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Blood biochemical constituents in normal and non-infectious repeat breeding crossbred cows

Amit Kumar, Madhu Shivhare, Ranjit Aich, Rahul Chourasia and Sudarshan Kumar

Abstract
The present study was conducted on 36 crossbred cows with history of repeat insemination at different intervals, randomly categorized in infectious and non-infectious groups on basis of positive or negative reaction to Whiteside test belonging to the Dairy farm of College of Veterinary science and Animal Husbandry, Mhow and clinical cases of progressive farmers brought for artificial insemination to teaching veterinary clinical complex and at the doorstep of farmers in nearby villages. These selected animals divided into 6 groups GnRH analogue(Gp I), GnRH + HCG(Gp II), HCG alone (Gp III), HCG + GnRH (Gp IV), Progesterone (Gp V) and Control Group (Gp VI). He found mean value of total plasma cholesterol, total plasma protein and total albumin globulin ration were significantly higher ($P<0.05$) on day 12 and day 30.

Keywords: blood biochemical constituents, normal, non-infectious, crossbred cows

Introduction
Repeat breeding has been defined as failure to conceive from 3 or more regularly spaced services in the absence of detectable abnormalities (Zemjanis, 1980) [34].
Repeat breeding is one of the major gynaecological problem and an important cause of infertility in cattle that results in delayed conception and increased calving interval, loss of milk production, reduction in calf crop, increased cost of treatment and culling of useful breeding animals leading to heavy economic losses to the dairy producers (Bhatt and Bhattacharyya, 2012) [3]. The cause of repeat breeding may originate either during early stages of follicular maturation and / or during pre-ovulatory period.
In dairy cows, luteal insufficiency and lower progesterone concentrations are known as a cause of embryonic mortality and reduce the pregnancy rates during early embryonic development (Howard *et al*., 2006) [13].
It has been hypothesized that increasing peripheral progesterone concentrations during the diestrus after insemination may improve embryo development and may suppress luteolysis, resulting in reduced embryonic loss.
GnRH/hCG injection causes a predictable release of LH hence, administration of GnRH and/or hCG before, during and post-insemination would more precisely synchronize ovulation with estrus, increased the pregnancy rates and decreased the early embryonic deaths (Das *et al*., 2007) [8].

Materials and Methods
Location of work
The proposed work was carried out on 36 clinical cases brought to the AI (Artificial Insemination) center of Department of Veterinary Gynaecology & Obstetrics, College of Veterinary Science and Animal Husbandry, Mhow and in nearby villages of Mhow.

Experimental design and treatment protocol
All experimental protocols and animal care met Institutional Animal Ethical Committee regulations.

Screening of animals
More than 200 animals (>90 days postpartum) with history of repeat insemination at different intervals were screened and examined to comprise the material of study and were broadly

categorized in infectious and non-infectious groups on basis of positive or negative reaction to Whiteside test (Pateria and Rawal, 1990) [22].
On the basis of above examination, the animals (non-infectious repeat breeder) were assigned into following groups (Table 01)

Group 1
Animals in this group (n=6) were treated with GnRH analogue injection buserelin acetate @ 10 µg (2.5 ml) intramuscularly at the time of Artificial Insemination (AI) and it was followed by 12th day after AI.

Group 2
Animals in this group (n=6) were treated with GnRH analogue injection buserelin acetate @ 10 µg (2.5 ml) intramuscularly at the time of AI and injection hCG @ 1500 IU intramuscularly on 12th day after the AI.

Group 3
Animals in this group (n=6) were treated with injection hCG @ 1500 IU intramuscularly at the time of AI followed by 12th day after the AI.

Group 4
Animals in this group (n=6) were treated with injection hCG @ 1500 IU intramuscularly at the time of AI and GnRH analogue injection buserelin acetate @ 10 mcg (2.5 ml) intramuscularly on 12th day after the AI.

Group 5
Animals in this group (n=6) were treated with injection hydroxyl progesterone 500 mg I/M on 4th day of estrum after first insemination.

Group 6 (Control group)
Animals in this group (n=6) were non-infectious repeat breeder crossbred cows and treated with injection normal saline (2ml) intramuscularly at the time of AI and on 12th day after AI as placebo.

Table 1: Grouping of animals

Groups	No. of Animals	Type of Animal	Treatment given
Group 1	6	Repeat breeder	GnRH at AI + 12th day
Group 2	6	Repeat breeder	GnRH at AI + hCG on 12th day
Group 3	6	Repeat breeder	hCG at AI + 12th day
Group 4	6	Repeat breeder	hCG at AI + GnRH on 12th day
Group 5	6	Repeat breeder	P_4 on 4th day after AI
Group 6 (Control group)	6	Repeat breeder	Normal saline at AI + 12th day

Blood sampling schedule
The blood samples (10 ml) were collected from jugular vein (at day 12 and 30 days of oestrus) in heparinized vials and were immediately centrifuged for 15 minutes at 3000 rpm. After centrifugation, the plasma samples were separated and collected in sterilized vials and stored at -20 ºC pending analysis.

Blood Biochemical parameters
Blood plasma was estimated for:

Total plasma cholesterol
Cholesterol was estimated by CHOD-PAP method using Erba manheim kit as described by Richmond (1973) [25] and values were expressed in (mg/dl).

Total plasma protein
Total protein was estimated by Biuret method using Erba manheim kit as described by Tietz (1986) [32] and values were expressed in (g/dl).

Albumin-Globulin ratio
This was determined by dividing the albumin value with the globulin value.

Statistical analysis
Data analysis was done as per the standard statistical method by application of Factorial Completely Randomized Design (Snedecor and Cochran, 1994) [29]

Result and Discussion
Total plasma cholesterol
the concentration of total plasma cholesterol (mg/dl) observed in conceived and non-conceived repeat breeding crossbred cows in the present study under GnRH+GnRH, GnRH+hCG, hCG+hCG, hCG+GnRH, Inj. Progesterone (P_4) and placebo+placebo treated group are presented in table 4.07 and figure 09.
The mean total plasma cholesterol concentration (mg/dl) in 3 conceived repeat breeding crossbred cows on day 12 of GnRH +GnRH injection was found to be 176.50±1.91 mg/dl which decreased slightly to 168.52±1.39 mg/dl on day 30 with an overall mean of 172.51±2.82 mg/ml. The corresponding values for 3 non-conceived repeat breeding crossbred cows were found to be 236.10±3.80 mg/dl on day 12 which decreased to 221.00±5.00 mg/dl on day 30 with an overall mean of 228.50±5.35 mg/dl.
The mean total plasma cholesterol concentration (mg/dl) in 4 conceived repeat breeding crossbred cows on day 12 of GnRH+ hCG injection was found to be 216.52±0.61 mg/dl which decreased to 209.81±0.61 mg/dl on day 30 with an overall mean of 213.10 ±2.37 mg/dl. The corresponding values for 2 non-conceived repeat breeding crossbred cows were found to be 217.46±2.14 mg/dl on day 12 which decreased to 212.00±2.31 mg/dl on day 30 with an overall mean of 214.70 ±1.93 mg/dl.
The mean plasma total cholesterol concentration (mg/dl) in 4 conceived repeat breeding crossbred cows on day 12 of hCG+ hCG injection was found to be 179.22±6.21 mg/dl which decreased to 170.23 ±3.37 mg/dl on day 30 with an overall mean of 174.70 ±3.18 mg/dl. The corresponding values for 2 non-conceived repeat breeding crossbred cows were found to be 200.13±9.03 mg/dl on day 12 which decreased to 191.20±06.12 mg/dl on day 30 with an overall mean of 195.60±3.16 mg/dl.
The mean plasma total cholesterol concentration (mg/dl) in 3 conceived repeat breeding crossbred cows on day 12 of hCG+ GnRH protocol was found to be 178.61±1.17 mg/dl which

decreased to 169.10±0.56 mg/dl on day 30 with an overall mean of 173.80±3.37 mg/dl. The corresponding values for 3 non-conceived repeat breeding crossbred cows were found to be 212.50±2.39 mg/dl on day 12 which decreased to 209.15±3.13 mg/dl on day 30 with an overall mean of 210.80±1.18 mg/dl.

The mean plasma total cholesterol concentration (mg/dl) in 4 conceived repeat breeding crossbred cows on day 12 of Inj. Progesterone (P_4) protocol was found to be 188.70±4.05 mg/dl which decreased to 179.10±3.14 mg/dl on day 30 with an overall mean of 183.90±3.40 mg/dl. The corresponding values for 2 non-conceived repeat breeding crossbred cows were found to be 201.10±2.49 mg/ dl on day 12 which decreased to 188.20±1.42 mg/dl on day 30 with an overall mean of 194.60±4.57 mg/dl.

The mean plasma total cholesterol concentration (mg/dl)in 3 conceived repeat breeding crossbred cows on day 12 of Placebo + Placebo treated cows was found to be 181.25±1.22mg/dl which decreased to 172.40±2.23mg/dl on day 30 with an overall mean of 176.80±3.13 mg/dl. The corresponding values for 3 non-conceived repeat breeding crossbred cows were found to be 210.18±2.82mg/dl on day 12 which decreased to 201.56±2.13 mg/dl on day 30 with an overall mean of 205.80 ±3.05 mg/dl.

In the present study, the total serum cholesterol concentrations in repeat breeding cows were significantly (P<0.05) lower when compared to normal cycling cows. This is in agreement with the reports of Patel *et al.* (2005) [21], Pandey *et al.* (2009) [19], Khan *et al.* (2010) [14] and Amle *et al.* (2014) [1]. Whereas non- significant difference (p>0.05) was recorded by Kumar *et al.* (2009a) [15].

This might be due to inadequate availability of metabolites and metabolic hormones in the central nervous system causing low level of GnRH secretion from the hypothalamus and also the gonadotrophic hormones from the anterior pituitary gland. Arosh *et al.* (1998) [2] also suggested the association of low level of cholesterol with reduced level of steroid Cholesterol is the most important sterol, in serum or in plasma, which originates from diet and from biosynthesis from acetyl CoA by decarboxylation of pyruvate and β-oxidation of fatty acids. About 70 per cent of cholesterol is present as cholesterol esters of fatty acids and the remaining 30 per cent is in free form. It is an essential precursor for steroid hormone synthesis of testis, ovary and adrenal cortex (Taylor *et al*., 1966 and Robinson, 1977) [31, 27]. Cholesterol utilized for steroid synthesis by ovarian tissue may be derived from de novo synthesis or cellular uptake of lipoprotein cholesterol (Grummer and Carrol, 1988) [11]. A high blood cholesterol concentration in cattle reflects a high concentration of HDL, which is directly related with the ovarian steriodogenesis. The higher level of cholesterol with advancement of lactation was a physiological adjustment to meet the lactation requirements. The current evidence makes it clear that at least for the ovary, placenta and adrenal, uptake of lipoprotein is the principle means of synthesis of the gonadal hormones (Sutaria, 2017) [30].

The comparison of the data presented in Table 4.07 indicate that there were no significant differences in the plasma total cholesterol profiles between days from treatment/AI inj. GnRH +GnRH, GnRH+hCG, hCG+hCG, hCG+GnRH, Inj. Progesterone (P_4) and placebo+ placebo treated group cows and also between conceived and non-conceived animals of the same groups, but the mean values (mg/dl) at day 30 post-AI in non- conceived cows in GnRH +GnRH (221.00±5.00), GnRH+hCG (212.00±2.31), hCG+hCG (191.20±06.12), hCG+GnRH (209.15±3.13), inj. Progesterone (P_4) (188.20±1.42) and placebo+ placebo treated group cows (201.56±2.13) were apparently higher as compared to those obtained in conceived groups (168.52±1.39, 209.81±0.61, 170.23±3.37, 169.10±0.56, 179.10±3.14 and 172.40±2.23 mg/dl, respectively.

The mean plasma total cholesterol concentration (mg/dl) values in conceived and non-conceived crossbred cows on day 12 and day 30 differ significantly (P<0.05) between all groups.

Noble *et al.* (1977) [17] and Chandrakar *et al.* (2003) [5] observed low plasma total cholesterol level during fertile estrus as compared to infertile estrus in buffaloes and cows, respectively, which corroborated with the present findings. The findings documented by Cetin *et al.* (2002) [4] were also very much similar to the present observations. Panchal (1995) [18] reported that the overall mean plasma total cholesterol was higher in inj. Duraprogen treated repeat breeding buffaloes than in control, which is in contrast to the present findings. Hadiya (2006) [12] reported that the average plasma cholesterol (mg per cent) in the experimental animals was 145.61 ± 2.33 (P>0.05).

Chaudhari (2012) [6] observed no significant difference in between conceived and non-conceived crossbred cows in GnRH protocol which is corroborated well with the present findings.

Rathod (2016) [24] reported that the mean total plasma cholesterol level (mg/dl) found in the normal cycling cows was 220.36 ± 7.88 while in repeat breeder cows, it was 190.56 ± 4.96. In the present study, the total serum cholesterol concentration values in repeat breeding cows were significantly (P<0.05) lower when compared to normal cycling cows. This is in agreement with the reports of Patel *et al.* (2005a) [21]; Pandey *et al.* (2009) [19]; Khan *et al.* (2010) [14] and Amle *et al.* (2014) [1], Whereas, non- significant difference (p>0.05) was recorded by Kumar *et al.* (2009b) [16].

This might be due to inadequate availability of metabolites and metabolic hormones in the central nervous system causing low level of GnRH secretion from the hypothalamus and also the gonadotrophic hormones from the anterior pituitary gland. Pareek and Aminuddeen (1985) [20] and Arosh *et al.* (1998) [2] also suggested the association of low level of cholesterol with reduced level of steroidogenesis.

The low levels of cholesterol in repeat breeder cows are indicative of subnormal nutritional status which is known to influence the pituitary function and reduce the secretion of gonadotrophin due to which follicles fail to develop and undergo atresia and subsequent development of early embryos is affected (Chauhan, 1981) [7]. Furthermore, poor nutrition and inanition which otherwise cannot be avoided in field situations also decrease the release of hormones that are unable to sustain complete reproductive response (Ramakrishna, 1996) [23].

Table 1: Mean plasma total cholesterol concentration (mg/dl) in non- infectious repeat breeding crossbred cows following different hormonal therapies

Treatment protocol	Status	No.	Days from treatment / AI		
			D-12	D-30	Overall
GnRH +GnRH	Conceived	3	176.50±1.91	168.52±1.39	172.51± 2.82
	Non conceived	3	236.10±3.80	221.00±5.00	228.5 ± 5.35
GnRH+hCG	Conceived	4	216.52±0.61	209.81±0.61	213.1± 2.37
	Non conceived	2	217.46±2.14	212.00±2.31	214.7 ± 1.93
hCG+hCG	Conceived	4	179.22±6.21	170.23±3.37	174.7 ± 3.18
	Non conceived	2	200.13±9.03	191.20±06.12	195.6 ± 3.16
hCG+GnRH	Conceived	3	178.61±1.17	169.10±0.56	173.8 ± 3.37
	Non conceived	3	212.50±2.39	209.15±3.13	210.8 ± 1.18
Inj.Progesterone	Conceived	4	188.70±4.05	179.10±3.14	183.9 ± 3.40
	Non conceived	2	201.10±2.49	188.20±1.42	194.6 ± 4.57
Placebo+Placebo	Conceived	3	181.25±1.22^{p}	172.40±2.23^{p}	176.8 ± 3.13
	Non conceived	3	210.18±2.82^{q}	201.56±2.13^{q}	205.8 ± 3.05

Total plasma protein

Details of the concentration of total protein (g/dl) observed in conceived and non-conceived crossbred cows in the present study under GnRH+GnRH, GnRH+hCG, hCG+hCG, hCG+GnRH, Inj. Progesterone (P_4) and placebo+ placebo treated group are presented in table 4.08 and figure 10.

The mean plasma total protein concentration (g/dl) in 3 conceived repeat breeding crossbred cows on day 12 of GnRH+GnRH injection were found to be 5.41±0.05 g/dl which increased slightly to 6.87±0.04 g/dl on day 30 with an overall mean of 6.14±0.51 g/ml. The corresponding values for 3 non-conceived repeat breeding crossbred cows were found to be 6.29±0.10 g/dl on day 12 which increased to 6.43±0.20 g/dl on day 30 with an overall mean of 6.36±0.04 g/dl.

The mean plasma total protein concentration (g/dl) in 4 conceived repeat breeding crossbred cows on day 12 of GnRH+hCG injection were found to be 6.58±0.04 g/dl which increased to 6.88±0.04 g/dl on day 30 with an overall mean of 6.73±0.10 g/dl. The corresponding values for 2 non-conceived repeat breeding crossbred cows were found to be 6.25±0.02 g/dl on day 12 which increased to 6.51±0.12 g/dl on day 30 with an overall mean of 6.38±0.09 g/dl.

The mean plasma total protein concentration (g/dl) in 4 conceived repeat breeding crossbred cows on day 12 of hCG +hCG protocol was found to be 6.88±0.14 g/dl which increased to 6.99±0.02 g/dl on day 30 with an overall mean of 6.93±0.03 g/dl. The corresponding values for 2 non-conceived repeat breeding crossbred cows were found to be 6.23±0.45 g/dl on day 12 which increased to 6.76±0.05 g/dl on day 30 with an overall mean of 6.49±0.18 g/dl.

The mean plasma total protein concentration (g/dl) in 3 conceived repeat breeding crossbred cows on day 12 of hCG+GnRH protocol was found to be 6.35±0.08 g/dl which increased to 6.83±0.09 g/dl on day 30 with an overall mean of 6.59±0.17 g/dl. The corresponding values for 3 non-conceived repeat breeding crossbred cows were found to be 6.41±0.20 g/dl on day 12 which increased to 6.75±0.09 g/dl on day 30 with an overall mean of 6.58±0.12 g/dl.

The mean plasma total protein concentration (g/dl)in 4 conceived repeat breeding crossbred cows on day 12 of Inj. Progesterone (P_4) protocol was found to be 6.66±0.11 g/dl which increased to 7.64±0.32 g/dl on day 30 with an overall mean of 7.15 ± 0.34 g/dl. The corresponding values for 2 non-conceived repeat breeding crossbred cows were found to be 6.24±0.04 g/ dl on day 12 which increased to 6.65±0.18 g/dl on day 30 with an overall mean of 6.24±0.14 g/dl.

The mean plasma total protein concentration (g/dl) in 3 conceived repeat breeding crossbred cows on day 12 of Placebo +Placebo treated protocol was found to be 6.46±0.10 g/dl which increased to 6.98±0.07 g/dl on day 30 with an overall mean of 6.72 ± 0.18 g/dl. The corresponding values for 3 non-conceived repeat breeding crossbred cows were found to be 6.44±0.04 g/ dl on day 12 which increased to 6.77±0.06 g/dl on day 30 with an overall mean of 6.60±0.11 g/dl.

The mean plasma total protein concentrations (g/dl) in conceived and non-conceived crossbred cows on day 12 and day 30 differed significantly (P<0.05) between all groups.

Repeat breeder cows treated with various hormonal therapies had relatively higher values of plasma total protein compared with placebo + Plasebo treated cows. Significant differences were observed in plasma total protein profiles between periods of the treatment. Higher concentration was observed in between conceived cows treated under inj. GnRH +GnRH, GnRH+hCG, hCG+hCG, hCG+GnRH, Inj. Progesterone (P_4) protocol.

Cetin *et al*. (2002) [4] found relatively identical serum total protein levels at estrus in repeat breeder than the fertile cows (8.26±0.13vs.8.50± 0.13 g per cent) which is higher than the findings obtained in present study.

Dhoble *et al.* (2004) [9] found significantly higher levels of plasma total protein in pregnant (7.92g/dl) than non-pregnant cows (7.71g/dl), which is comparable with the present findings in cows in normal cyclic control, hCG and inj. Progesterone supplementation protocol. Plasma total protein was lower in conceived animals than non-conceived ones under GnRH and Mid- cycle PGF2α protocol.

Rathod (2016) [24] reported that the mean total plasma protein (g/dl) level found in the normal cycling cows was 10.02 ± 0.40 while in repeat breeder cows, it was 9.14 ± 0.20 and the levels were higher than those observed in the present study. However Pandey *et al.* (2009) [19] and Khan *et al.* (2010) [14] found significantly lower (P <0.05) concentration of total plasma protein in the repeat breeding cows in comparison with the normally cycling cows.

Low level of plasma protein resulted in the deficiency of certain amino acids required for the biosynthesis of gonadotropins and gonadal hormones (Vohra *et al.*, 1995 and Arosh *et al.*, 1998) [2] which might cause reproductive hormonal disturbances in animals leading to inactive ovaries (Roberts, 1971) [26].

Albumin globulin ratio

Details of mean Albumin-Globulin ratio observed in conceived and non-conceived repeat breeding crossbred cows in the present study under GnRH+GnRH, GnRH+hCG,

hCG+hCG, hCG+GnRH, Inj. Progesterone (P_4) and placebo+ placebo treated group are presented in table 4.09 and figure 11

The mean plasma albumin–globulin ratio in 3 conceived repeat breeding crossbred cows on 12th day of GnRH+GnRH injection was found to be 0.69±0.02 which increased to 0.74±0.02 on day 30 with an overall mean of 0.69±0.01. The corresponding values for 3 non-conceived repeat breeding crossbred cows were found to be 0.67±0.04 on 12th day which increased to 0.73±0.04 on 30th day with an overall mean of 0.70±0.02.

The mean plasma albumin–globulin ratio in 4 conceived repeat breeding crossbred cows on day 12 of GnRH+ hCG injection was found to be 0.54±0.02 which slightly increased to 0.60±0.04 on day 30 with an overall mean of 0.57±0.02. The corresponding values for 2 non-conceived repeat breeding crossbred cows were found to be 0.66±0.02 on day 12 which increased to 0.72±0.02 on day 30 with an overall mean of 0.69±0.02.

The mean plasma albumin–globulin ratio in 4 conceived repeat breeding crossbred cows on day 12 of hCG+hCG protocol was found to be 0.67±0.04 which increased to 0.79±0.02 on day 30 with an overall mean of 0.73±0.04. The corresponding values for 2 non-conceived repeat breeding crossbred cows were found to be 0.62±0.09 on day 12 which increased to 0.71±0.07 on day 30 with an overall mean of 0.66±0.03.

The mean plasma albumin–globulin ratio in 3 conceived repeat breeding crossbred cows on day 12 hCG+GnRH protocol was found to be 0.78±0.02 which decreased to 0.63±0.04 on day 30 with an overall mean of 0.70±0.05. The corresponding values for 3 non-conceived repeat breeding crossbred cows were found to be 0.51±0.02 on day 12 which increased to 0.53± 0.05 on day 30 with an overall mean of 0.52±0.00.

The mean plasma albumin–globulin ratio in 4 conceived repeat breeding crossbred cows on day 12 of Inj. Progesterone (P_4) was found to be 0.89±0.06 which decreased to 0.75±0.02 on day 30 with an overall mean of 0.82 ±0.04. The corresponding values for 2 non-conceived repeat breeding crossbred cows were found to be 0.88±0.06 on day 12 which decreased to 0.79±0.02 on day 30 with an overall mean of 0.83±0.03.

The mean plasma albumin–globulin ratio in 3 conceived repeat breeding crossbred cows on day 12 of Placebo +Placebo treated cows was found to be 0.71±0.04 which decreased to 0.69±0.04 on day 30 with an overall mean of 0.70 ±0.00. The corresponding values for 3 non-conceived repeat breeding crossbred cows were found to be 0.58±0.06 on day 12 which increased to 0.65±0.06 on day 30 with an overall mean of 0.61±0.02.

The mean plasma albumin-globulin ratio in conceived and non-conceived crossbred cows on day 12 and day 30 differed significantly ($P<0.05$) between all groups.

Biochemical analysis of El-Sabaie *et al.* (1988) [10] revealed that repeat breeder dairy animals had higher A/G ratio than controls and the cows with endometritis had decreased A/G ratio.

Biochemical studies of Sahadev *et al.* (2007) [28] revealed non-significantly lower values of albumin/ globulin ratio as 0.68:1 ± 0.01 in cows with endometritis as compared to 0.70:1 ± 0.03 in healthy cows.

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