

## **Relation of various physiological blood parameters with the postpartum reproductive efficiency in cattle**

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### **Abstract**

Certain blood parameters indicate the postpartum reproductive performance in cattle. In the present study, blood samples were collected from 25 lactating animals on 10<sup>th</sup> day of calving and thereafter at weekly intervals up to 12 weeks postpartum. The samples were analyzed for glucose, cholesterol, serum urea nitrogen (SUN) and total proteins, which were correlated with the postpartum reproductive efficiency in cattle. Animals with more amount of blood glucose (55.49mg/dl) came to heat within 2 months compared to those with lesser blood glucose (50.20mg/dl), which came to heat after 2 months postpartum. There was no significant difference in the blood total protein concentration between the two groups of animal's i.e. which conceived within 3 months and another group which conceived after 3 months. Significantly more blood cholesterol (181.8mg) was present in animals which came to heat within 2 months than animals with cholesterol (159.9mg/dl) which came to heat after 2 months postpartum. Animals with lesser BUN (14.74 mg/dl) came to heat early compared to those with more amount of BUN (15.69 mg/dl). There was significant correlation of blood glucose, cholesterol and BUN with first postpartum heat.

**Keywords:** Blood glucose, BUN, Cholesterol, parameters and postpartum

### **1. Introduction**

Reproductive efficiency of a female is assessed by its ability to produce a viable offspring at the expected intervals. The postpartum period is a vulnerable period for diseases in the reproductive life of the female [1]. In farm animals, the postpartum period terminates with the exhibition of first postpartum estrus at which the pregnancy can be established. By this time the hypothalamo-hypophysial-gonadal-axis is expected to assume normal function so as to permit estrus, ovulation, conception, corpus luteum (CL) maintenance and pregnancy. The protein and energy balance is very important to keep the urea nitrogen levels in the animal system under check. Higher urea nitrogen levels in the body are known to affect uterine environment leading to failure of conception [2]. It has been put forth that negative energy balance affects gonadotropin secretion, ovarian physiology and ovarian hormone production which in turn

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adversely affect reproductive performance of cattle during postpartum period [3]. In view of this situation, Francisco *et al* [21] has concluded that blood glucose act as significant indicators or predictors of postpartum ovulations. There are many factors affecting duration of postpartum intervals like age, body weight, body condition, nutritional status, blood and milk parameters, seasonal effects, milk yield, suckling effects etc. Some of the recent studies conducted abroad indicate that certain blood parameters can indicate the postpartum reproductive performance in exotic cattle [4] [5]. But, such studies are lacking in our country. In spite of various reports available to overcome repeat breeding problem, understanding of the actual indicators for identifying of the cause for the same remains complex. Hence, in the present study, the blood samples from postpartum cattle were analyzed for glucose, cholesterol, blood

urea nitrogen (BUN) and total proteins to correlate them with the postpartum reproductive efficiency.

## 2. Materials and Methods

This study was conducted at the Military Dairy Farm located in Bangalore city. Throughout the experimental period, cows were fed a diet to meet the maintenance, growth and lactation requirements. The farm has herd strength of around 600 cattle and has been maintained under strict veterinary care and management. The animals were regularly screened for Tuberculosis, Johnes disease and Brucellosis to eliminate the reactors and keep the herd free from above diseases, in addition to being periodically vaccinated against Rinderpest, Foot and Mouth disease, Haemorrhagic septicemia, Black quarter and Anthrax.

Following calving, the animals were carefully observed twice daily for signs of behavioral estrus and the day of first postpartum estrus were duly recorded. However, artificial insemination was not carried out until the animal had clear vaginal discharge. Freshly thawed frozen semen of proven fertility was used for artificial insemination.

### 2.1 Experimental animals

Twenty-five crossbred (12 HF and 13 Frieswal) cows were randomly selected among the animals calved at Military Dairy Farm, Hebbal, Bangalore.

Following selection of the animals, a detailed history was obtained with regard to:

- (a) Age of the animal in years.
- (b) Parity.
- (c) Milk yield in liters / day on the day of selection.
- (d) Calving history (normal or abnormal calving).
- (e) Immediate postparturient complications such as eversion of uterus, RFM, hypocalcaemia, ketosis, Downer cow syndrome and septic metritis.

### 2.2 Experimental protocol

Periodical blood samples were collected from all the 25 cows after examining for from 10<sup>th</sup> day postpartum period then onwards, every 7 days upto 12 weeks. With respect to observations on first postpartum heat, the cows were considered as the two groups i.e 1) Cows which came to heat within two months postpartum and 2) Cows which came to heat after two months postpartum. And with respect to conception, the animals were considered as the two groups i.e. 1) the animals, which conceived within three months postpartum and 2) Animals, which conceived after three months postpartum. Blood samples were collected aseptically from jugular vein on specified days and allowed to clot. The serum was collected by centrifuging the clotted blood @ 2500

rpm for 10 min and the samples were stored at  $-20^{\circ}\text{C}$  till analyzed. Serum samples were analyzed for Glucose and Total Cholesterol using Autopack kit, Total Protein by Biuret method and Serum urea nitrogen by Diacetyl monoxime method.

### 2.3 Statistical analysis

The relation between various blood parameters and reproductive parameters was analyzed by Pearson correlation. The difference in these parameters between the groups was estimated by t-test. Differences between the mean values were considered significant when the P values were less than 0.05. MS Excel programme and GraphPad Prism software were used for statistical analysis.

## 3. Results

The relationship of various physiological parameters with postpartum fertility in dairy cattle was analyzed in this study. Out of 25 animals, the incidence of postpartum anestrus was 18 per cent and the incidence of repeat breeding was 13.6 per cent.

### 3.1 Glucose

The mean serum glucose levels for the Group 1 and Group 2 were  $55.49 \pm 0.59$  mg/dl and  $50.20 \pm 0.43$  mg/dl, respectively. Significantly ( $P < 0.0001$ ) more amount of blood glucose was present in animals which came to estrus within 2 months compared to those which came to estrus after 2 months postpartum. There was a significant ( $P < 0.05$ ) negative correlation ( $r = -0.44$ ) between blood glucose concentration and day of occurrence of first postpartum estrus (Table 1). There was a non-significant positive correlation ( $r = 0.01$ ) between glucose and day of conception. There was no significant difference ( $P > 0.05$ ) in the plasma glucose values of first three months postpartum between the two groups of animals i.e. one group, which conceived before 3 months and the other group which conceived after 3 months postpartum ( $54.22 \pm 0.68$  and  $53.71 \pm 0.79$  mg per cent, respectively).

### 3.2 Total protein

There was a non-significant ( $P > 0.05$ ) negative correlation ( $r = -0.04$ ) between protein and day of first estrus (Table 2). There was no significant difference ( $P > 0.05$ ) in the protein concentration between the two groups of animals i.e. the group which came to estrus within 2 months and the other group comprising animals that came to estrus after 2 months ( $7.14 \pm 0.09$  g per cent vs  $7.08 \pm 0.08$  g per cent, respectively). There was a non significant ( $P > 0.05$ ) negative correlation ( $r = -0.11$ ) between the total protein concentration and day of conception postpartum. There was no significant ( $P > 0.05$ ) difference in the blood total protein concentration

between the group of animals, which conceived within 3 months and the other group of animals that conceived after 3 months ( $7.18 \pm 0.09$  g per cent vs  $7.09 \pm 0.10$  g per cent).

### 3.3 Total Cholesterol

Significantly ( $P < 0.0001$ ) more amount of blood cholesterol was present in animals which came to estrus within 2 months compared to those which did not come to estrus within 2 months postpartum ( $181.8 \pm 2.80$  vs  $159.9 \pm 1.78$ ). There was a significant ( $P < 0.05$ ) negative correlation ( $r = -0.36$ ) between blood cholesterol concentration and day of occurrence of first postpartum estrus. There was no significant difference ( $P > 0.05$ ) in the blood cholesterol concentration between the two groups viz. the group of animals which conceived within 3 months and the other group of animals which conceived after 3 months postpartum ( $177.0 \pm 3.34$

and  $172.6 \pm 3.96$ , respectively). There was a non significant ( $P > 0.05$ ) negative correlation ( $r = -0.11$ ) between blood cholesterol concentration over a period of 3 months postpartum and day of conception.

### 3.4 Serum urea nitrogen (SUN)

There was a significant ( $P > 0.05$ ) difference in the SUN level between the two categories i.e. those came to estrus within 2 months postpartum and those did not come to estrus before 2 months ( $14.74 \pm 0.22$  vs  $15.69 \pm 0.33$  mg per cent, respectively). There was a non significant ( $P > 0.05$ ) positive correlation ( $r = 0.12$ ) between the SUN and day of conception. There was no significant ( $P > 0.05$ ) difference in the SUN level between the two groups of animals which conceived within 3 months and the groups which conceived after 3 months of postpartum ( $15.05 \pm 0.24$  vs  $15.77 \pm 0.40$  mg per cent).

**Table 1: Mean  $\pm$  SE values of blood and milk parameters during first 3 months of postpartum (n = 25)**

| Days postpartum         | 10                | 17                | 24                | 31                | 38                | 45                | 52                | 59               | 66                | 74                | 81                |
|-------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------------------|-------------------|-------------------|-------------------|
| <b>Blood parameters</b> |                   |                   |                   |                   |                   |                   |                   |                  |                   |                   |                   |
| 1) Glucose (mg %)       | 42.35 $\pm$ 0.96  | 47.37 $\pm$ 0.9   | 50.09 $\pm$ 1.03  | 53.65 $\pm$ 1.17  | 53.41 $\pm$ 0.65  | 55.02 $\pm$ 0.84  | 55.92 $\pm$ 1.06  | 56.10 $\pm$ 1.13 | 56.33 $\pm$ 1.11  | 54.17 $\pm$ 0.68  | 54.40 $\pm$ 0.69  |
| 2) Protein (g %)        | 5.73 $\pm$ 0.09   | 6.43 $\pm$ 0.11   | 7.35 $\pm$ 0.11   | 7.90 $\pm$ 0.09   | 8.67 $\pm$ 0.08   | 8.91 $\pm$ 0.08   | 9.38 $\pm$ 0.07   | 10.04 $\pm$ 0.07 | 10.54 $\pm$ 0.08  | 10.44 $\pm$ 0.08  | 10.84 $\pm$ 0.09  |
| 3) Cholesterol (mg %)   | 149.48 $\pm$ 4.06 | 148.95 $\pm$ 2.86 | 159.78 $\pm$ 4.94 | 162.87 $\pm$ 4.84 | 165.72 $\pm$ 5.07 | 168.83 $\pm$ 4.42 | 175.25 $\pm$ 5.86 | 178.51 $\pm$ 5.4 | 180.82 $\pm$ 5.34 | 182.13 $\pm$ 5.45 | 185.21 $\pm$ 6.21 |
| 4) BUN (mg %)           | 11.28 $\pm$ 1.021 | 12.71 $\pm$ 1.05  | 14.01 $\pm$ 1.04  | 15.03 $\pm$ 0.91  | 16.28 $\pm$ 1.00  | 16.84 $\pm$ 1.08  | 16.92 $\pm$ 0.93  | 17.06 $\pm$ 1.01 | 16.62 $\pm$ 1.01  | 17.09 $\pm$ 0.96  | 17.23 $\pm$ 1.01  |
| <b>Milk Parameters</b>  |                   |                   |                   |                   |                   |                   |                   |                  |                   |                   |                   |
| 1) MUN (mg %)           | 10.25 $\pm$ 0.42  | 11.44 $\pm$ 0.39  | 12.55 $\pm$ 0.37  | 13.50 $\pm$ 0.29  | 14.46 $\pm$ 0.35  | 15.15 $\pm$ 0.33  | 15.50 $\pm$ 0.30  | 15.60 $\pm$ 0.27 | 15.34 $\pm$ 0.24  | 15.98 $\pm$ 0.27  | 15.94 $\pm$ 0.25  |
| 2) Lactose (g %)        | 3.48 $\pm$ 0.0    | 3.54 $\pm$ 0.0    | 3.73 $\pm$ 0.05   | 3.78 $\pm$ 0.04   | 3.92 $\pm$ 0.06   | 4.04 $\pm$ 0.06   | 4.17 $\pm$ 0.05   | 4.22 $\pm$ 0.05  | 4.314 $\pm$ 0.05  | 4.34 $\pm$ 0.04   | 4.38 $\pm$ 0.05   |
| 3) Milk yield (L)       | 11.56 $\pm$ 1.02  | 12.86 $\pm$ 1.05  | 13.62 $\pm$ 1.04  | 13.34 $\pm$ 0.91  | 13.78 $\pm$ 1.00  | 14.14 $\pm$ 1.08  | 14.38 $\pm$ 1.08  | 14.28 $\pm$ 0.93 | 13.9 $\pm$ 1.01   | 13.84 $\pm$ 0.96  | 14.24 $\pm$ 1.01  |

**Table 2: Mean  $\pm$  SE values for uterine involution interval and exhibition of first postpartum estrus in crossbred cows**

| Parameters                          | Exhibition of first postpartum estrus before 2 months (n = 12) | Exhibition of first postpartum estrus after 2 months (n = 13) |
|-------------------------------------|--|---|
| Uterine involution (days)           | 31.16 $\pm$ 2.15   | 27.53 $\pm$ 2.22  |
| First postpartum estrus (days)      | 43.33 $\pm$ 3.25   | 96.14 $\pm$ 10.19   |
| Exhibition of postpartum estrus (%) | 48   | 52  |

**Table 3: Mean  $\pm$  SE values of physiological and biochemical parameters recorded during first postpartum period in crossbred cows**

|                             | Overall mean $\pm$ SE | Animals came to estrus within 2 months | Animals came to estrus after 2 months | Animals conceived within 3 months | Animals conceived after 3 months |
|-----------------------------|-----------------------|--|---------------------------------------|-----------------------------------|----------------------------------|
| BCS                         | 3.00 $\pm$ 0.06       | 3.18 $\pm$ 0.04                        | 3.17 $\pm$ 0.04                       | 2.97 $\pm$ 0.03                   | 3.12 $\pm$ 0.05                  |
| <b>Serum parameters</b>     |                       |  |                                       |                                   |                                  |
| 1) Glucose (mg %)           | 52.62 $\pm$ 1.31      | 55.49 $\pm$ 0.59                       | 50.02 $\pm$ 0.59                      | 54.22 $\pm$ 0.68                  | 53.71 $\pm$ 0.79                 |
| 2) Protein (g %)            | 8.75 $\pm$ 0.51       | 7.14 $\pm$ 0.09                        | 7.08 $\pm$ 0.08                       | 7.18 $\pm$ 0.09                   | 7.09 $\pm$ 0.10                  |
| 3) Total cholesterol (mg %) | 168.9 $\pm$ 3.83      | 181.8 $\pm$ 2.80                       | 159.9 $\pm$ 1.78                      | 177.0 $\pm$ 3.34                  | 172.6 $\pm$ 3.96                 |
| 4) Urea nitrogen (mg %)     | 15.56 $\pm$ 0.61      | 14.74 $\pm$ 0.22                       | 15.69 $\pm$ 0.33                      | 15.05 $\pm$ 0.24                  | 15.77 $\pm$ 0.40                 |
| <b>Milk parameters</b>      |                       |  |                                       |                                   |                                  |
| 1) Milk yield (L)           | 13.63 $\pm$ 0.24      | 13.89 $\pm$ 0.49                       | 14.54 $\pm$ 0.38                      | 13.60 $\pm$ 0.58                  | 14.33 $\pm$ 0.54                 |
| 2) Lactose (g %)            | 3.10 $\pm$ 0.19       | 4.00 $\pm$ 0.04                        | 3.98 $\pm$ 0.04                       | 3.95 $\pm$ 0.04                   | 4.15 $\pm$ 0.04                  |
| 3) MUN (mg %)               | 14.16 $\pm$ 0.51      | 13.77 $\pm$ 0.20                       | 14.64 $\pm$ 0.25                      | 14.12 $\pm$ 0.23                  | 14.36 $\pm$ 0.32                 |

**Table 4: Correlation coefficients of physiological parameters with postpartum fertility**

|                              | First estrus | Conception |
|------------------------------|--------------|------------|
| BCS (loss of BCS at calving) | 0.19         | –          |
| <b>Serum parameters</b>      | -0.44*       | 0.011      |
| 1) Glucose                   | -0.04        | -0.11      |
| 2) Protein                   | -0.36*       | -0.11      |
| 3) Total Cholesterol         | 0.24         | 0.12       |
| 4) BUN                       |              |            |
| <b>Milk parameters</b>       | 0.20*        | 0.04       |
| 1) MUN                       | -0.05        | 0.24       |
| 2) Milk lactose              | 0.17*        | 0.04       |
| 3) Milk yield                |              |            |

\* P &lt; 0.05

## 4. Discussion

### 4.1 Glucose

Energy is the first limiting dietary factor for cows in early lactation. Energy demands in the form of milk output and body maintenance exceeds energy inputs in early postpartum. Cows mobilize energy stores from their body reserve to make up the difference between energy intake and energy output. This puts cows into a negative energy state. Length of time cows spend on negative energy states vary quite a bit, depending mainly on their ability to increase dry matter intake (DMI) rapidly. Indeed, DMI is much more closely correlated to energy status than milk [6]. Negative energy status of a cow is one of the most important, affecting days to first ovulation. Cows with poor body conditions (negative energy) may not show cycle until 60 days postpartum, increasing open days.

Lucy *et al* [7] opined that there exist direct relationship between positive energy status at early postpartum and diameter of largest follicle on day 10 postpartum. These positive effects of energy on reproduction might be due of increase in Leutinizing hormone (LH) / Follicle stimulating hormone (FSH), insulin, Growth hormone, Insulin like growth factor (IGF) and other yet to be determined compounds as activated by improving energy status. In order to avoid large negative energy, energy balance, and maximize dry matter intake (DMI), well-known conventional management practices are recommended. In the present study, significantly ( $P < 0.0001$ ) more amount of blood glucose was present in animals which came to estrus within 2 months compared to those which came to estrus after 2 months postpartum ( $55.49 \pm 0.59$  mg per cent vs  $50.20 \pm 0.43$  mg per cent). However, Singh *et al* [8] reported that the level of glucose did not influence early or late occurrence of postpartum estrus in buffaloes.

Rabiee and Lean [9] found that there was a positive and highly significant cross correlation between the uptake of glucose and cholesterol and suggested that glucose may promote cholesterol uptake into ovarian cells or vice versa. In the present study there was a statistical positive correlation of blood glucose and blood cholesterol. These results are in agreement with the observations of Morrow (1969). Reist *et al* [10] reported that the plasma glucose may be involved in steroid synthesis in the ovary and luteal function and thus, might strongly influence the interval from calving to conception.

### 4.2 Total protein

Protein is required for maintenance, growth, lactation, and reproduction. NRC (1989) recommends dietary concentration of 19 per cent crude protein in milch cattle diet during the first 3 weeks post partum and 16-18 per cent thereafter, depending upon the amount of milk being produced. The reason for higher recommendation (19 per cent) just after calving is low DMI. It is also during this period that cows undergo tremendous metabolic changes, e.g. shifting from a homeostatic to homeorhetic state to prioritize milk production. Demand for glucose is intense and gluconeogenesis is prominent. Protein reserves in the body and tissue are called upon to supply carbon skeletons for glucose synthesis. About 11.4 to 16.0 kg of protein are often mobilized during the first 60 days of lactation [NRC, 1989]. In this until 6-10 weeks after calving conversion of amino acids (AA) to glucose, ammonia is liberated and must be transformed into urea by the liver to prevent ammonia toxicity. Detoxification of ammonia by the liver to urea is an energy consuming process. These processes continue in concert until all protein and energy requirements can be met by diet consumption; this usually does not occur

In the present study the mean values of the plasma protein levels in both groups did not differ significantly i.e. the groups which came to estrus within 2 months and the other group comprising animals that came to estrus after 2 months ( $7.14 \pm 0.09$  g per cent vs  $7.08 \pm 0.08$  g per cent, respectively). These values are well within the range reported by Larson *et al* [11], Agarwal *et al* [12], Quayam *et al* [13], in cows and buffaloes. And the total protein in blood in the present study did not reveal any significant difference between group of animals which conceived within 3 months and other groups animals that conceived after 3 months ( $7.18 \pm 0.09$  g per cent vs  $7.09 \pm 0.10$  g per cent). However Kavani *et al* [14] reported higher protein levels during fertile cycles. The average serum total proteins levels were significantly higher in normal

cycling cows ( $8.62 \pm 0.13$ g/dl) than in anestrus cows ( $6.82 \pm 0.040$  g/dl) [15][16][17]. The role of proteins in reproduction of ruminants is equivocal. Diets deficient in protein have resulted in weak expression of estrus, cessation of estrus, repeat breeding, etc. However, total proteins in circulation represent a balance between the biosynthesis and catabolism or mechanical loss [18]. The lower level of serum proteins may cause deficiency of certain amino acids required for the synthesis proteins in the body.

#### 4.3 Total cholesterol

In the present study, there was a significant negative correlation between blood cholesterol concentration and day of occurrence of first postpartum heat, which is in agreement with an earlier report. Henricks *et al* [19] in this regard purports to the view that the highest adrenal cholesterol values occur at estrus when females are under estrogen dominance eventually facing a decline later on when the progesterone phase sets in. In our study, significantly ( $P < 0.0001$ ) more amount of blood cholesterol was present in animals which came to estrus within 2 months compared to those which did not come to estrus before 2 months postpartum ( $181.8 \pm 2.80$  vs  $159.9 \pm 1.78$  mg/dl). Plasma cholesterol concentrations were consistently important in predicting nutritional status of lactating and nonlactating dairy cows. Plasma insulin and IGF-I concentrations increase concomitantly with plasma cholesterol during early lactation [20][21][22].

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