FTAI) in CIDR, Ovsynch and Cosynch protocol were 50.00, 50.00 and 33.33 %, respectively. The corresponding overall conception rates of 2 cycles post-treatment were 83.33 (5/6), 66.66 (4/6) and 83.33 % (5/6). In control group, the first service and overall conception rates were 33.33 and 66.66 (4/6) %, respectively. The overall pooled mean values of total protein, cholesterol and triglyceride varied between intervals from calving till 90 days postpartum in the range of 5.82 ± 0.19 to 6.44 ± 0.15 g/dl, 79.66 ± 2.70 to 190.57 ± 8.84 mg/dl and 16.31 ± 0.86 to 21.53 ± 1.59 mg/dl, respectively. The values of cholesterol varied significantly between periods in all the 4 groups, including pooled values and that of triglycerides showed significant difference only in CIDR group. None of the three parameters were influenced by estrus induction/ synchronization protocols, during treatment or till 40 days post-AI. The biochemical investigations helped to conclude that the selected animals in all hormone protocols and control group were healthy and were maintained under optimum nutritional regime, and this was probably the reason, why their profile was not influenced by the various estrus induction and synchronization protocols used.

**Key words:** Gir cows, Puerperal period, Estrus synchronization, Plasma protein, Cholesterol, Triglycerides

## INTRODUCTION

The postpartum period is regarded as important in the reproductive life of the cow because of its effect upon future fertility. It determines productive and reproductive responses during lactation and is therefore, a pivotal time in the production cycle of the cow. Prolonged interval between calving and onset of ovarian function is regarded as one of the most important gynaecological problems responsible for failure to maintain optimum reproductive efficiency which in turn causes economic loss to the dairy farmers (Shamsuddin *et al.,* 2006; Kamal, 2010). In the recent years, considerable attention has been focused on biochemical constituents of blood that have great diagnostic value in evaluating the physiological status as well as in the clinical practice to improve postpartum fertility in female bovines. The puerperal events and reproductive performance without and with estrus synchronization treatments in Gir cows, an internationally reputed Zebu milch breed, have recently been documented by us (Ammu and Dhami, 2012) This study was aimed to measure the mean plasma profiles of total protein, cholesterol and triglycerides at 10 days interval from the day of calving till 90 days post-partum, during estrus synchronization treatments (CIDR, Ovsynch and Cosynch), at estrus/AI and then upto 40 days post-AI in Gir cows on an organized farm just to monitor the postpartum period and to evaluate the effect of hormonal therapies on blood biochemical profile.

## MATERIALS AND METHODS

### Experimental Animals

Twenty four postpartum suckled Gir cows of 2nd - 4th parity were selected for this study from calving till 140 days postpartum on an organized farm of Livestock Research Station, AAU, Anand. The reproductive/ovarian status in cows not expressing behavioural estrus were assessed around day 70 postpartum by per rectal palpation of the genitalia on three occasions, each at 10 days interval. Based on the reproductive status, the anoestrus and suboestrus cows were randomly distributed at day 90-92 postpartum into following 3 groups of estrus induction/synchronization protocols, each with 6 animals. Six normal cyclic cows exhibiting first estrus within 90 days postpartum and inseminated served as control.

### Treatment Groups

| Group | No. of cows | Reprod. Status pre-treatment | Treatment & AI details |
|---|---|---|---|
| I (Control) | 6 | All cyclic cows | No treatment, Normal cyclic control, only AI at spontaneous estrus. |
| II (CIDR) | 6 | 3 anoestrus and 3 suboestrus | CIDR insert (Pfizer) I/Vg for 7 days, Inj. $PGF_2a$ (cyclix, 2 ml) on day 7 and FTAI 48 hrs later. |
| III (Ovsynch) | 6 | 2 anoestrus and 4 suboestrus | Inj. GnRH (Receptal) 20 µg I/M on day 0, Inj. $PGF_2a$ (cyclix, 2 ml) on day 7, $2^{nd}$ GnRH Inj on day 9 and FTAI 22 hrs later. |
| IV (Cosynch) | 6 | 2 anoestrus and 4 suboestrus | Inj. GnRH (Receptal) 20 µg I/M on day 0, Inj. $PGF_2a$ (cyclix, 2 ml) on day 7 and $2^{nd}$ GnRH Inj on day 9 with simultaneous AI soon. |

The estrus induction response following various treatments and conception rates at induced estrus and overall of two cycles were recorded. Good quality frozen-thawed semen was used for AI.

### Blood Collection

Blood samples were collected from jugular vein on the day of calving and thereafter at 10 days intervals up to the initiation of treatment protocol (around day 90 postpartum) in anoestrus/ suboestrus cows or AI in case of control animals. The blood sampling was later rescheduled based on the treatment protocols or AI, i.e. on day of first treatment (day 0), day of PGF2$\alpha$ injection (Day 7), day of induced/natural estrus (day 9/10, AI), and then on day 10, 20, 30 and 40 post-AI. Tubes containing blood were immediately centrifuged at 3000 rpm for 15 minutes. The plasma was carefully aspirated with a pipette and stored with a drop of 0.01 % merthiolate at -20° C until analyzed.

### Analysis of Plasma Samples

The levels of plasma total protein, total cholesterol and triglycerides were estimated by Biuret (Doumas, 1975) CHOD/PAP (Trinder, 1969) and GPO/PAP (Trinder, 1969) methods, respectively, using standard procedures and kits procured from Crest Biosystems, Goa, on Chemistry Analyser (Mindray, BS 120). The data so generated were analyzed statistically using CRD, Duncan's NMRT and 't' test (Snedecor and Cochran, 1994).

## RESULTS AND DISCUSSION

### Estrus Induction Response and Conception Rate

Three estrus induction/synchronization protocols, viz., CIDR, Ovsynch, and Cosynch used on six anoestrus/suboestrus Gir cows each revealed that though cent per cent cows expressed synchronized estrus clinically, with some 17 % having silent estrus in first two protocols, the first service CR varied between them with values of 50.00, 50.00 and 33.33 %, respectively, as compared to 33.33 % in normal cyclic - control - cows. The corresponding overall conception rates of two cycles over the 25 days period following induced estrus were 83.33, 66.66 and 83.33 %, respectively, as against 66.66 % in normal cyclic group. The CIDR and Cosynch protocols were superior over Ovsynch protocol and normal cyclic -control- group. These results corroborated with those of Cevik *et al.* (2010), Keskin *et al.* (2011) and Bhoraniya *et al.* (2012) in cows.

### Plasma Total Protein

The mean plasma protein concentrations fluctuated non-significantly from calving until day 90 postpartum in all 4 groups, except at day 60-70, where they were significantly higher in Ovsynch group. The pooled mean plasma total protein concentration, irrespective of groups, also varied non-significantly between intervals postpartum from 5.82 ±0.19 to 6.51 ±0.15 g/dl (Table 1). The non-significant differences observed in the levels of total plasma protein between intervals postpartum within the group or between groups at any of the intervals postpartum upto 90 days compared favourably with the report of Tegegne *et al.* (1993) and Patel and Dhami (2006). They also failed to see significant difference in total protein concentrations of cyclic - acyclic cows and anoestrus - suboestrus cows between weeks or even between groups at any of the intervals postpartum, whereas Setia *et al.* (1992) and Onita and Colibar (2009) found significantly ($P<0.05$) lower levels of total protein on the day of parturition and it showed significant rise with advancement of lactation in cows. Khasatiya (2003) not only found significant variation in postpartum plasma protein profile, but the conceived buffaloes had higher plasma protein levels throughout the 15 weeks of postpartum. Neither estrus induction/ synchronization protocols (CIDR, Ovsynch and Cosynch) nor post-AI periods influenced the mean plasma protein levels significantly (Table 1). Bhoraniya *et al.* (2010) also noted similar trend with Ovsynch and CIDR treatment protocols in anoestrus Kankrej cows. Khasatiya (2003), however, observed insignificant influence of GnRH and PGF2$\alpha$ treatment on plasma protein levels in anoestrous and suboestrus buffaloes.

### Plasma Total Cholesterol

The mean cholesterol concentration differed significantly ($P<0.01$) between different intervals postpartum in all the four groups including overall pooled means. The overall pooled mean cholesterol level was lowest 79.66 ±2.70 mg/dl on the day of calving, which then increased gradually and significantly from day 10 (113.97 ±6.79 mg/dl) to day 50 (189.07 ±7.48 mg/ml) postpartum and then remained more or less static till day 90 and even upto day 140 postpartum. The cholesterol levels on the day of calving were lowest in all 4 groups reached to peak around day 60-80 postpartum and then fluctuated unevenly upto day 90 postpartum. There was no significant difference in mean values between the four groups at any of the intervals upto 90 days postpartum, though the values from day 30 onwards postpartum were apparently lower in animals of normal cyclic control group as compared to the corresponding values in anoestrous/suboestrus animals of all three synchronization treatment groups, indicating its utilization in steroidogenesis in cyclic animals (Table 1).

**Table 1: Postpartum plasma total protein and cholesterol levels at 10 day interval in different groups of Gir Cows before and after various estrus synchronization protocols (Mean ±SE)**

| Postpartum Period(days) | Group-I (Control,) | Group-II (CIDR) | Group-III (Ovsynch) | Group-IV (Cosynch) | Overall Pooled |
|---|---|---|---|---|---|
| ***Plasma total protein***(g/dl) | | | | | |
| 0$ | 6.20±0.41 | 5.53±0.35 | 6.06±0.39$^{d}$ | 5.50±3.39 | 5.82±0.19 |
| 10 | 5.82±0.29 | 6.38±0.18 | 6.32±0.17$^{cd}$ | 6.22±0.29 | 6.18±0.12 |
| 20 | 5.98±0.31 | 6.52±0.18 | 6.37±0.11$^{cd}$ | 6.09±0.54 | 6.24±0.16 |
| 30 | 6.03±0.26 | 5.97±0.42 | 6.38±0.20$^{cd}$ | 6.49±0.55 | 6.22±0.18 |
| 40 | 5.95±0.35 | 6.40±0.46 | 6.67±0.12$^{abcd}$ | 6.59±0.49 | 6.40±0.19 |
| 50 | 6.17±0.40 | 5.97±0.16 | 6.66±0.26$^{abcd}$ | 6.57±0.33 | 6.34±0.15 |
| 60 | 5.95±0.28 | 6.27±0.23 | 7.18±0.14$^{a}$ | 6.65±0.32 | 6.51±0.15 |
| 70 | 6.08±0.25 y | 6.01±0.14y | 7.15±0.23$^{ab}$ x | 6.52±0.33xy | 6.44±0.15 |
| 80 | 6.28±0.29 | 6.27±0.19 | 6.81±0.21$^{abc}$ | 6.01±0.36 | 6.34±0.14 |
| 90 | -- | 5.98±0.13 | 6.65±0.24$^{abcd}$ | 6.47±0.34 | 6.31±0.12 |
| Day 0* | -- | 5.98±0.22 | 6.45±0.33$^{bcd}$ | 6.45±0.42 | 6.29±0.19 |
| Day 7** | -- | 6.40±0.31 | 6.64±0.17$^{abcd}$ | 6.41±0.24 | 6.48±0.13 |
| Day 9/10-AI | 6.13±0.31 | 6.70±0.16 | 6.16±0.20$^{cd}$ | 6.53±0.24 | 6.39±0.12 |
| 10 Post-AI | 6.41±0.19 | 6.18±0.07 | 6.24±0.18$^{cd}$ | 6.30±0.36 | 6.28±0.11 |
| 20 Post-AI | 6.09±0.24 | 6.72±0.25 | 6.37±0.24$^{cd}$ | 6.06±0.27 | 6.32±0.13 |
| 30 Post-AI | 6.11±0.32 | 6.32±0.13 | 6.55±0.16$^{abcd}$ | 6.16±0.33 | 6.29±0.12 |
| 40 Post-AI | 6.10±0.27 | 6.30±0.13 | 6.51±0.10$^{abcd}$ | 5.97±0.37 | 6.22±0.12 |
| ***Plasma total cholesterol (mg/dl)*** | | | | | |
| 0$ | 88.84±6.60$^{d}$ | 75.38±3.45$^{d}$ | 75.07±5.37$^{d}$ | 79.35±5.03$^{e}$ | 79.66±2.70$^{f}$ |
| 10 | 107.20±3.17$^{cd}$ | 105.17±7.76$^{cd}$ | 111.40±13.79$^{cd}$ | 132.14±22.26$^{cd}$ | 113.97±6.79$^{e}$ |
| 20 | 133.43±10.71$^{bc}$ | 138.99±9.18$^{bc}$ | 124.21±9.35$^{c}$ | 124.47±14.52$^{de}$ | 130.27±5.36$^{de}$ |
| 30 | 148.76±12.57$^{ab}$ | 141.15±5.19$^{bc}$ | 153.11±11.86$^{bc}$ | 149.26±15.80$^{bcd}$ | 148.07±5.67$^{cd}$ |
| 40 | 147.97±10.49$^{ab}$ | 174.95±14.45$^{ab}$ | 174.38±13.59$^{ab}$ | 179.80±20.19$^{abc}$ | 169.28±7.50$^{cd}$ |
| 50 | 155.34±14.31$^{ab}$ | 189.46±11.05$^{a}$ | 184.71±17.97$^{ab}$ | 191.67±17.37$^{ab}$ | 180.30±7.81$^{bc}$ |
| 60 | 153.43±8.41$^{ab}$ | 193.84±8.67$^{a}$ | 197.26±14.24$^{ab}$ | 211.74±17.53$^{a}$ | 189.07±7.48$^{ab}$ |
| 70 | 156.01±8.12$^{ab}$ | 189.69±9.37$^{a}$ | 194.97±19.63$^{ab}$ | 221.60±21.52$^{a}$ | 190.57±8.84$^{ab}$ |
| 80 | 179.61±11.10$^{a}$ | 186.86±9.86$^{a}$ | 175.49±19.30$^{ab}$ | 210.46±26.66$^{abc}$ | 187.35±8.46$^{ab}$ |
| 90 | -- | 180.83±9.91$^{ab}$ | 181.68±13.73$^{ab}$ | 221.92±16.54$^{a}$ | 189.57±7.81$^{ab}$ |
| Day 0* | -- | 183.36±20.37$^{ab}$ | 190.96±12.77$^{ab}$ | 218.26±18.90$^{a}$ | 197.53±10.24$^{a}$ |
| Day 7** | -- | 192.43±17.34$^{a}$ | 206.90±16.91$^{a}$ | 216.54±19.94$^{a}$ | 205.29±10.11$^{a}$ |
| Day 9/10-AI | 170.76±18.61$^{ab}$ | 205.63±25.23$^{a}$ | 192.66±18.80$^{ab}$ | 216.42±17.25$^{a}$ | 197.48±10.12$^{a}$ |
| 10 Post-AI | 175.95±16.34$^{a}$ | 193.05±20.98$^{a}$ | 202.61±19.82$^{a}$ | 219.49±17.58$^{a}$ | 198.73±9.45$^{a}$ |
| 20 Post-AI | 167.81±17.07$^{ab}$ | 203.81±20.53$^{a}$ | 213.60±20.48$^{a}$ | 202.06±14.67$^{ab}$ | 198.08±9.31$^{a}$ |
| 30 Post-AI | 164.81±14.66$^{ab}$ | 208.47±16.43$^{a}$ | 212.80±15.26$^{a}$ | 216.63±10.97$^{a}$ | 202.24±7.97$^{a}$ |
| 40 Post-AI | 154.86±11.12$^{ab}$y | 205.11±12.30$^{a}$ x | 215.06±16.38$^{a}$x | 208.35±9.21$^{a}$ x | 197.63±7.65$^{a}$ |

0$ = day of calving, * day of first treatment, ** day of PG injection. Synchronization treatment was initiated on day 92-93 postpartum.

Means bearing uncommon superscripts within the column (a,b) / row (x,y) differ significantly (P<0.05).

The increasing trend of plasma total cholesterol concentration seen from the day of calving to subsequent weeks postpartum was associated with the initiation of ovarian activity and establishment of estrous cyclicity postpartum in some animals with silent ovulation as detected by plasma progesterone profile (Ammu and Dhami, 2012). Prakash and Tandon (1979) and Onita and Colibar (2009) noted steep rise in the postpartum concentration of serum cholesterol during first 6 weeks in crossbred cows, while Khasatiya (2003) noted significant weekly variation with gradual increasing trends from calving till 15 weeks postpartum in Surti buffaloes, but without significant influence of GnRH or PG treatment on day 42 postpartum. Setia *et al.* (1992) also observed the lowest serum cholesterol value on the day of parturition in cows, which increased afterwards. Guedon *et al.* (1999) found a linear and significant increase in serum cholesterol concentration from 2 to 9 weeks after calving which then declined slightly. Further, none of the synchronization treatments significantly influenced the plasma total cholesterol, although the levels in CIDR and Ovsynch groups were slightly elevated during and after therapies. In contrast the levels in control group declined insignificantly over the 40 days post-AI period (Table 1). Jain and Pandita (1995) reported significantly higher mean serum cholesterol level in normal cyclic cows and at PGF2$\alpha$ treated induced estrus cows. Mesaric *et al.* (1997) however noted significantly higher cholesterol in cows at 225 days (pregnant) than at 70 or 150 days postpartum. Bhoraniya *et al.* (2010) also reported non-significant variations in the cholesterol levels between days for Ovsynch or CIDR treated anoestrus Kankrej cows. Present findings thus to some extent corroborate with these reports.

**Table 2: Postpartum plasma triglyceride levels (mg/dl) at 10 day interval in different groups of Gir Cows before and after various estrus synchronization protocols (Mean ±SE)**

| Postpartum Period(days) | Group-I (Control, ) | Group-II (CIDR) | Group-III (Ovsynch) | Group-IV (Cosynch) | Overall Pooled |
|---|---|---|---|---|---|
| 0$ | 14.98±1.10 | 14.61±1.01$^{c}$ | 17.59±2.35 | 19.88±4.56 | 16.77±1.32$^{cd}$ |
| 10 | 15.02±1.47 | 15.25±2.00$^{c}$ | 16.44±1.96 | 18.54±1.45 | 16.31±0.86$^{d}$ |
| 20 | 16.87±1.32 | 16.73±1.44$^{bc}$ | 16.52±1.12 | 19.25±1.88 | 17.34±0.72$^{bcd}$ |
| 30 | 20.34±6.29 | 18.03±2.12$^{abc}$ | 17.07±0.75 | 22.01±2.19 | 19.61±1.73$^{abcd}$ |
| 40 | 18.59±3.06 | 18.58±1.87$^{abc}$ | 19.12±1.63 | 23.34±3.37 | 19.91±1.28$^{abcd}$ |
| 50 | 20.70±3.70 | 18.52±1.46$^{abc}$ | 18.27±1.09 | 24.94±4.62 | 20.61±1.55$^{abcd}$ |
| 60 | 18.59±3.10 | 20.30±1.24$^{abc}$ | 20.66±1.77 | 26.26±3.43 | 21.45±1.33$^{abc}$ |
| 70 | 19.57±3.40 | 20.00±1.45$^{abc}$ | 18.94±2.60 | 27.62±4.01 | 21.53±1.59$^{abc}$ |
| 80 | 22.04±6.13 | 19.24±1.25$^{abc}$ | 17.00±1.63 | 25.47±4.03 | 21.34±1.91$^{abc}$ |
| 90 | -- | 18.20±1.00$^{abc}$ | 16.44±1.65 | 25.21±3.22 | 19.69±1.30$^{abcd}$ |
| Day 0* | -- | 20.34±1.70$^{abc}$ y | 16.49±1.17 y | 26.00±1.85 x | 20.95±1.29$^{abcd}$ |
| Day 7** | -- | 22.21±2.32$^{abc}$ | 17.47±1.64 | 24.31±2.89 | 21.33±1.45$^{abc}$ |
| Day 9/10-AI | 21.78±4.39 | 23.02±2.55$^{ab}$ | 18.55±2.25 | 26.24±2.89 | 22.43±1.52$^{a}$ |
| 10 Post-AI | 21.31±2.72 | 23.99±2.40$^{ab}$ | 18.65±2.57 | 25.58±3.15 | 21.65±1.38$^{ab}$ |
| 20 Post-AI | 20.69±3.20 | 25.74±4.51$^{a}$ | 19.48±2.22 | 23.13±2.98 | 22.33±1.64$^{a}$ |
| 30 Post-AI | 19.61±2.09 | 23.77±3.12$^{ab}$ | 21.61±1.48 | 24.68±2.50 | 22.54±1.19$^{a}$ |
| 40 Post-AI | 18.90±3.46 | 23.45±3.84$^{ab}$ | 22.59±3.91 | 25.02±3.47 | 22.65±1.78$^{a}$ |

0$ = day of calving, * day of first treatment, ** day of PG injection. Synchronization treatment was initiated on day 92-93 postpartum.

Means bearing uncommon superscripts within the column (a,b) / row (x,y) differ significantly (P<0.05).

**Plasma Triglycerides**

The plasma triglycerides concentrations varied insignificantly between different intervals till 90 days postpartum in all groups. The values in CIDR group varied significantly ($P<0.05$) between periods that however included treatment and post-treatment/post-AI intervals also. The values fluctuated unevenly in all the groups at different intervals postpartum with increasing trend from calving till 60-70 days postpartum. The values were apparently higher in Ovsynch and Cosynch groups at all intervals postpartum (Table 2). Marques and Castillo (1996) and Mesaric *et al.* (1997) also noted lower levels of plasma triglycerides at the beginning of lactation as compared to mid and late lactation or pregnancy in Holstein cows. Khasatiya *et al.* (2005) and Patel and Dhami (2005), however, found gradual and significant rise in weekly concentration of triglycerides during early postpartum period in Surti buffaloes and HF cows. Guedon *et al.* (1999) opined that the plasma triglycerides levels were influenced by physiological status of animal and were higher during last 10 weeks of pregnancy than at calving and early postpartum. None of the estrus induction and synchronization protocols used, viz., CIDR, Ovsynch and Cosynch influenced the plasma triglyceride levels, and values in all the treated and control group were almost same before, during and after treatment/AI (Table 2). Khasatiya (2003) in Surti buffaloes and Patel and Dhami (2005) in HF cows noted insignificant effect of GnRH and PG therapies around day 42-49 postpartum on plasma triglycerides profile. Ahlawat and Derashri (2009) did not find significant variation in the levels of plasma triglycerides between cyclic and anoestrus cows or even conceiving and non-conceiving cows.

The present findings indicate that the three traits studied were within physiological limits and were neither influenced by postpartum period nor by hormonal therapies in cows.

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