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THE RELATIONSHIP BETWEEN PHYSIOLOGICAL BLOOD PARAMETERS AND FERTILITY IN HOLSTEIN COWS

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ABSTRACT

Relationships between physiological blood parameters and postpartum (PP) fertility (days open, and the number of artificial inseminations required per conception, AIPC) were studied in 15 Holstein cows. Serum concentrations of total proteins, globulin, aspartate aminotransferase (AAT) and phosphorus varied significantly during the first 95 days postpartum. Number of days open and AIPC were not significantly affected by the uterine health and parity, but younger animals tended to have shorter days open, and required fewer AIPC ($P<0.08$). Serum phosphorus levels were significantly higher in younger cows, and phosphorus concentration on d 35 PP showed a significant but negative correlation ($r=-0.70$) with days open and AIPC. For the cows with endometritis (n=8), the correlation coefficients of days open with serum glucose ($r=0.89$ on d 5 PP), AAT ($r=0.76$ on d 20 PP) and phosphorus ($r=-0.72$ on d 35 PP) were significant. For normal cows, the correlation coefficients of days open with serum glucose ($r=-0.80$ on d 95 PP), AAT ($r=-0.75$ on d 35 PP), phosphorus ($r=-0.81$ on d 35 PP), and blood hemoglobin ($r=0.80$ on d 20 to 50 PP) were significant ($P<0.05$). The only parameter which was significantly correlated

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with days open, regardless of the uterine health status, was serum phosphorus on d 35 PP. Regression equations of days open on physiological parameters were constructed. Although the number of animals in this experiment was small, but the regression equations were promising and it is suggested that more comprehensive studies should be undertaken for a better understanding of the relationship between PP fertility and physiological parameters.

Key words: Dairy cows, Days open, Physiological parameters, Post partum period.

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رابطه بین فراسنجه های (پارامترهای) فیزیولوژیک خون و باروری در ماده گاوهای هولشتین

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چکیده

رابطه بین فراسنجه های فیزیولوژیک خون وباروری در دوره پس از زایش (شمار روزهای باز و شمار تلقیح مصنوعی برای آبستن شدن) در ۱۵ ماده گاو هولشتین، بررسی شد. غلظت پروتئین

تام، گلوبولین، آنزیم آسپارتات آمینوترانسفراز(AAT) و فسفر تغییر معنی داری را در ۹۵ روز نخستین پس از زایش نشان داد. شمارروزهای باز و شمار تلقیح مصنوعی برای آبستن شدن (AIPC) تحت تاثیر وضعیت رحم (سالم و عفونی) و شمار دوره های آبستنی قرار نگرفت ولی ماده گاوهای جوان تر، با شمار کمتری تلقیح مصنوعی، آبستن شدند($P<0.08$). غلظت فسفر در سرم خون گاوهای جوان تر بیشتر بود. غلظت فسفر سرم خون در روز ۳۵ پس از زایش، همبستگی منفی و معنی داری($r=-0.70$) با شمار روزهای باز و AIPC نشان داد. برای گاوهایی که دچار اندومتریت پس از زایش بودند ($n=8$)، همبستگی شمار روزهای باز با غلظت های گلوکز سرم در روز پنجم($r=0.89$)،AAT در روز بیستم($r=0.76$) و فسفردرروز سی وپنجم ($r=-0.75$)، فسفر در روز سی و پنجم ($r=-0.81$) و هموگلوبین خون در روزهای بیستم تا پنجاه و پنجم ($r=0.80$) پس از زایش معنی دار بود. تنها فراسنجه ای که بدون توجه به وضعیت رحم، همبستگی معنی داری با شمار روزهای باز داشت، غلظت فسفردرروزسی و پنجم پس از زایش بود. معادله های رگرسیون شمار روزهای باز روی فراسنجه های فیزیولوژیک خون گزارش شده اند. هرچند که شمارگاوهای به کار برده شده در این آزمایش چندان زیاد نبود اما معادله های رگرسیون به دست آمده، این نوید را می دهند که با انجام آزمایش های گسترده تری بتوان به فراسنجه های فیزیولوژیک دست یافت که بتوانند باروری در دوره پس از زایش گاو را پیش بینی کنند.

INTRODUCTION

Attempts have been made to relate the postpartum (PP) fertility to nutritional status in dairy cattle (9). Clinical and biochemical parameters have been measured as indicators of nutritional energy balance, and have been related to fertility. Some studies indicate that low blood glucose (14, 23), low serum albumin (26), increased ketone bodies (2), high blood urea (3, 6), high or low serum phosphorus (19), energy deficit (1, 4, 9, 31), reduced liver function (21) and overfeeding with protein (9) in early

lactation may be related to fertility problems. Number of services required per conception was positively correlated with globulin concentration, but was negatively correlated with potassium and packed-cell volume (25).

Forshell *et al.* (7) reported that plasma glucose concentrations at first insemination were significantly different between cows that became pregnant and those that did not; however, total protein, albumin, globulin, aspartate aminotransferase (AAT), bilirubin, bile acids, calcium, magnesium and phosphorus in blood and urea in milk were not significantly related to pregnancy rate. They believed it is possible to evaluate the risk of reduced fertility by measuring blood glucose or milk acetone few weeks after calving. Zurek *et al.* (32) studied the relationship between metabolic status and the interval to first ovulation in PP dairy cows; plasma AAT decreased significantly and the proportion of tyrosine to total large neutral amino acids significantly increased during the 12 days prior to first ovulation and both were correlated with luteinizing hormone (LH) secretion. The relationship between ketone body status in the early postpartum period and first ovulation of dairy cows was reported recently by Reist *et al.* (22). Concentrations of ketone bodies in serum and milk during the first 6 wk of lactation were higher in cows in which postpartum ovarian cyclicity occurred within 30 d as compared with those in which postpartum ovarian cyclicity occurred after 30 d. Concentrations of plasma glucose, nonesterified fatty acids and milk urea nitrogen (MUN) did not differ between these groups.

Nutrition in most Iranian dairy herds is seldom optimal and long PP intervals are often recorded, part of which could be due to nutritional imbalances. Physiologic parameters related to fertility may be helpful in designing proper nutritional strategies for dairy herds in Iran. In most studies, few parameters have been investigated and it is necessary to consider more varied parameters for a better understanding of the factors that are related to fertility problems in dairy cows. Therefore, the purpose of this experiment was to investigate the relationship between a number of physiologic parameters and fertility (days open and the number of services per conception) in Iranian Holstein cattle.

MATERIALS AND METHODS

The study was carried out during the spring and summer of 1999 at the Animal Research Station of the College of Agriculture, Shiraz University, 15 km north of Shiraz. The experimental animals were kept in a loose-housing barn with an open lot. Fifteen recently calved Holstein cows (2- to 7- year old) were used in the experiment. Their mean (±SD) daily milk yield during the experimental period was 24.2±2.0 kg. A ration balanced for the highest producing cow in the group, and consisting of alfalfa hay, corn silage and a concentrate mixture (corn, barley, wheat grain, wheat bran, cottonseed meal, dicalcium phosphate, common salt, and mineral-vitamin mix) was fed *ad lib.* to all cows. The ration supplied (DM basis) 1.5 Mcal NE_l per kg DM, 15% crude protein, 0.5% calcium, 0.35% phosphorus and crude protein content was determined from measuremnets of nitrogen content, and other ingredients were calculated from published values in tables of feed composition. The cows were milked thrice daily, and milk production was recorded weekly. Reproductive tract condition was assessed by rectal palpation. Eight cows developed endometritis, and were treated accordingly. Estrus was detected by using a penile-deviated teaser. The cows were artificially inseminated at least 60 days postpartum, and pregnancy was diagnosed by rectal palpation on day 60 after insemination.

Blood and milk samples were obtained at morning milking, over a 95-day period at 15-day intervals starting on day 5 postpartum. Blood was collected from the coccygeal vein and a sample was transferred into an EDTA-containing tube. Another blood sample was transferred into a tube without anti-coagulant, and the serum was separated and frozen to -20° C until analysis. Hematocrit value (micromethod) and blood hemoglobin concentration (cyanomethemoglobin method) were determined according to Jain (11). Concentrations of total protein (Biuret method) and albumin (bromocresol green) were determined according to Johnson *et al.* (12). Bilirubin (Vandenberg method) concentration was determined according to Tolman and Rej (30) Blood serum (BUN) and milk (MUN) urea nitrogen (diacetylmonoxime method) concentrations were determined as described by

Newman and Price (16). Concentrations of calcium (colorimetric method) and phosphorus (ammonium molybdate method) were measured as described by Endres and Rude (5). Serum AAT levels (Reitman-Frankel method) were determined according to Moss and Henderson (15). Concentrations of glucose (27) and cholesterol (24) were determined enzymatically. Serum globulin values were estimated by subtracting albumin from total protein values.

Due to variations in age and parity, and small numbers within all age groups, the cows were classified into two age groups (2 to less than 4, and 4 to 7 years), and two parity groups (parity=1 and parity ≥2) for data analysis. Eight cows developed endometritis and were treated; therefore, the uterine health was considered in data analysis. Data were analyzed by appropriate procedures of the Statistical Analysis System (28). Repeated measures ANOVA was used to test within animal variations in measured values for various physiological parameters. Data for days open (number of days from calving to pregnancy as determined by rectal palpation) were analyzed using the GLM procedure. Number of artificial inseminations (AI) per conception (AIPC) was analyzed using the Kruskal-Wallis H test. Correlation coefficients of days open and AIPC with each parameter were determined. Regression equations were constructed using the Forward procedure with open days as the dependent variable and physiological parameters as the independent variables. Mean comparison was performed using Duncan's multiple range test (28).

RESULTS AND DISCUSSION

Uterine health, cow age and parity did not affect most of the parameters under investigation ($P>0.05$), except for serum phosphorus concentration which was significantly ($P<0.002$) greater in cows less than 4 yr as compared with those greater than 4 yr of age (8.53 vs. 7.21 mg d l^{-1}). Serum concentrations of total proteins ($P<0.03$), globulin ($P<0.03$), AAT ($P<0.05$), and phosphorus ($P<0.05$), varied significantly (Table 1) during the first 95 days postpartum. Concentration of glucose (range: 55-63 mg d l^{-1}), albumin (range: 5.4-6.3 g d l^{-1}), bilirubin (range: 0.17-0.41 mg d l^{-1}),

cholesterol (range:159-205 mg d l^{-1}) and calcium (range: 7.5-10.8 mg d l^{-1}) in the serum, blood hemoglobin levels (range: 8.2-8.9 g d l^{-1}), BUN concentration (range: 7.8-8.6 mg d l^{-1}), calcium-to-phosphorus ratio (range: 0.94-1.26) in serum, hematocrit value (range: 25.5-26.7%), and MUN concentration (range: 11.6-14.5 mg d l^{-1}) did not vary significantly during this period. None of the interaction terms was significant.

Table 1. Serum concentrations (mean±SD; n=15 cows) of physiological blood parameters which changed significantly during the postpartum period (PP).

Days PP	Total proteins (g dl^{-1})	Globulins (g dl^{-1})	AAT† (IU l^{-1})	Phosphorus (mg dl^{-1})
5	$5.41^{b§}$ ±0.87	2.46^{b} ±0.73	81.0^{a} ±22.1	8.44^{ab} ±1.29
20	5.80^{ab} ±0.79	2.46^{b} ±1.06	77.4^{a} ±18.3	7.89^{ab} ±1.82
35	6.06^{a} ±0.73	2.93^{ab} ±0.78	71.5^{b} ±16.6	7.19^{b} ±1.44
50	6.29^{a} ±0.75	3.20^{a} ±0.69	76.3^{a} ±12.8	7.81^{ab} ±1.58
65	6.02^{ab} ±0.56	3.03^{ab} ±0.50	73.2^{b} ±16.5	7.22^{b} ±1.78
80	5.82^{ab} ±0.90	2.55^{b} ±0.97	69.4^{b} ±13.1	8.16^{ab} ±1.55
95	5.98^{ab} ±0.88	2.69^{ab} ±0.67	65.6^{b} ±13.1	8.59^{a} ±1.95

† AAT: Aspartate aminotransferase.

§ Within each column, means with the same superscript (s) are not statistically different (Duncan's multiple range test; P>0.05).

Butler *et al.* (3) reported that BUN and MUN concentrations greater than 19 mg d l^{-1} were associated with decreased pregnancy rate in dairy cattle, with plasma and milk yielding similar results for monitoring urea concentration in dairy cows. Rajala-Schultz *et al.* (20) reported on the association between MUN and fertility in 24 dairy herd in Ohio. They observed that cows with MUN levels bellow 10.0 were 2.4 times more likely and cows with MUN levels between 10.0 and 12.7 mg d l^{-1} were 1.4 times more likely to be confirmed pregnant than cows with MUN values above 15.4 mg d l^{-1} Their results also suggested that the levels of MUN that adversely affect fertility may be lower than those reported earlier. According to Godden *et al.* (8) MUN values are affected by season, month, parity group, stage of lactation, and sample type. The low association between MUN and BUN values in the present experiment was probably due to experimental

procedure for blood and milk sampling, and the smaller number of cows used in this experiment.

Number of days open was not significantly affected by the uterine health, and parity, but younger animals tended to have shorter open days ($P<0.08$; Table 2). Cows with endometritis were treated immediately after diagnosis, which can explain why their open days were not longer than for cows with normal uteri; however, it must be noted that only moderate and severe endometritis can be diagnosed by rectal palpation. AIPC was not significantly affected by uterine health and parity, but there was a tendency for younger cows to require less AI ($P<0.08$) to become pregnant (1.25 vs. 1.71). Serum phosphorus levels were significantly higher in younger cows, and phosphorus concentration on d 35 PP showed a significant but negative correlation ($r=-0.70$) with days open and AIPC. The correlation coefficient between AIPC and days open in this study was 0.74 ($P<0.02$). A high percentage of older cows are not able to sustain their pregnancies, especially after 4th lactation; this could be due to implantation failure as a result of delayed uterine involution (18).

Table 2. Days open (mean±SD) in experimental cows according to uterine health, age and parity.

		Mean±SD	n	P
Uterine health	Endometritis	106.6 ± 24.0	8	NS
	Normal	98.7 ± 26.6	7	
Age (yr)	2 to < 4	92.6 ± 26.9	8	0.08
	≥ 4 to 7	114.7 ± 16.6	7	
Parity	1	95.0 ± 33.6	6	NS
	≥2	108.2 ± 16.8	9	

Several blood parameters showed significant correlation with days open and AIPC. The correlation coefficients of these parameters with days open and AIPC were in the same order. However, the significantly correlated parameters were different for healthy cows (6 parameters) and those with endometritis (3 parameters). For the cows with endometritis, the correlation coefficients of days open with serum glucose ($r=0.89$; $P<0.1$; on d 5 PP), AAT ($r=0.76$; $P<0.05$; on d 20 PP) and phosphorus ($r=-0.72$; $P<0.05$; on d 35 PP) were significant. For normal cows, the correlation

coefficients of days open with serum glucose (r=-0.80; P<0.05; on d 95 PP), AAT (r=-0.75; P<0.05; on d 35 PP), phosphorus (r=-0.81; P<0.05; on d 35 PP), and blood hemoglobin (r=0.80; P<0.05; on d 20 to 50 PP) were significant. The only parameter which was significantly correlated with days open, regardless of the uterine health status, was serum phosphorus on d 35 PP The positive correlation of AAT with days open in cows with endometritis, is consistent with the data of Zurek *et al.* (32) who found significant decreases in plasma AAT during the 12 days prior to ovulation. An increase in AAT was highly correlated with a decrease in pituitary responsiveness to GnRH stimulation in postpartum cows (17); however, this explanation can not be applied to normal cows with a negative correlation between AAT levels and days open.

The regression equation of days open on physiological blood parameters for normal cows was:

$$\text{Days open} = 42.66 + 51.16Hb_{20} - 41.91Hb_{50} + 2.39MUN_{65}$$

where, Hb_{20} and Hb_{50} are blood hemoglobin levels (g d l^{-1}) on d 20 and 50 PP, respectively, and MUN_{65} is milk urea nitrogen concentration (mg d l^{-1}) on d 65 PP. They accounted for 77.6%, 18.5% and 3.7% of total variations in days open, respectively (R^2=0.998).

The regression equation of open-days on physiological blood parameters for cows with endometritis was:

$$\text{Days open} = 210.80 - 2.91Gluc_5 + 0.93Gluc_{35} + 1.83BUN_{35}$$

in which, $Gluc_5$ and $Gluc_{35}$ are serum glucose concentrations (mg d l^{-1}) on d 5 and 35 PP, respectively, and BUN_{35} is serum urea nitrogen concentration (mg d l^{-1}) on d 35 PP. They accounted for 78.7%, 18.4% and 2.3% of total variations in open-days, respectively (R^2=0.993).

The relationship of hemoglobin levels with days open of normal cows in the present work is interesting, and despite the relatively small number of animals in this study, it is worthy of further considerations. Rowlands *et al.* (25) found an inverse relationship between packed cell volume and the number of services required for conception. The contribution of urea nitrogen levels to regression equation was modest, but it is consistent with the belief that they may have a deleterious effect on fertility. The MUN

values (11.6 to 14.5 mg d l^{-1}) are within the range of values that were associated with lower pregnancy rates in Ohio dairy cows (20).

Larson *et al.* (13) found no relationship between reproductive performance of dairy cattle and blood concentration of calcium, phosphorus, magnesium, copper, zinc, and selenium on day 14-21 and 38-45 after calving; however, selenium concentration on day 14-21 showed a positive and significant correlation with the number of services per conception and days-open. In a study by Forshell *et al.* (7), no relationships were found between conception rates and concentrations of total protein, albumin, globulin, AAT, bilirubin, bile acids, magnesium, calcium, phosphorus, and MUN. Glucose concentration on weeks 4-7 PP was higher in cows that conceived as compared with those that did not. Acetone concentration in milk of infertile cows was higher on weeks 3-5 PP. Kappell *et al.* (10) concluded that blood cholesterol and possibly glucose levels during the first 40 to 60 days PP would increase the calving to conception interval in dairy cows.

Although considerable amount of published works has addressed the relationships between physiological parameters and postpartum fertility in dairy cows, it is evident that more comprehensive studies, encompassing more varied parameters, are needed with large number of cows before a definite conclusion can be reached with regard to the most reliable and applicable physiological criteria of fertility in dairy cattle.

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