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Postpartum plasma profile of total cholesterol and triglycerides in Holstein Friesian cows with and without hormone therapy under tropical climate*

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ABSTRACT

Twenty-four HF cows of University farm were monitored through clinical diagnosis and weekly plasma profile of total cholesterol and triglycerides from the day of calving till 21st week postpartum. This was done in conceived and non-conceived animals, and with and without GnRH (Receptal) and $PGF_2\alpha$ (Lutalyse) treatment at 7th week postpartum in anoestrus and suboestrus cows (6 each), respectively. The mean cholesterol concentration of GnRH and $PGF_2\alpha$ groups did not differ significantly (93.98±1.87 vs 99.89±1.90 mg/dl) between weeks or between groups at any of the intervals postpartum. It was significantly higher in GnRH treated group than the control group at 10th to 14th week postpartum. Further, the concentration in GnRH treatment, control and pooled groups was low at calving, which declined a little at 1st week, and then increased linearly and significantly to reach the highest level by 14th-17th week postpartum in three groups, and then declined insignificantly till 21st week postpartum, however in $PGF_2\alpha$ groups, the weekly means fluctuated insignificantly. Moreover, the values of cholesterol were significantly higher in PGF2α treated than control group only at 3rd and 4th week postpartum. The weekly mean cholesterol concentrations of conceived and non-conceived cows and their pooled group fluctuated highly significantly ($P < 0.01$) between weeks postpartum from 62.40±9.19 to 115.27±6.79 mg/dl, but not between groups at any of the intervals postpartum. The animals that conceived had relatively low cholesterol during early postpartum period and it increased significantly by 4th week and further by 19th week postpartum compared to non-conceived group. The weekly mean plasma triglycerides levels in cows of GnRH group were higher than those of $PGF_2\alpha$ group almost throughout the postpartum period with significant ($P < 0.05$) difference at 1st, 15th and 19th week postpartum, including the overall means (52.68±1.17 vs 43.15±1.43 mg/dl). However in GnRH and $PGF_2\alpha$ treatment, control and their pooled groups as well as in conceived and non-conceived groups it neither varied significantly between weeks nor between groups at any of the intervals postpartum. Triglycerides levels were little low at calving, then got slightly elevated from 5th-6th week postpartum and again showed slight declining values from 13th-14th week till 21st week postpartum in all the groups. Moreover, the levels were higher in $PGF_2\alpha$ control than the treatment group throughout postpartum period, but varied significantly only at 3rd, 4th and 8th week. The overall mean triglycerides concentrations of GnRH and PGF2α treated cows were significantly lower ($P < 0.01$) than their controls (48.18±1.01 vs 56.96±2.08 and 33.48±1.43 vs 53.06±2.26 mg/dl), but the values of conceived and non-conceived groups did not vary significantly (50.36±1.59 vs 46.94±1.18 mg/dl).

Key words: Holstein Friesian cows, hormone therapy, cholesterol, triglyceride, postpartum period

The economy of dairy industry is influenced by the reproductive efficiency particularly calving interval of dairy animals. Early postpartum period exerts biological and physiological stress on the dam resulting into negative energy balance, altered blood metabolic profile (Setia *et al.*, 1992), delayed conception and thereby extended calving interval (Butler *et al.*, 2000). There are certain biochemical and metabolic constituents, which directly reflect the nutritional status and influence the reproductive performance of the animal either by acting as precursor of hormone synthesis or by stimulating response of target tissues for their action. Such biochemical, particularly glucose, cholesterol and triglycerides, profiles have been studied in postpartum fertile & infertile buffaloes (Setia *et al.*, 1992; Shah *et al.*, 2003a) and even in exotic cattle from abroad (Kappel *et al.*, 1984; Mesaric *et al.*, 1997). The plasma profile of

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triglycerides reflects lipid metabolism particularly in mammary glands of ruminants (Guedon *et al.*, 1999), while cholesterol being a constituent of plasma lipoproteins involved in lipid transport system of the body (Taylor *et al.*, 1966) and an essential precursor of steroid hormone synthesis in gonads and adrenal cortex (Rowlands *et al.*, 1980), can be a good probe to guide clinician towards improving postpartum fertility in female bovines. However, no literature was available on monitoring the postpartum plasma profile of cholesterol and triglycerides in exotic cattle born and reared under tropical climate. Hence, the present study was aimed to monitor these parameters during early postpartum period with and without GnRH and PGF2á treatment in HF cows under tropical farm management.

MATERIALS AND METHODS

This study was carried out over first 150 days postpartum on 24 healthy normally calved cows managed under routine feeding and housing protocol of HF Project, GAU, Anand, during the period from November 2002 to June 2003, with the object of monitoring postpartum period through clinical diagnosis and weekly plasma profile of certain metabolites with and without GnRH and $PGF_2\alpha$ treatment at 7th week postpartum. A group of 6 animals having small inactive ovaries till day 49 postpartum were treated on that day with the single i/m injection of 0.02 mg (5 ml) Buserelin acetate (GnRH analogue, Receptal®, Intervet India Pvt. Ltd) and 6 animals of similar nature were kept as control without treatment. Another group of 6 animals having established normal cyclicity, but without manifestation of clear pronounced signs of oestrus were treated with single i/m injection of 25 mg (5 ml) Dinoprost tromethamin, THAM salt ($PGF_2\alpha$, Lutalyse®, Pharmacia & Upjohn), between day 48 and 55 postpartum after confirming the presence of mature corpus luteum on either of the ovaries and 6 animals of similar nature were kept as untreated control.

All the animals of above two groups and their controls were followed and compared for their clinical response, conception and weekly plasma profile of total cholesterol and triglycerides till day 150 postpartum. Cows exhibiting signs of oestrus were bred only after 50 days of calving by AI using frozen-thawed semen and were palpated per rectum for pregnancy 45 days later. Heparinized venous blood samples were collected from all 24 cows at regular weekly interval from the day of calving till at least 150 days (21 weeks) postpartum. The plasma samples were stored at -20°C and were used for estimation of total cholesterol and triglycerides profiles using standard assay kits and an autoanalyzer. The data were analyzed statistically using CRD, Duncan's NMRT and Student's 't' test to know the weekly/ group variation, if any (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

The findings on weekly mean plasma profile of total cholesterol and triglycerides recorded from the day of calving till 21st week postpartum in HF cows of GnRH and $PGF_2\alpha$ treatment and their control groups as well as of conceived, non-conceived and overall pooled groups are depicted in Figure 1 and Table 1.

Total cholesterol : The mean plasma cholesterol concentration in GnRH treatment group was 56.25±5.75 mg/dl on the day of calving, which decreased a little at 1st week postpartum (53.23±2.41 mg/dl), and then increased linearly and significantly to 79.05±5.10 mg/dl by 3rd week and 129.33±13.01 mg/dl by 11th week postpartum reaching to the highest level of 133.82±4.98 mg/dl at 14th week postpartum and then again declined gradually till 21st week postpartum. In GnRH control group the total cholesterol level on the day of calving was 82.78±20.12 mg/dl and it declined to 73.73±14.36 mg/dl at 1st week postpartum, which then fluctuated non-significantly with the highest level of 103.02±14.60 mg/dl at 17th week postpartum. The total cholesterol level in GnRH treated cows was higher in comparison to its controls throughout the postpartum period, except first three weeks, but differed significantly only at 10th to 14th week postpartum, including the overall means (101.97±1.74 vs 86.36±2.87 mg/dl). Moreover in GnRH pooled group, the cholesterol level was lowest by 1st week postpartum (63.48±7.60 mg/dl), which increased significantly by 7th week (96.33±6.78 mg/dl) with further rise up to 106.83±11.08 mg/dl by 11th week postpartum, and thereafter fluctuated insignificantly till 21st week postpartum (Fig. 1).

Unlike GnRH, in $PGF_2\alpha$ treatment, control and pooled groups, the weekly mean plasma cholesterol concentrations fluctuated insignificantly in the range of 66.73±10.82 to 128.13±12.46 mg/dl. The levels were low during early postpartum period and high around 10th to 19th week postpartum in all three groups. The weekly as well as overall mean (103.57±2.52 vs 96.11±2.88 mg/dl) cholesterol concentration of $PGF_2\alpha$ treated and control groups varied insignificantly at most intervals, except at

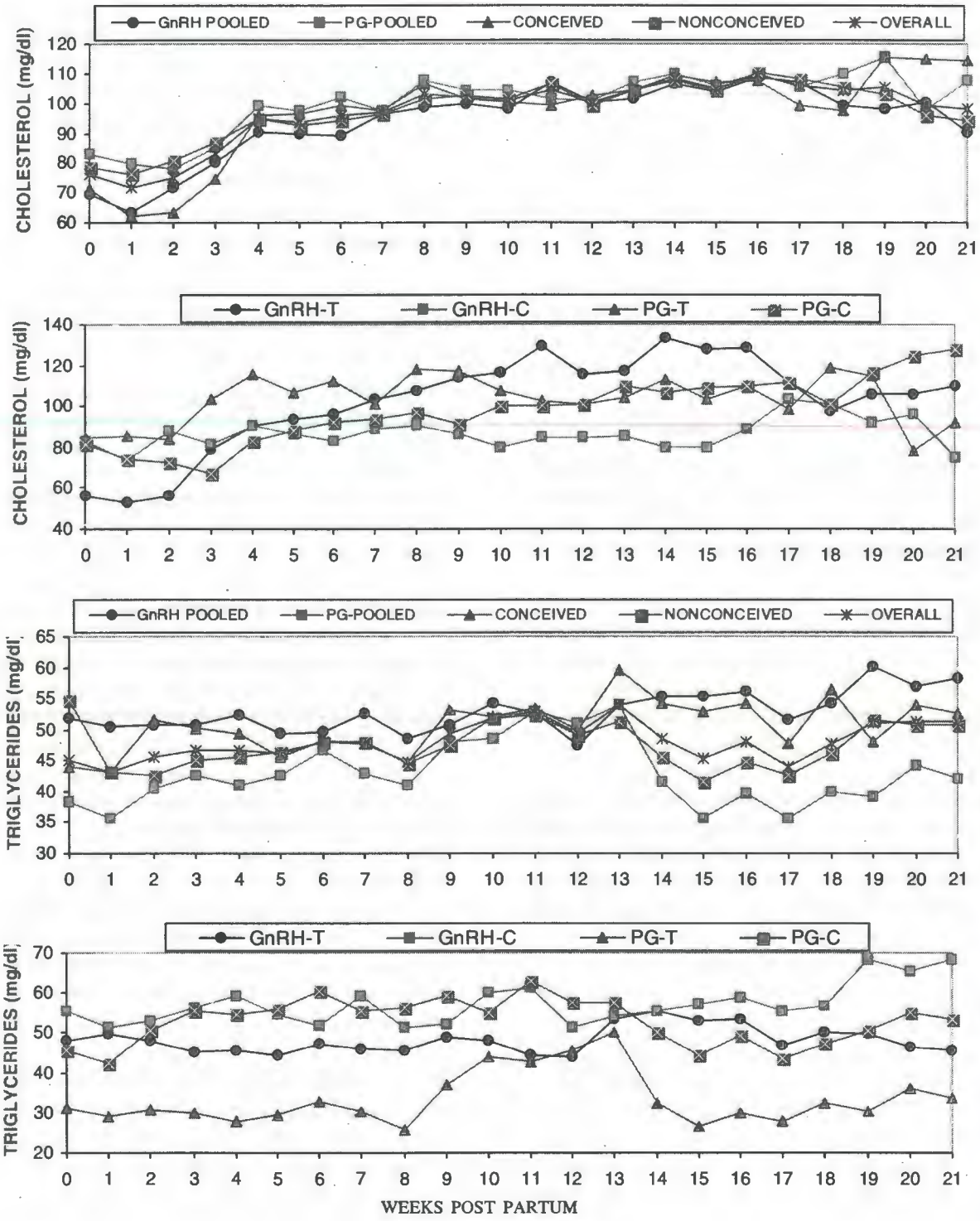

Fig. 1. Postpartum weekly plasma total cholesterol and triglycerides profile under GnRH and PGF2á regimes and in conceived and non-conceived HF cows

Table 1. Weekly (mean ± SE) plasma cholesterol and triglycerides profile in conceived and non-conceived (by day 150 PP) HF cows

Weeks post-partum	Total cholesterol (mg/dl)			Triglycerides (mg/dl)		
	Conceived (n=8)	Non-conceived (n=16)	Overall (n=24)	Conceived (n=8)	Non-conceived (n=16)	Overall (n=24)
0	71.39±9.91^{cd}	78.68±9.61^{cd}	76.25±7.12^{efg}	43.97±3.28	45.67±5.94	45.11±4.06
1	62.40±9.19^{d}	76.21±8.04^{d}	71.60±6.21^{g}	43.11±4.99	43.16±5.14	43.14±3.74
2	63.31±8.78^{d}	81.01±8.55^{bcd}	75.11±6.53^{fg}	51.64±7.45	42.68±4.88	45.67±4.09
3	74.63±12.07^{bcd}	86.79±6.26^{abcd}	82.73±5.77^{defg}	50.09±6.93	44.98±4.80	46.69±3.89
4	95.73±10.54^{abc}	94.51±6.18^{abcd}	94.91±5.28^{abcde}	49.29±8.58	45.57±5.15	46.74±4.37
5	96.45±6.56^{abc}	92.30±5.62^{abcd}	93.68±4.27^{abcdef}	45.56±6.70	46.48±5.80	46.11±4.38
6	99.06±10.69^{abc}	94.09±6.01^{abcd}	95.75±5.25^{abcde}	48.36±8.38	48.02±4.78	48.13±4.13
7 #	97.51±8.51^{abc}	96.64±5.44^{abcd}	96.93±4.50^{abcd}	47.77±8.80	47.94±4.88	47.88±4.27
8	106.66±7.90^{abc}	101.59±7.66^{abcd}	103.28±5.66^{abcd}	44.94±8.02	44.64±5.00	44.74±4.17
9	101.30±8.35^{abc}	102.66±8.44^{abcd}	102.21±6.17^{abcd}	53.13±8.40	47.46±4.90	49.35±4.23
10	100.46±9.81^{abc}	101.41±8.87^{abcd}	101.09±6.63^{abcd}	52.20±7.09	51.68±5.67	51.85±4.37
11	99.15±11.23^{abc}	106.49±8.66^{ab}	104.05±6.78^{abc}	53.40±7.56	52.45±6.72	52.77±5.04
12	102.81±9.70^{abc}	99.53±7.23^{abcd}	100.62±5.69^{abcd}	49.90±4.78	49.05±5.93	49.34±4.20
13	104.16±6.25^{abc}	104.16±7.83^{abc}	104.16±5.54^{abc}	59.49±4.41	51.26±4.92	54.00±3.63
14	109.39±8.42^{ab}	107.66±8.72^{ab}	108.23±6.35^{a}	54.31±5.97	45.61±5.55	48.51±4.21
15	106.96±9.20^{abc}	104.02±6.83^{abc}	105.00±5.38^{a}	52.95±6.22	41.59±5.71	45.38±4.40
16	107.50±9.08^{ab}	110.42±9.04^{a}	109.45±6.63^{a}	54.17±6.92	44.83±6.25	47.94±4.76
17	99.50±5.64^{abc}	108.27±7.34^{ab}	106.18±5.74^{a}	47.75±8.20	42.74±5.49	43.93±4.55
18	97.73±2.02^{abc}	105.45±9.09^{abc}	104.16±7.57^{abc}	56.47±15.79	46.08±5.56	47.81±5.19
19	115.27±6.79^{a}	103.44±9.52^{abc}	105.66±7.84l	48.09±12.51	51.53±5.81	50.88±5.10
20	114.87±8.40^{a}	95.80±11.34^{abcd}	99.38±9.43^{abcd}	53.88±10.47	50.81±6.14	51.39±5.23
21	114.20±9.67^{a}	94.34±8.13^{abcd}	98.06±7.02^{abcd}	52.65±12.73	50.84±6.13	51.18±5.34
Overall	**95.52±2.01**	**97.49±1.69**	**96.88±1.32**	**50.36±1.59**	**46.94±1.18**	**48.00±0.94**

0 = Day of calving; # Treatment day 49 Postpartum; * $P < 0.05$; ** $P < 0.01$ between subgroups.
Means bearing superscript in common within a column do not differ significantly ($P > 0.05$)

3rd (103.42±12.20 vs 66.73±10.82 mg/dl) and 4th week (115.93±12.47 vs 83.02±7.78 mg/dl) postpartum, where it was significantly higher in treatment than the control group. Similarly, the weekly mean cholesterol levels of GnRH and $PGF_2\alpha$ pooled groups also did not differ significantly at any of the intervals postpartum, except the overall means (93.98±1.87 vs 99.89±1.90 mg/dl), being higher in suboestrus cows (Fig. 1).

The weekly mean cholesterol concentration of conceived, non-conceived cows and their pooled group fluctuated significantly ($P < 0.01$) among weeks postpartum from 62.40±9.19 to 115.27±6.79, 76.21±8.04 to 110.42±9.04 and 71.60±6.21 to 109.45±6.63 mg/dl, respectively. The values increased gradually and significantly with advancing postpartum period. The animals that conceived had relatively low cholesterol during early postpartum period and it increased significantly by 4th week and further by 19th week postpartum compared to non-conceived group, where such rise was delayed beyond 11th week postpartum and it further declined by 20th week postpartum. However, there was no significant difference between conceived and non-conceived groups at any of the intervals postpartum, including overall means (95.52±2.01 vs 97.47±1.69 mg/dl; Table 1, Fig. 1).

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 oestrus cyclicity postpartum. These findings were in agreement with the reports of Belyea *et al.* (1975) and Sato (1978) in dairy cows and of Shah *et al.* (2003a,b) in buffaloes. Rowlands *et al.* (1980) observed 2.5 fold increase in the cholesterol level during first 8 weeks postpartum in non-suckled HF cows, but it had no relation with conception rate. Setia *et al.* (1992) observed lowest cholesterol value on the day of parturition in cows, which increased throughout the lactation. Guedon *et al.* (1999) recorded gradual and significant rise in serum cholesterol level till 10th week postpartum and then decline. Tainturier *et al.* (1984) observed a significant drop in serum cholesterol concentration at calving as compared to gestational values and then a steep rise leading to 2-3-fold increase by second month of lactation. This change may be attributed to drop in its circulatory levels through increased coupling with oestrogen and thyroxine after parturition, which normally inhibit cholesterogenesis. Lactation probably also affect the level of serum cholesterol, which act as a fatty acid carrier in the form of cholesterol ester for milk synthesis, as a result there is gradual increase in serum cholesterol level with advancing lactation. These reports and our findings clearly proved that plasma total cholesterol, being precursor of steroid hormones, is closely associated with physiological status of the animal and reproduction in particular.

A gradual increase in total cholesterol level with minor fluctuations seen in cows following GnRH treatment was, however, in contradiction to report of Sonawane *et al.* (1994), who observed continuous drop in serum cholesterol level for 10 days after Receptal treatment, although Jain and Pandita (1995) did not find such change in total cholesterol at induced oestrus following $PGF_2\alpha$ treatment in cows. As seen in our study, Vohra *et al.* (1995) recorded almost identical values of cholesterol in cyclic and anoestrus cows, while Joe *et al.* (1998) observed significantly higher serum cholesterol in oestrus cows than in anoestrus cows. Moreover, the weekly sampling adopted following hormone therapy in the present study was probably too long to reflect it's real endocrine action on blood profile, as the action of GnRH, prostaglandins and gonadotropins is very short-lived and rather immediate (Sonawane *et al.*, 1994). The findings on conceived and non-conceived groups were in agreement with the reports of Srivastava and Sahni (2000) and Shah *et al.* (2003a). Kappel *et al.* (1984) indicated that decreased blood cholesterol concentration during first 40 to 60 days postpartum delayed the conception in non-suckled Holstein cows.

There was a parallel trend between plasma progesterone and plasma total cholesterol (Patel, 2004). This was conceivable, as cholesterol is an essential precursor for biosynthesis of steroid hormones. The plasma cholesterol levels had significant ($P < 0.05$) positive correlations with plasma P4, glucose, calcium, phosphorus, Ca:P ratio, magnesium, and negative correlations with plasma total protein, triglycerides and zinc levels in conceived group and in the GnRH group (Patel, 2004), indicating its role in physiology of reproduction in association with carbohydrates and macro-minerals.

Plasma triglycerides : The weekly plasma triglycerides concentrations in GnRH treatment, control and pooled

groups varied insignificantly between weeks postpartum from 43.89±3.26 to 68.48±8.41 mg/dl. The values on the day of calving were 48.29±2.64 and 55.37±11.81 mg/dl in GnRH treatment and control groups, and they did not vary at any of the intervals postpartum (Fig. 1), although the overall mean value of GnRH treatment group was significantly lower ($P < 0.01$) than the control group (48.18±1.01 vs 56.96±2.08 mg/dl).

Like GnRH, the triglycerides concentrations in $PGF_2\alpha$ treatment, control and pooled groups also varied non-significantly between weeks postpartum in the range of 25.86±2.64 to 62.82±12.41 mg/dl. The levels were little low at calving then got slightly elevated from 5th-6th week postpartum and again showed slight declining trend from 13th-14th week till 21st week postpartum in all the three groups. The weekly mean triglycerides levels were higher in $PGF_2\alpha$ control than the treatment group throughout the postpartum period, but varied significantly only at 3rd, 4th and 8th week, including the overall pooled means (53.06±2.26 vs 33.48±1.43 mg/dl). Further, the cows of GnRH group in comparison to $PGF_2\alpha$ had significantly higher ($P < 0.05$) weekly mean triglycerides concentrations almost through out the postpartum period with significant differences at 1st, 15th and 19th week postpartum and also in the overall pooled means (52.68±1.17 vs 43.15±1.43 mg/dl).

The weekly mean triglycerides profile in conceived, non-conceived and their pooled group also fluctuated non-significantly between different weeks postpartum in the range of 41.59±5.71 to 59.49±4.41 mg/dl. There was no significant variation in the values of conceived and non-conceived cows at any of the intervals postpartum, including the overall means (50.36±1.59 vs 46.94±1.18 mg/dl; Table 1; Fig. 1). The plasma profile of triglycerides had significant ($P < 0.05$) negative correlations with plasma progesterone, glucose, total cholesterol, calcium, phosphorus and magnesium, and negative correlations with all micro-minerals studied, except zinc, in all groups (Patel, 2004).

The present findings of almost constant weekly profile of triglycerides coincided with the report of Khasatiya (2003) in buffaloes, but did not agree with the results of Marques and Castillo (1996), who reported decrease in triglycerides levels at the beginning of lactation in Holstein cows. Guedon *et al.* (1999) observed that the plasma triglycerides levels were influenced by physiological status of animal and were higher during last 10 weeks of pregnancy than at or after calving. Mesaric *et al.* (1997) also did not find significant variation in plasma triglycerides levels of cows at 70, 150 and 225 days postpartum i.e. in cows with conceived and non-conceived status. The rapid increase in the triglycerides during early postpartum/lactation may be attributed to increased demand of the udder for fatty acids synthesis for milk fat, and also to lowest level of circulatory oestrogen and thyroxine profile, which influence the lipid metabolism (Tainturier *et al.*, 1984). Our observations of slight rise in triglycerides levels at 4-6 week postpartum compared to early or late phase to some extent goes with this opinion. Khasatiya (2003) also reported gradual and non-significant rise in plasma triglycerides levels after parturition in Surti buffaloes with or without GnRH and $PGF_2\alpha$ treatment at day 40-42 postpartum.

Thus, it was concluded that a gradual rise in total cholesterol with advancing postpartum period particularly in conceived and GnRH treatment group was associated with folliculogenesis, oestrus, ovulation and pregnancy, as it is a precursor of steroid hormones, although triglycerides did not reflect any specific trend.

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