Changes in blood biochemical constituents in cows with normal and impaired fertility

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Abstract
The study assessed the influence of certain haematological and blood biochemical parameters in crossbred cows. The levels of Haemoglobin, PCV, Serum phosphorous, blood glucose, total serum protein and total serum cholesterol were $10.06\pm0.61$, $31.00\pm1.33$, 4.85$\pm$0.34, 46.10$\pm$3.19, 7.00$\pm$0.99 and 95.09$\pm$8.67 in normally fertile cows. In cows with impaired fertility which were deficient in different constituents, the corresponding levels were 6.86$\pm$0.075, 21.50$\pm$0.50, 3.65$\pm$0.068, 31.13$\pm$0.726, 4.57$\pm$0.30 and 71.36$\pm$1.66 respectively, all of which were significantly lower than their corresponding levels in normally fertile cows ($P < 0.10$). The overall conception rate was 81.80 percent in normal group, whereas that was 11.11 only in impaired fertility group. Conception level of cows with lower level of Hb was significantly lower than that of cows with normal level ($P < 0.05$). Similarly, conception rate of cows with low level of PCV was also significantly lower compared to that of normal cows. There was statistically significant reduction in conception rate of animals with low levels of serum phosphorous ($P < 0.01$), blood glucose and cholesterol ($P < 0.01$). The results of present study proved that certain haematological and blood biochemical parameters have definite influence on fertility in cows.

Keywords: Serum phosphorous, blood glucose level, cholesterol, crossbred cows

Introduction
The production capacity of cattle, to a very great extent depends on the reproductive efficiency as measured by its ability to conceive and deliver a viable calf each year during its life span. In this context the scientific aspect of reproductive management is of extreme relevance [1]. Any deviation or prolongation in the breeding rhythm results in a progressive economic loss due to widening of dry periods, reduced calving and lactation. In Kerala, it is estimated that if the calving interval could be shortened from 19.9 months to 15 months, 18.5 per cent more cows would calve every year, contributing to an additional 2.6 lakh metric tonnes of milk worth Rs. 90 crores [2].

The reasons of reproductive inefficiency include anatomical and genetic defects, physiological, pathological and managemental factors. Over the last many decades there has been a noticeable trend in the causes of infertility in cattle [3]. Although, nonspecific infections due to opportunistic pathogens are still important [4], by far the greatest cause of infertility is poor management of herds. Nutritional status of the animals during the initial lactation period play significant role in the postpartum reproductive performance. There are several reports to indicate that dairy cows deficient in nutrition exhibit low fertility; the main reason being the ration deficient both qualitatively and quantitatively. If the ration is deficient in Metabolisable energy the cow mobilises glycogen from muscle resulting in decline of body weight leading to poor fertility. Afterall, reproduction is considered as luxury function by the animal. Superimposed on energy and protein deficiency there can also be multiple deficiencies of phosphorous, trace elements and certain vitamins. However there appears to be no evidence to show that any single nutrient is required specially for reproduction.

Nutritional errors do not always produce similar clinical signs in all animals in a herd and in certain circumstances individual animal may be specially affected. High yielding cows may not be able to maintain body weight and conceive on a diet which enables low yielding animals to achieve this objective. The relationship between supply of nutrients and reproductive performance is a difficult problem [5]. This results in a risk that the breakdown point in a system, so far to its supply of nutrients for reproduction is considered, may not be recognised, until too late.
Application of the knowledge of the nutritional parameters influencing reproduction under these systems could be expected to increase the efficiency of management. Unfortunately, the data on these lines are scanty, particularly in crossbred cows and therefore research on these lines should receive greater emphasis.

Although there are several reports to indicate the relationship between the levels of these metabolites and other haematological profile and fertility in purebred and indigenous cows, literature reveals paucity of such information in Sumandini crossbred cows. Hence the present study was conducted to analyse the role of important haematological and biochemical parameters in crossbred cows with normal and impaired fertility with ultimate object of assessing their influence on fertility.

Materials and Methods
The study was conducted in animals brought for insemination at the artificial insemination centre and University Livestock Farm Mannuthy, Thrissur, Kerala. Breeding history and feeding practices of all these animals were collected in details and were subjected to detailed gynaeco-clinical examination and were divided primarily into two groups.

Group I consisted of eleven animals which had apparently normal breeding history and which conceived with one or two inseminations were included in group I and were inseminated and followed up. Group II consisted of animals that were hard to settle and free from any genital infection but apparently deficient in nutrition. Fifty-two such animals were selected to group II.

Ten ml of blood was collected from the jugular vein of all the cows in the groups in test tubes, and was allowed to colt. The serum was separated and collected (Sample A). Another 10 ml of blood was collected in test tubes containing 10 mg of EDTA (Sample B).

Using serum from sample A, glucose was estimated by O-Toludine method using Stangen Glucose Kit. Five ml of reagent was mixed with 0.05 ml of standard (S) and serum (T) and heated for 10 minutes in boiling water bath. Then it was cooled and absorbance (A) was read at 630 nm against deionised water (Blank) in a spectrophotometer. Then glucose was estimated as the ratio of absorbance of Test to that of Sample expressed in percentage.

Total protein in serum was estimated by Biuret method [8]. Serum protein level below 5.7 g per cent was considered deficient [7].

Serum phosphorous was estimated by modified Metol method using Stangen Phosphorous Kit. 0.1 ml of serum (T), Standard (S) and de-ionised water (Blank) were pipetted into clean dry test tubes, and 1 ml of Metol reagent were added to all the test tubes. Mixed well and kept in room temperature for 5 minutes. Absorbance (A) was measured on a spectrophotometer at 680 nm within 30 minutes. Then phosphorous concentration was estimated as the ratio of absorbance of Test to that of Sample multiplied by five.

Total cholesterol was estimated by Wybenga and Pileggi’s method [8] using Stangen Cholesterol Kit. 0.05 ml of serum (T), Standard (S) and Blank (deionised water) were pipetted out into clean dry test tubes. 5 ml of cholesterol reagent was added to each of the test tubes. Mixed well and immediately placed in a boiling water for exactly 90 seconds. Cooled immediately in running tap water and measured the absorbance (A) of serum and standard against blank on a spectrophotometer at 560 nm within 15 minutes. Then total cholesterol was estimated as the ratio of absorbance of Test to that of Sample multiplied by 200.

Blood was taken from sample B and haemoglobin was estimated by Sahlis acid hematin method [9] and Packed Cell Volume (PCV) was determined by Wintrobe haematocrit method [10]. Hb level below 7 g per cent and PCV level below 24 per cent were considered as deficient [7].

The data recorded were analysed statistically [9], using statistical software SPSS (SPSS, USA).

Results and Discussion
The haematological parameters of animals with normal fertility (Group I) revealed that the Hb values ranged from 6.6 to 13 g percent with an average of 10.06 g percent while the PCV ranged from 22 percent to 38 percent with a mean of 31 percent. Among the biochemical parameters of cows in group I, the level of serum phosphorous ranged from 4 mg per cent to 6.85 mg per cent with a mean of 4.85 mg per cent; serum protein from 5.62 g percent to 10.33 g percent with a mean of 7.00 g percent, blood glucose from 29 mg percent to 63.63 percent with a mean of 46.10 mg percent and total cholesterol levels from 48 mg percent to 140 mg percent with a mean of 95.09 mg percent respectively.

Perusal of the data regarding the haematological parameters of cows with impaired fertility (Group II) revealed that the level of Hb ranged from 6 g percent to 12.5 percent with a mean of 8.085 and PCV from 20 to 36 percent with a mean of 28.52. In cows with impaired fertility, it was observed that the level of phosphorous ranged from 2.57 to 8.85 mg percent with a mean of 4.175 mg percent; protein from 3.75 to 11.6 g percent with a mean of 6.915 mg percent, glucose from 22 to 81.81 mg percent with a mean of 47.27 percent and cholesterol value from 48 to 184 mg percent with a mean of 93.35 mg percent respectively.

A comparative statement of various blood parameters of animals with normal fertility and animals with impaired fertility deficient in various parameters are presented in Table 1. Animals with normal fertility recorded and average of 10.06 ± 0.618 g percent of Hb while animals with impaired fertility registered a level of 6.86±0.075 g percent. Similarly, the mean level of PCV averaged 31.00±1.33 percent in animals with normal fertility while that of animals with impaired fertility, it was only 21.50 ±0.50 percent. The data on serum phosphorous level of animals with normal fertility and impaired fertility were 4.85±0.343 mg percent and 3.65±0.068 mg percent respectively. The total serum protein of animals with normal fertility and impaired fertility was 7.00±0.99 g percent and 4.57±0.30 g percent respectively. The blood glucose level of animals with normal fertility was 46.10±3.19 mg percent while that of animals with impaired fertility was 31.13±0.726 mg percent. The mean total serum cholesterol for cows with normal fertility was 95.09±8.67 mg percent while that of animals with impaired fertility was 71.36±1.66 mg percent. All these parameters were significantly higher (P < 0.01) in animals with normal fertility that in animals with impaired fertility.

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Overall conception rate of animals with normal fertility (Group I) is presented in Table 2. It was observed from the table that 9 out of 11 cows conceived registering a conception rate of 81.8 percent. Overall conception rate of animals with impaired fertility without any treatment (Groups II) is presented in Table 2. Perusal of data revealed that out of 29 animals, 18 were inseminated and only two animals conceived giving conception rate of 11.1 percent.

Table 2: Overall conception rate of animals with normal fertility (Group I) and animals with impaired fertility (Group II)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Group</th>
<th>No. observed</th>
<th>No. inseminated</th>
<th>No. conceived</th>
<th>Conception rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal fertility (GI)</td>
<td>11</td>
<td>11</td>
<td>9</td>
<td>81.80</td>
</tr>
<tr>
<td>2</td>
<td>Impaired fertility (GI)</td>
<td>29</td>
<td>18</td>
<td>2</td>
<td>11.11</td>
</tr>
</tbody>
</table>

The conception rate of animals with normal levels of haemoglobin and animals with low levels of haemoglobin are presented in Table 3. Perusal of data revealed that out of 22 animals with normal level of Hb, 11 animals conceived registering a conception rate of 50 percent while none of the 7 animals with low level of Hb conceived. The difference in conception rate was significant between the groups (P< 0.10). Whereas, there was no significant difference observed in conception rate of cows with normal values of packed cell volume and lower levels of PCV, though three animals with low levels of PCV did not conceive.

There was significant difference observed between the conception rate of cows with normal values of serum phosphorous, blood glucose and total serum cholesterol with that of cows with lower values (P< 0.01).

Table 3: Conception rate of cows with normal and low levels of different haematological and blood biochemical constituents

<table>
<thead>
<tr>
<th>S. No</th>
<th>Blood constituents</th>
<th>Normal</th>
<th>Low</th>
<th>X² value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inseminated</td>
<td>Conceived</td>
<td>CR</td>
</tr>
<tr>
<td>1</td>
<td>Hemoglobin</td>
<td>22</td>
<td>11</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Packed cell volume (%)</td>
<td>27</td>
<td>11</td>
<td>40.75</td>
</tr>
<tr>
<td>3</td>
<td>Serum Phosphorous (mg%)</td>
<td>14</td>
<td>10</td>
<td>71.4</td>
</tr>
<tr>
<td>4</td>
<td>Total serum protein (g%)</td>
<td>15</td>
<td>7</td>
<td>46.7</td>
</tr>
<tr>
<td>5</td>
<td>Blood Glucose (mg%)</td>
<td>10</td>
<td>7</td>
<td>70</td>
</tr>
<tr>
<td>6</td>
<td>Total serum cholesterol</td>
<td>13</td>
<td>9</td>
<td>69.2</td>
</tr>
</tbody>
</table>

*significant at 5% level; **significant at 1% level.

In dairy animals, blood glucose level is an indication of the gluconeogenesis (synthesis from precursors) and glycogenolysis (mobilization of stored glucose). Glucose is the chief source of energy for the primary reproductive organs in the body of cattle [11]. It exerts substantial effect on thecal cell steroidogenesis invitro and responsible for achieving early ovulation in postpartum cows [12]. Blood glucose levels modulate the hypothalamic-hypophysial-ovarian axis, there by promoting the synthesis and secretion of gonadotropins and stimulating the growth of graffian follicles [13]. It is well documented that a positive correlation exists between the glucose uptake and cholesterol levels, which prove that glucose is essential for cholesterol uptake in to ovarian cells [14]. It is also reported that glucose is essential for the development of embryos post blastulation [15]. In dairy animals, under nutrition and reduced blood glucose levels usually leads to delayed onset of postpartum cyclicity [16]. Previously, it was established that during the fertile phase of oestrus, usually the glucose levels were found to be elevated in normally cycling animals than anoestrus or suboestrus animals [17]. Similarly, a reduced concentration of blood glucose in non-fertile animals, an indication of subnormal energy status of cows is reported earlier [18]. It was previously reported observed [19] that serum glucose level (mg/dl) were lower in repeat breeder cows (44.71±5.17) compared to fertile cows (65.00±6.27).

Cholesterol is the chief precursor for the biosynthesis of progesterone by the luteal cells of ovary, which helps for the implantation of embryos and responsible for maintenance of pregnancy [20, 21]. Previously, higher levels of blood cholesterol levels reflected in reproduction parameters of high producing cows. It is reported that after calving, the mean serum total cholesterol levels exhibit a linear path of increasing [22]. Phosphorous is generally associated with reproductive performance of dairy animals. Reduced levels of phosphorous leads to impaired fertility, irregular/delayed oestrus, and prolonged conception interval [23, 24]. But there are not enough studies available that excess feeding of phosphorous improves reproductive efficiency. The results of present study also emphasize that phosphorous is an important component for reproductive performance of crossbred cows.

Conclusion
The results of present study revealed that certain haematological and biochemical parameters have definite influence on the fertility in crossbred cows. The assessment of these parameters as an index of impending fertility in dairy cows would need further detailed study based on larger number of animals.

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Table 1: Haematological and blood biochemical parameters of cows with normal and impaired fertility

<table>
<thead>
<tr>
<th>S. No</th>
<th>Blood constituents</th>
<th>Cows with normal fertility</th>
<th>Cows with impaired fertility</th>
<th>t value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hemoglobin</td>
<td>10.06±0.618 (11)</td>
<td>6.86±0.075 (12)</td>
<td>6.0124*</td>
</tr>
<tr>
<td>2</td>
<td>Packed cell volume (%)</td>
<td>31.00±13.3 (11)</td>
<td>21.50±0.50 (6)</td>
<td>6.6947*</td>
</tr>
<tr>
<td>3</td>
<td>Serum Phosphorous (mg%)</td>
<td>4.85±0.34 (11)</td>
<td>3.65±0.068 (32)</td>
<td>4.6690*</td>
</tr>
<tr>
<td>4</td>
<td>Total serum protein (g%)</td>
<td>7.00±0.99 (11)</td>
<td>4.57±0.30 (6)</td>
<td>3.731*</td>
</tr>
<tr>
<td>5</td>
<td>Blood Glucose (mg%)</td>
<td>46.10±3.19 (11)</td>
<td>31.13±0.726 (28)</td>
<td>4.5127*</td>
</tr>
<tr>
<td>6</td>
<td>Total serum cholesterol</td>
<td>95.09±8.67 (11)</td>
<td>71.36±1.66 (33)</td>
<td>2.7146</td>
</tr>
</tbody>
</table>

Numbers in parenthesis indicate number of observations; *significant at 10% level
References