Global Veterinaria 9 (6): 726-731, 2012
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2012.9.6.65109

Association Between Blood Metabolites and Pregnancy Success at First Service in Iranian Dairy Cows

[1]M. Karimi Dehkordi, [2]M. Bolourchi, [1]S. Safi and [3]A. Rahimi Foroushani

[1]Department of Clinical Pathology, Faculty of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Iran
[3]Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Abstract: This study examines the relationship between some metabolites and reproductive performance of Holstein dairy cows around parturition and testes the hypothesis whether assessment of these metabolites can be used to predict the occurrence of pregnancy at first service in dairy cows. The data were generated from 97 Holstein cows rose on a large commercial farm and fed the same diet as total mixed ration. Serum concentrations of glucose, β-hydroxybutyrate and Non-esterified fatty acids were measured in all cows on 10 days prepartum and weeks 1, 2, 4 and 6 postpartum. Following estrus detection, the cows were artificially inseminated and examined by transrectal ultrasonography approximately 30 days after service to determine pregnancy status. Animals were divided into two groups, conceived and failed to conceive to first service, for statistical analyses. Regarding the interaction between the week of sample collection and pregnancy outcome, the cows that conceived and the cows that didn't conceive to first service did not differ significantly in blood concentrations of glucose and β-hydroxybutyrate at all times of sampling. Non-pregnant cows had significantly higher circulating Non-esterified fatty acid concentrations in the fourth and sixth weeks after calving than pregnant cows after first artificial insemination.

Key words: Glucose · β-hydroxybutyrate (BHBA) · Non-esterified Fatty Acids (NEFA) · First Service Conception Rate · Periparturition Periods

INTRODUCTION

Economically, reproduction is one of the major traits in dairy production. In this regard, the conception rate to first service, i.e. the percentage of cows that conceive after first service (PR/ FAI), is crucial. Fertility in dairy cows has decreased continuously over the last 40 years as milk production has increased [1]. This condition has been observed in several countries. In France, the decline in fertility has been observed in the past 20 years [2]. There are also accounts from USA of declining fertility in dairy cows [3, 4]. In England, this decrease has been approximately 1% per year [1].

The decrease in fertility associated with increasing milk production is related to negative energy balance post-calving and metabolic disorders. Therefore, changes in blood metabolite concentrations may be valuable predictors of conception to a specific insemination (AI). Reist *et al.* [5] and Walsh *et al.* [6] stated that cows not conceiving to first service had lower serum glucose concentrations than cows conceiving to first service. Lack of glucose may be detrimental to ovarian function, resulting in follicles and oocytes of poorer quality. There are contrasting researches about the effect of hyperketonemia on reproductive performance. Koller *et al.* [7] reported incremented concentrations of ketone bodies in the first 6 weeks post calving delayed conception. Walsh *et al.* [6] found that the first service conception risk had declined by 50% in cows with serum β-hydroxybutyrate (BHBA) concentrations ≥1400 µmol/L

Corresponding Author: Karimi Dehkordi, Department of Clinical Pathology, Student of Veterinary Clinical Pathology, Faculty of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.

in the second week postpartum. Some researchers have reported no effect of subclinical ketosis on cow's fertility [8, 9].

Some recent reports refer to an association between Non-esterified fatty acids (NEFA) and decreased fertility; however, the findings are controversial. Some studies reported that cows with higher concentration of NEFA had lower fertility [10]. A few such as Reist *et al.* [5] and Snijder *et al.* [9], however, have not reported any significant effects of blood NEFA concentrations on reproductive performance. One possible reason why earlier investigations may not always have detected significant relationships between metabolic traits and fertility is that cows may not have been sampled at the most relevant time point. To overcome such limitation, it needs to be evaluated these relationships at several stages in lactation. The objective of the current study was to determine whether concentrations of glucose, BHBA and NEFA measured at 10 days prepartum and in each of the first, second, fourth and sixth weeks postpartum could be used at herd level to predict success of conception to first service and in which times relative to calving, were most influential in predicting fertility.

MATERIALS AND METHODS

The study was conducted on 97 lactating *Holstein* cows of parities 2 to 5 in a large commercial dairy herd, in Chaharmahal and Bakhtiari province of Iran. Seasonal effects were minimized as most of the cows on farm calved during a one-hundred-day period from August until November in 2011. Blood samples were collected at 5 to 6 a.m. (before feeding) on day 10 prepartum and weeks 1, 2, 4 and 6 postpartum, via the tail vein into a glass tube. Blood samples were left to clot at room temperature for approximately 30 min and then centrifuged at 2000×g. The obtained serum samples were stored at -20°C until analyzed for glucose, BHBA and NEFA concentrations.

Blood glucose was analyzed according to colorimetric methodology of glucose oxidase. For this determination, commercial kit (Parsazmoon, Tehran, Iran) was used by an automatic biochemical analyzer (Biotecnica, Targa 3000, Rome, Italy). β-Hydroxybutyrate (BHBA) and Non-esterified fatty acids (NEFA) were determined by a D-3-hydroxybutyrate kit and a NEFA Kit (Randox Laboratories Ltd, Ardmore, UK).

The cows were inseminated by an expert inseminator when standing heat was observed. Pregnancy diagnosis was performed by ultrasonography 30 to 40 days after service and the second palpation was done two weeks later to validate the pregnancy. The cows were divided into two categories; those which conceived at first insemination and those which need two or more inseminations. First service conception rate (FSCR) was defined as the number of first AI followed by calving or confirmed late pregnancy per all first AI. A dependent dichotomous variable was coded as 1 (conception) and 0 (failure to conceive) for each lactation record. The means of blood metabolites for significant (P<0.05) differences were compared at each of sampling times, separately. 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

Conception rate to first service was 29 per cent (28/97). The mean±SD of serum glucose, BHBA and NEFA concentrations were measured at each sampling time for pregnant and non-pregnant cows and compared adjusting for repeated measures within cow and are shown in Table 1. The present study revealed several relationship estimates between traits and fertility that were statistically significant (P<0.05) or tended to be significant (P<0.1).

Table 1: Peripartum week-specific estimates of concentrations of blood metabolites (means±SE) for cows that conceived and those that did not conceive to first service

Pregnancy status	Not conceived	Conceived
Before parturition		
Glucose (mg/dl)	50.58±1.12	50.55±2.22
β-hydroxybutyrate (μmol/L)	496.16±18.2^{c}	442.83±20.82^{d}
Non-esterified fatty acids (μmol/L)	287.15±34.62	305.48±51.78
Week 1		
Glucose (mg/dl)	42.75±1.67	42.28±1.77
β-hydroxybutyrate (μmol/L)	711.52±46.16	862.35±168.50
Non-esterified fatty acids (μmol/L)	756.02±72.07	737.59±97.14
Week 2		
Glucose (mg/dl)	41.37±1.57	40.85±2.13
β-hydroxybutyrate (μmol/L)	636.82±63.25	698.8±97.56
Non-esterified fatty acids (μmol/L)	603.69±47.52	615.59±80.20
Week 4		
Glucose (mg/dl)	45.23±1.24	45.25±1.75
β-hydroxybutyrate (μmol/L)	483.29±19.86	506.95±30.87
Non-esterified fatty acids (μmol/L)	248.98±15.08^{a}	202.45±21.12^{b}
Week 6		
Glucose (mg/dl)	48.73±1.38	46.57±2.01
β-hydroxybutyrate (μmol/L)	545.56±39.36	508.38±25.47
Non-esterified fatty acids (μmol/L)	213.81±12.33^{a}	175.52±8.90^{b}

Mean values with different superscripts within rows differ a,b: P<0.05 and c,d: P<0.1

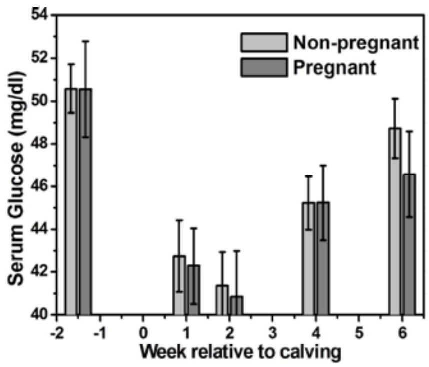

Fig. 1: Mean serum glucose concentrations and standard error in Holstein cows that were subsequently diagnosed pregnant or non-pregnant after first insemination. Each cow was sampled once in 10 days before calving, in each of the first 2 weeks and again in the fourth and sixth weeks after calving. * $P<0.05$

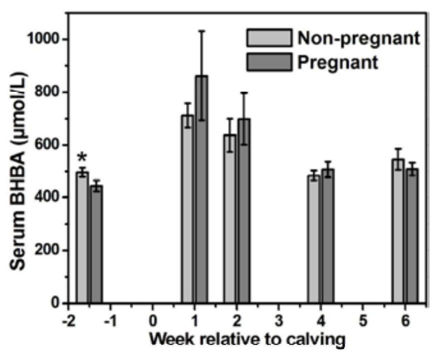

Fig. 2: Mean serum BHB concentrations and standard error in Holstein cows that were subsequently diagnosed pregnant or non-pregnant after first insemination. Each cow was sampled once in 10 days before calving, in each of the first 2 weeks and again in the fourth and sixth weeks after calving. * $P<0.1$

Sex and calf weight did not affect reproductive performance in our study ($P>0.05$). There were no significant differences between the cows did not conceive and those that conceived to first service in the mean plasma glucose concentration at all times of sampling ($P>0.05$; Figure1). BHBA values tended to be significantly lower in the cows conceived to first service (442.83±20.82 µmol/L vs. 496.16±18.20 µmol/L) before parturition ($P<0.1$). The comparison of blood BHBA concentration between two groups measured at the other times of sampling did not reveal any significant difference (Figure 2). The cows that didn't conceive to first service had higher plasma concentrations of NEFA during the 4th and 6th week of lactation (248.98±15.08 µmol/L vs. 202.45±21.12 µmol/L and 213.81±12.33 µmol/L vs. 175.52±8.90 µmol/L, respectively) than those that conceived to this service ($P=0.03$; Figure 3).

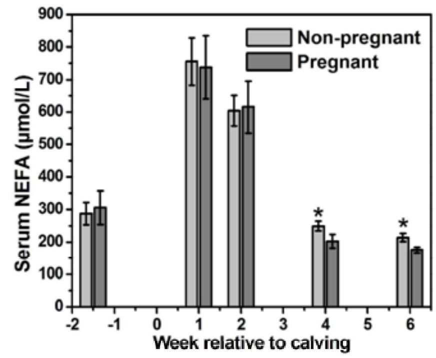

Fig. 3: Mean serum NEFA concentrations and standard error in Holstein cows that were subsequently diagnosed pregnant or non-pregnant after first insemination. Each cow was sampled once in 10 days before calving, in each of the first 2 weeks and again in the fourth and sixth weeks after calving. * $P<0.05$

DISCUSSION

This study was carried out in a commercial dairy farm (about 1450 lactating dairy cows) in a mountainous region in Iran. Conception rate to first service on our farm (29%) was higher than that of (27%) reported recently for 87 Iranian dairy cows by Kadivar *et al.* [11]. In Japan, the same result was obtained by analyzing data from lactation dairy cows [12]. Different results were found in Iran by Ansari-Lari *et al.* [13] in multiparous Holstein dairy cows, the mean PR/FAI in that study was 41.6%. These different reports from various countries have collected by different veterinarians. Regarding previous research, however, it is difficult to specify a general first service conception risk in cows, since the farming systems and breeders' techniques for artificial insemination (AI) differ greatly between studies and have noticeable negative effects on the first service conception rate.

In the present study, blood glucose concentration was not significantly different between the cows that conceived and those that did not conceive to first service in all sampling times ($P>0.05$). This shows the measurement of glucose concentration may not be a dependable indicator for defining conception status after the first service at periparturition period. There is evidence that glucose is the main energy source for the ovary [14], so glucose deficiency may impair ovarian function and causes poor quality of follicles and oocytes. It has been reported previously [15, 16] that pregnancy rate was higher in cows with high plasma glucose concentrations. Mean glucose values for both groups of the cows in the present study were above the average value (40.5 mg/dl) for cows which did not become pregnant in the study by Plym-Forshell *et al.* [15]. However, in the present study, as reported by Hayhurst *et al*. [17] and Kadivar *et al.* [11], glucose concentrations were not related to reproductive performance. In dairy cows, glucose concentrations are under tight homeostatic control and a great energy demand to hold milk production is provided through gluconeogenesis [18]. Therefore, glucose may be a poor analysis for following and investigating dairy cows' problems.

Plasma concentrations of BHBA did not significantly differ between the cows that did not conceive to first service and those that conceived to this service in both prepartum and postpartum. But, there was a tendency for increased circulating BHBA concentration measured shortly before calving, in non-pregnant cows after first AI relative to cows diagnosed pregnant (P=0.09). Wathes *et al.* [19] showed that in both multiparous and primiparous cows, concentrations of BHBA were significantly greater in cows having delayed ovulation. Similarly the effect of an increased BHBA concentration on prolonged interval calving to conception was reported by Koller *et al.* [7]. Oikonomou *et al.* [20] presented a significantly negative genetic correlation between serum BHBA concentrations with first-service conception rate in the first week postpartum. Walsh *et al.* [6] defined BHBA concentration thresholds for the prediction of probability of pregnancy after the first insemination early in lactation. They showed that cows with serum BHBA$\geq$1000 µmol/L in the first week or $\geq$1400 µmol/L in the second week were significantly less likely to be diagnosed pregnant after first insemination. The same relation between fertility and BHBA concentration is reported by Ospina *et al.* [10]. In their study, in animals sampled postpartum, the risk of pregnancy within 70 days post-voluntary waiting period (VWP) was reduced by 13% when BHBA concentrations were $\geq$970 µmol/L.

In the current study, mean BHBA concentration in all sampling times were lower than threshold values reported by Walsh *et al.* [6] and Ospina *et al.* [10] that above this, risk of pregnancy after first service is reduced. This can explain why BHBA in this study did not affect the probability of pregnancy. Consistent with our results, Fahey *et al.* [21], Waters *et al.* [22] and Falkenberg *et al.* [23] did not reveal any significant relationship between peripartum BHBA concentrations and reproductive parameters. Furthermore, detrimental effects of ketone bodies on reproductive success depend on the longevity of their increased levels [24]. In this study, the duration of increasing BHBA concentration may be too short to exert a negative effect on fertility.

In the present study, including the interaction between the week of sample collection and pregnancy outcome, non-pregnant cows had higher circulating NEFA concentrations in the fourth and sixth weeks after calving than the cows diagnosed pregnant at first artificial insemination. This shows that postpartum NEFA concentration is a reliable indicator for defining conception status after the first service. A number of studies have focused on the relationship between NEFA concentrations and reproductive performance [17, 19, 25]. The negative impact of NEFA concentration on commencement of luteal activity postpartum was reported during the 4^{th} and 7^{th} week of lactation [19]. A delay in the resumption of ovulation limits the number of oestrous cycles before service, which may account for the reduced conception rates [26]. However, Reist *et al.* [5] and Fahey *et al.* [21] did not revealed any significant relationship between NEFA concentration and fertility.

Regarding the stage of sampling in which significant relationships were observed, we note that these relationships were found in the last weeks of sampling, when the majority of animals were already cyclic. This is quite sensible because this is the time that the most of the cows were inseminated for the first time and energy balance indicators are expected to be more informative in this period.

In one study by Ospina *et al*. [10], the risk of pregnancy within 70 d post-VWP was reduced by 16% when NEFA concentrations in the first 2 weeks were $\geq$720 µmol/L. Although NEFA concentrations in our study only in week 1 postpartum (did not conceive: 756.02±72.07 µmol/L and conceived: 737.59±97.14 µmol/L) were little higher than threshold value reported by Ospina *et al.* [10], the negative effects of elevated NEFA on reproductive performance have been masked in this time. However, this threshold is related to assess conception within 70 days post-voluntary waiting period (VWP) and the level at which elevated NEFA is associated with conception at first service was not evaluated.

Although elevated concentrations of both NEFA and BHBA decline the risk of conception, through direct toxic effect on the follicles with induction of cumulus cells apoptosis, necrosis and follicular development arrest

[27, 28], however, in our study, NEFA concentration was found to have the stronger relationship with reproductive performance than BHBA. This situation is likely because of the more direct physiological relationship between NEFA concentrations and negative energy balance [29].

CONCLUSION

The BHBA and NEFA concentrations are assumed to be the best indicators of a cow's energy balance [5]. Interestingly, in this study the mean values of BHBA and NEFA for both groups of the cows were within the normal range for dairy cows (NEFA<700 µmol/L and BHB<900 µmol/L) [30]. It indicates that our cows did not suffer from extensive and prolonged negative energy balance. So we can say the cows used in the present study were well-managed and likely had normal fertility. Given these results, a new theory is formulated. It proposes that the indicators used in the present study may have a higher or different predictive value in cases of poor nutritional or metabolic status. This means that risk factors for fertility should be evaluated in classifying animals as either being in negative energy balance or not.

REFERENCES

1. Royal, M.D., A.O. Darwash, A.P.F. Flint, R. Webb, J.A. Woolliams and G.E. Lamming, 2000. Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. Anim. Sci., 70: 487-501.
2. Boichard, D., A. Barbat and M. Briend, 1998. Genetic evaluation for fertility in French dairy cattle. Proc. of an international workshop on Genetic Improvement of Functional Traits in Cattle (GIFT), Fertility and reproduction, Grub, Germany, Nov., 1997. Interbull Bulletin, 18: 99-101.
3. Thompson, J.R., 1998. Worldwide Holstein breeding. Intermediate report workshop EU Concerted action Genetic Improvement of Functional Traits in Cattle; (GIFT). Warsaw, Poland. International Bull Evaluation Service. Bulletin, 19: 29-35.
4. Washburn, S.P., C.H. Brown, B.T. McDaniel and S.L. White, 2000. Reproductive trends among Southeastern dairy herds. J. Dairy Sci., 83(Suppl. 1): 229.
5. Reist, M., A. Koller, A. Busato, U. Kupfer and J.W. Blum, 2002. First ovulation and ketone body status in the early postpartum period of dairy cows. Theriogenology, 54: 685-701.
6. Walsh, R.B., J.S. Walton, D.F. Kelton, S.J. LeBlanc, K.E. Leslie and T.F. Duffield, 2007. The effect of subclinical ketosis in early lactation on reproductive performance of postpartum dairy cows. J. Dairy Sci., 90: 2788-2796.
7. Koller, A., M. Reist, J.W. Blum and U. Kupfer, 2003. Time empty and ketone body status in the early postpartum period of dairy cows. Reprod Dom Anim., 38: 41-49.
8. Andersson, L. and U. Emanuelson, 1985. An epidemiological study of hyperketonaemia in Swedish dairy cows; determinants and the relation to fertility. Preventative Veterinary Medicine, 3: 449-462.
9. Snijders, S.E.M., P.G. Dillon, K.J. O'Farrell, M. Diskin, A.R.G. Wylie, D. O'Callaghan, M. Rath and M.P. Boland, 2001. Genetic merit for milk production and reproductive success in dairy cows. Anim Reprod Sci., 65: 17-31.
10. Ospina, P.A., D.V. Nydam, T. Stokol and T.R. Overton, 2010. Associations of elevated nonesterified fatty acids and β-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J. Dairy Sci., 93: 1596-1603.
11. Kadivar, A., M.R. Ahmadi, H.A. Gheisari and S. Nazifi, 2010. Relationship among Insulin-Like Growth Factor I some metabolite concentrations in prepartum and Reproductive function in Holstein-Friesian dairy cows. Comparative clinical pathology, ANIREP-D-10-2561.
12. Shrestha, H.K., T. Nakao, T. Suzuki, T. Higaki and M. Akita, 2004. Effects of abnormal ovarian cycles duringpre-service period postpartum on subsequent reproductive performance of high-producing Holstein cows. Theriogenol., 61: 1559-1571.
13. Ansari-Lari, M., M. Kafi, M. Sokhtanlo and H.N. Ahmadi, 2010. Reproductive performance of Holstein dairy cows in Iran. Trop Anim Health Prod., 42(6): 1277-83.
14. Rabiee, A.R., I.J. Lean, J.M. Gooden and B.G. Miller, 1999. Relationships among metabolites influencing ovarian function in the dairy cow. J. Dairy Sci., 82: 39-44.
15. Plym Forshell, K., L. Andersson and B. Pehrson, 1991. The relationship between fertility of dairy cows and clinical biochemical measurements with special reference to plasma glucose and milk acetone. J. Vet. Med., A38: 608-616.

16. Westwood, C.T., I.J. Lean and J.K. Garvin, 2002. Factors influencing fertility of Holstein dairy cows: A multivariate analysis. J. Dairy Sci., 85: 3225-3237.
17. Hayhurst, C., M.K. Sorensen, M.D. Royal and P. Lovendahl, 2007. Metabolic regulation in Danish bull calves and the relationship to the fertility of their female offspring. J. Dairy Sci., 90: 3909-3916.
18. Herdt, T.H., 2000b. Variability characteristics and test selection in herd-level nutritional metabolic profile testing. Vet. Clin. North Am. Food Anim. Pract., 16: 387-403.
19. Wathes, D.C., N. Bourne, Z. Cheng, G.E. Mann, V.J. Taylor and M.P. Coffey, 2007. Multiple correlation analyses of metabolic and endocrine profiles with fertility in primiparous and multiparous cows. J. Dairy Sci., 90: 1310-1325.
20. Oikonomou, G., G. Arsenos, G.E. Valergakis, A. Tsiaras, D. Zygoyiannis and G. Banos, 2008. Genetic Relationship of Body Energy and Blood Metabolites with Reproduction in Holstein Cows. J. Dairy Sci., 91: 4323-4332.
21. Fahey, J., J. F.Mee and D. O'Callaghan, 2001. Can blood metabolites, body condition and milk production be used to predict reproductive performance in dairy cows? Ir Vet. J., 54: 572-577.
22. Waters, S., D.G. Morris and M.G. Diskin, 2006. The association between circulating metabolic hormones during the early postpartum period and subsequent fertility in dairy cow. Research report, RMIS No, 5679: 145-148.
23. Falkenberg, U., J. Haertel, K. Rotter, M. Iwersen, G. Arndt and W. Heuwieser, 2008. Relationships between the concentration of Insulin-Like Growth Factor-1 in serum in dairy cows in early lactation and reproductive performance and milk yield. J. Dairy Sci., 91: 3862-3868.
24. Miettinen, P.V., 1990. Metabolic balance and reproductive performance in Finnish dairy cows. Zentralbl Veterinarmed., 37: 417-424.
25. Burkhart, M., R. Youngquist, J. Spain, J. Sampson, J. Bader, R. Vogel, W. Lamberson and A. Garverick, 2005. NEFE and glucose levels in serum of periparturient dairy cows are indicative of pregnancy success at first service. J. Dairy Sci., 88(Suppl. 1): 299.
26. Butler, W.R. and R.D. Smith, 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci., 72: 767.
27. Friggens, N.C., 2003. Body lipid reserves and the reproductive cycle: towards a better understanding. Livest Prod. Sci., 83: 219-236.
28. Jorritsma, R., M.L. Cesar, J.T. Hermans, C.L.J.J.K. Ruitwagen, P.L.A.M. Vos and T.A.M. Kruip, 2004. Effects of non-esterified fatty acids on bovine granulosa cells and developmental potential of oocytes *in vitro*. Anim Reprod Sci., 81: 225-235.
29. Herdt, T.H., 2000a. Ruminant adaptation to negative energy balance. influences on the etiology of ketosis and fatty liver. Vet. Clin. North. Am. Food Anim. Pract., 16: 215-230.
30. Mee, J.F. and M. Nolan, 1994. Summer mini-metabolic profile of 50 spring-calving dairy herds. Teagasc, Moorepark Research and Development Division Research Report, pp: 40-41.