and cooperation.

The animals were Holstein breed and fed corn silage, concentrates and hay throughout the year. Diets fed before and after calving were formulated to meet or exceed National Research Council recommendations (NRC, 1989). Feed analysis was not performed, but the approximated nutrient contents of the diet were estimated during the sampling times (Table 1). The mean milk yields were taken from the milk records. The nearest milk yield relevant to the date of blood sampling was used (Table 2). In almost all cases, sampling was without apparently causing any untoward anxiety or stress to the animals.

Blood samples were obtained on 5 ± 3, 30±3 and 58±3 days postpartum. Blood was taken via jugular venipuncture into a plain glass tube in which the blood was allowed to coagulate. A heparinized microhaematocrit tube was used to collect blood for packed cell volume (PCV) determination. All samples were transported to the laboratory within two hours after collection. Serum was harvested after centrifugation frozen and stored at -20°C until analysis. Blood analysis was performed by a computerized auto-analyzer (Technicon RA-1000). Serum glucose, by glucose-oxidase method; urea nitrogen, by D-acetyl mono-oxim method; cholesterol, by CHOD-TAP method; albumin, by Bromcresol green method; total protein, by Biuret method; calcium, by orthocresol method; inorganic phosphorus, by phosphomolybdate method; and aspartate amino transferase (AST), by IFCC method were measured for each sample.

Cows were observed for estrus activity throughout day, also during handling for milking and feeding. The cows were inseminated by a local technician using the same batch of semen in the farm from six weeks postpartum. Pregnancy was evaluated 35–42 days after insemination and a second palpation was done two weeks later to confirm the pregnancy. Cows were divided into two categories; those which conceived at either first or second insemination (group 1) and those which were inseminated ≥3inseminations.

The normality of the data distribution was tested by Kolmogorov-Smirnov test. Difference between means of two normally-distributed independent groups was tested by independent samples Student's t-test, using SPSS program for Windows®. If the distribution of data was not normal, Mann-Whitney U-test was used, instead. A p value <0.05 was considered significant.

Results

Twenty-eight (70%) of 40 cows conceived at either the first or second insemination, while 12 (30%) of 40 needed three or more inseminations. Calcium and inorganic phosphorus values were significantly ($P<0.05$) higher in the group 1 cows at second stage of sampling. The concentrations of calcium in fertile (group 1) and infertile (group 2) cows were 2.34 and 2.17 mmol/l, respectively. The levels of inorganic phosphorus in groups 1 and 2 were 1.84 and 1.42 mmol/L, respectively (Table 3). The comparison of measured blood constituents between two groups at the first and third samplings did not reveal significant difference.

Discussion

Various reproductive traits have been measured and reported in investigations of the possible association between blood chemistry and reproductive performance. Conception rate has been considered as fertility indicator in this study. Nebel and McGilliard (1993) suggested, although influenced by many factors, conception rate or number of breeding is more inherently associated with physiological functions.

In this study calcium and inorganic phosphorus levels of fertile cows were significantly ($P<0.05$) higher than cows with low fertility.

Calcium ion has critical role in the metabolism of the cells of the reproductive system, but it has not still been shown to be involved in nutritional and metabolic fertility (McClure, 1994). In a study, calcium was lower in 106 sterile animals as compared with 96 normal (mean calcium concentration of 9.3 mg/dl as compared with 9.9 mg/dl) (Payne and Payne, 1987). A direct correlation between the feeding of calcium and conception rate has been found (Hurley nad Doane, 1989).

Calcium-dependent mechanisms are

Table 1: Estimated daily nutrients fed to the cows of the study at dry period and the 1st and 2nd month of lactation based on the number of insemination

Time of sampling	Number of insemination	Total weigh (kg)	Dry matter (kg)	Crude protein (%DM)	TDN (kg)	Energy (Mcal)	Calcium (g)	Phosphorus (g)
Dry period		18.18	10.40	17.6	11.12	24.32	84	41
1st month after calving	1st or 2nd	27.19	18.06	18.2	16.64	37.79	110	120
	3rd or more	27.54	18.79	18.2	16.29	38.42	110	120
2nd month after calving	1st or 2nd	26.61	18.05	19.8	18.42	40.95	100	100
	3rd or more	27.71	19.08	19.7	19.21	42.72	110	110

Table 2: The average of milk yield (kg) of the cows on based on the number of insemination

	1st month of lactation	2nd month of lactation	3rd month of lactation
Cows conceived at the 1st or 2nd insemination	22.31	25.54	25.52
Cows conceived at 3rd or more insemination	23.42	30.20	30.08

Table 3: The mean ± SD of blood constituents at different stages of sampling in two groups of the study

Blood constituents	Days 5 ± 3 postpartum		Days 30 ± 3 postpartum		Days 58 ± 3 postpartum	
	Group 1 [1]	Group 2 [2]	Group 1	Group 2	Group 1	Group 2
Glucose (mmol/L)	2.66 ± 0.46	2.38 ± 0.63	3.20 ± 0.43	3.10 ± 0.51	3.21 ± 0.51	3.46 ±0.49
SUN (mmol/L)	4.47 ± 1.85	4.15 ± 1.59	4.31 ± 1.25	3.57 ± 0.71	4.76 ± 1.52	3.75 ± 0.45
Cholesterol (mmol/L)	2.55 ± 0.7	2.23 ± 0.73	4.48 ± 1.04	3.91 ± 0.93	5.42 ± 1.41	5.41 ± 0.64
Albumin (g/L)	29.3 ± 5.2	28.9 ± 3.2	33.0 ± 5.4	27.6 ± 1.8	30.6 ± 3.8	30.5 ± 2.6
Calcium (mmol/L)	2.24 ± 0.17	2.25 ± 0.14	2.34 ±0.20[a]	2.17 ± 0.18[b]	2.23 ± 0.13	2.28 ± 0.12
Phosphorus (g/L)	1.71 ± 0.36	1.79 ± 0.26	1.84 ± 0.39[c]	1.42 ± 0.38[d]	1.94 ± 0.4	1.81 ±0.3
AST (U/L)	90.57 ± 27.94	91.63 ± 42.02	79.71 ± 13.73	70.25 ± 20. 73	64.04 ± 12.56	65.25 ± 14.28
PCV (%)	32.91 ± 2.51	33.88 ± 2.59	30.22 ± 2.59	30.13 ± 2.95	31.27 ± 2.39	31.33 ± 3.61
Total protein (g/L)	69.8 ± 5.30	69.3 ± 6.20	79.0 ± 4.10	80.7 ± 6.80	80.4 ± 5.7	79.2 ± 7.1

a and b values differed significantly (P>0.05); c and d values differed significantly (P>0.05)

[1]group 1: cows conceived at the 1st or 2nd insemination (n = 28)

[2]group 2: cows conceived after the 3rd or more insemination (n = 12)

involved in steroid biosynthesis in the adrenal glands and ovaries. Calcium also may have roles in steroidogensis by influencing delivery or utilization of cholesterol by mitochondria or by stimulating the conversion of pregnenolone to progesterone. Gonadotropin-releasing hormone stimulation of luteinizing hormone (LH) release from the pituitary gland also involves a calcium-dependent mechanism. No cAMP is involved and LH is not released in the absence of calcium or in the presence of calcium blocking agents (Hurley and Doane, 1989).

Phosphorus deficiency induces lowered conception rate, irregular estrus and anestrus, decreased ovarian activity, increased incidence of cystic follicles and generally depressed fertility (Morrow, 1980). In a study on 27 dairy heifers put on phosphorus-deficient diets, services per conception were 2.8. With phosphorus supplementation in 26 heifers, services per conception decreased to 1.3. An Oklahoma study on 48 dairy cows, suggested that an increased incidence of cystic follicles and more services per conception resulted after feeding cows with a diet of 0.4% phosphorus over two years as compared with 0.6% phosphorus (Morrow, 1980). In addition, there have been experiments which indicate that low serum phosphorus (Hewett, 1974; Pugh *et al.*, 1985) can be associated with fertility problems.

As phosphorus is an integral component of nucleic acids, nucleotides and some proteins, the hypophosphatemic state affects many of cells. It is required for transfer and utilization of energy and normal phospholipid metabolism, and is an integral part of many coenzymes. The involvement of phosphorus in phospholipid and cAMP synthesis may be a key to its effect on reproduction; the roles of calcium and phospholipid-dependent protein kinases and cAMP-dependent protein kinases may be crucial in mediating hormone actions (Hurely and Doane, 1989).

Despite the significant difference in calcium and inorganic phosphorus between the two groups of fertile and infertile cows, the calcium concentrations in both groups were within normal range. It is remained to be elucidated which of the two minerals may have more influential role on infertility and what level of calcium and phosphorus deficiency may substantially affect fertility.

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Short Paper

Relationships between fertility, serum calcium and inorganic phosphorus in dairy cows

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Summary

A prospective study was conducted to investigate the difference in levels of some blood constituents between fertile and infertile cows. A total of 40 dairy cows were selected at random from a dairy farm. Serum samples were collected three times on 5 ± 3, 30 ± 3 and 58 ± 3 days postpartum. Serum glucose, urea nitrogen (UN), cholesterol, albumin, total protein, calcium, inorganic phosphorus and aspartate aminotransferase (AST) were measured for each sample. Cows were divided into two categories: those which conceived at either the first or second insemination (group 1) and those which conceived ≥3 inseminations (group 2). Seventy percent (28 of 40) of the cows conceived at first or second insemination and 30% (12 0f 40) needed 3 or more inseminations. Results showed that calcium (2.34 *vs.* 2.17 mmol/L) and inorganic phosphorus (1.84 *vs.* 1.42 mmol/L) were significantly ($P<0.05$) higher in the group 1 cows at second stage of sampling.

Key words: Infertility, Metabolic profile test, Blood chemistry, Calcium, Inorganic phosphorus

Introduction

The multifactorial nature of infertility is an important reason why investigators have met varying success when turned to profile tests as a potential panacea for diagnosis of all reproductive problems. Many investigators link infertility with abnormalities of energy, protein and mineral status. There have been experiments, which indicate low glucose (McClure, 1968; McClure, 1970), increased ketone bodies (Andersson and Emanuelsson, 1985), low serum albumin (Rowlands *et al.*, 1980), high blood urea (Ferguson *et al.*, 1988), high or low serum phosphorus (Hewett, 1974; Pugh *et al.*, 1985), other mineral imbalances (Pugh *et al.*, 1985), reduced liver function (Reid *et al.*, 1979), energy deficit (Villa-Goday *et al.*, 1988; Butler and Smith, 1989) and overfeeding with protein (Hewett, 1974; Chalupa, 1984) can all be related to fertility problems. Recently, Reist *et al*, (2003) showed that high plasma concentrations of glucose and cholesterol were associated with a short calving to conception interval. In another study, delayed ovulated animals had higher betahydroxybutyrate and lower glucose levels in the pre-service period than normal profile cows (Taylor *et al.*, 2003). The conclusions about these relationships have usually been on blood and milk samples taken at different times during the early phases of lactation. It seems that the concentrations at the time of insemination have been overlooked (Plym Forshell *et al.*, 1991).

The aim of this study was to compare the concentrations of some blood constituents of fertile and infertile cows by taking measurements at early lactation and the time of insemination in a dairy herd.

Materials and Methods

A total of 40 dairy cows (aged 3–8 years) were selected at random from a dairy farm in Mashhad, Northeast of Iran. The farm was selected because of proper data recording and relatively good management