Iranian Journal of Applied Animal Science

Investigation of Differences in Biochemical and Mineral Composition of Urine between Pregnant and Non-Pregnant Crossbred Dairy Cattle

Research Article

M.M. Khan[1], S. Prasad[1], F.A. Khan[1*] and H.P. Gupta[1]

[1] Department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Science, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Received on: 26 Dec 2014
Revised on: 12 Mar 2015
Accepted on: 15 Mar 2015
Online Published on: Dec 2015

*Correspondence E-mail: firdous@uoguelph.ca

Online version is available on: www.ijas.ir

ABSTRACT

Dairy farmers have been longing for simple and economical methods of early pregnancy diagnosis. Urine based biochemical and mineral assays could be one of those methods, owing to the ease of sample collection and relatively simple and economical assays involved. However, basic information on changes in urinary composition during early pregnancy in cattle is lacking. This study was designed to evaluate the differences in biochemical and mineral composition of urine between pregnant and non-pregnant dairy cattle. Urine samples were obtained from crossbred dairy cows, classified retrospectively as pregnant or non-pregnant (n=12 in each group), based on transrectal palpation and ultrasonography at 60 days post insemination, and assayed for glucose, total protein, calcium, phosphorus, magnesium, iron, zinc and copper. Sampling was done every 3 days beginning on the day of insemination (d 0) until d 27. Analysis of data on concentrations of the various variables between pregnant and non-pregnant cows indicated significant ($P<0.05$) differences in urinary concentrations of total protein, glucose, calcium, phosphorus, iron, zinc and copper between the two groups at one or more of the sampling days. Moreover, within each group, there were significant differences in concentrations of the studied components across various days of the estrous cycle or pregnancy. Results of the present study indicate marked quantitative differences in biochemical and mineral composition of urine between pregnant and non-pregnant cattle. This information can serve as a basis for devising simple, easy-to-use and cost effective pregnancy diagnosis kits for use in dairy cattle.

KEY WORDS biochemical, dairy cattle, mineral, pregnancy, urine.

INTRODUCTION

Reproductive management constitutes an integral part of a dairy enterprise irrespective of whether it is an intensively managed large scale dairy farm or a small scale backyard dairy unit. In many developing countries including India, the latter is more common with each household maintaining either one or a few dairy cows. Early diagnosis of pregnancy in cattle is very important for an efficient reproductive management. Early identification of non-pregnant cows helps the farmer make a decision to rebreed, treat, or cull them (Fricke and Lamb, 2005), thus reducing the inter-breeding intervals (Stevenson, 2005) or losses incurred on maintenance of infertile animals. Various direct and indirect methods of pregnancy diagnosis have been used in cattle (Purohit, 2010). Transrectal palpation of the reproductive tract has been the most commonly used method worldwide and is traditionally performed at 40 to 60 days of gestation (Lucy *et al.* 2011). However, due to its increased sensitivity and ability to allow earlier diagnosis of pregnancy, ultrasonography has become more popular and has replaced transrectal palpation as a tool for early pregnancy diagnosis, especially in developed countries (Fricke, 2002).

Although the transrectal palpation and ultrasonographic methods of pregnancy diagnosis work well for reproductive management, there are some practical limitations. Both the methods require a specially trained person who, in most of the cases, is a veterinarian and in rare instances, a technician or trained farm staff (Lucy *et al.* 2011). The shortage of large animal veterinarians in certain parts of the world (Jensen *et al.* 2009) and the inability of small scale farmers in developing countries to bear the expenses of a veterinarian's visit are some of the problems that limit the use of these techniques. This prompted the quest for alternative methods of pregnancy diagnosis and over the years, different methods based on early pregnancy factor (EPF), milk or blood progesterone, interferon stimulated genes and pregnancy associated glycoproteins (PAGs) were developed. Of these, only PAG assay is reliable and allows pregnancy diagnosis at day 28 of gestation (Lucy *et al.* 2011). However, PAG test involves blood collection and ELISA, which are again limiting factors, especially for a small scale farmer with no technical expertise. Therefore, development of an easy-to-perform and economical cow-side test for early diagnosis of pregnancy would be of immense practical value to livestock farmers, especially in developing countries. A simple urine based biochemical and / or mineral test would be an ideal method for diagnosing pregnancy, owing to the ease of sample collection and test procedure. However, there is lack of basic information on differences in biochemical and mineral composition of urine between pregnant and non-pregnant cattle. Therefore, the objective of this study was to investigate the differences in concentration of biochemical and mineral constituents of urine between pregnant and non-pregnant crossbred dairy cattle. Specific parameters included glucose, total protein, calcium, magnesium, phosphorus, iron, zinc and copper.

MATERIALS AND METHODS

This study was conducted at Instructional Dairy Farm, G.B. Pant University of Agriculture and Technology, Pantnagar, which is located in the Tarai region of Uttaranchal, India. Animal handling and sample collection during the study were done in compliance with the Institutional Animal Ethics Committee. A total of 40 crossbred dairy cows with mean age of 8.2 ± 0.41 years and mean parity of 4.6 ± 0.38 were used. These cows were free from any apparent abnormalities of the reproductive tract, based on history and transrectal palpation and were maintained under uniform housing, feeding and other management conditions throughout the period of this study. The cows were observed twice daily, in the morning and in the evening, for signs of estrus and those in estrus were inseminated about 12 hours after the observed onset of estrus.

Urine samples were collected every three days beginning on the day of insemination (d 0) until d 27. Perineal region was washed to prevent the contamination of urine samples with fecal material. Urine was collected either by massaging the skin below the vulva (escutcheon) or by catheterization of the urethra. About 10 mL of urine were collected from each animal in sterilized plastic vials, transferred immediately to laboratory on ice, and stored at -20 ˚C till further processing.

Pregnancy diagnosis was done on d 60 by transrectal palpation and ultrasonography and the animals were retrospectively classified into pregnant (n=12) or non-pregnant (n=28). Owing to financial and logistic constraints, urine samples from only 12 of the 28 non-pregnant cows were used for biochemical and mineral assays and the selection of these 12 cows was completely random.

Urine samples were thawed at room temperature and assayed for various biochemical and mineral components using commercial kits and atomic absorption spectrophotometer. Glucose and total protein concentrations were determined colorimetrically by glucose oxidase-peroxidase (GOD-POD) and modified Biuret end point assays, respectively, using commercial diagnostic kits (Span diagnostics Ltd., India).

Calcium, magnesium, and phosphorus concentrations were determined by O-Cresolphthalein Complexone, Calmagite, and UV Molybdate end point assays, respectively, using commercial diagnostic kits (calcium and phosphorus: Span diagnostics Ltd., India, magnesium: Crest Biosystem Ltd., India). For determination of iron, zinc, and copper concentrations, urine samples were digested and subjected to atomic absorption spectrophotometry. Urine samples were digested as per procedure described by Kolmer *et al.* (1951).

About 3 mL of urine sample were mixed with equal volume of concentrated nitric acid in a digestion tube. The mixture was kept overnight at room temperature followed by digestion on low heat (70-80 ˚C) using heat bench (digestion bench), until the volume of samples was reduced to about 1 mL.

To this, 3 mL of double acid mixture (3 parts concentrated nitric acid and 1 part 70% perchloric acid) were added and low heat digestion continued until the digested samples became watery clear and emitted white fumes. Further heating was continued to reduce the volume to approximately 0.5 mL. Final volume of filtrate was made up to 10 mL with triple distilled deionized water after luke warming the solution.

Atomic absorption spectrophotometry was performed using a spectrophotometer (Model No. AAS 4141) manufactured by Electronic Corporation of India Ltd., Hyderabad, India. It uses a double beam with a wave length range of 190-900 nm. Separate hollow UV lamps for each mineral were used and air/acetylene flame was used as fuel. Sample analysis was done and concentrations of the assayed minerals were expressed in parts per million (ppm).

Data were analyzed by generalized linear model for the main effects of group and day and their interaction (group-by-day) using SPSS software version 16.0, (SPSS, 2007). In case of a significant interaction, the differences between groups within day were analyzed for statistical significance by independent samples t-test and the differences between days within group were analyzed for statistical significance by one-way ANOVA followed by Tukey's HSD (Honestly Significant Difference) test. Differences were considered significant at P < 0.05. Data are presented as Mean ± SEM, unless otherwise stated.

RESULTS AND DISCUSSION

Owing to the significant difference on d 0 between pregnant and non-pregnant cattle in concentrations of glucose, total protein, calcium, and iron, the data on these parameters were transformed to percent change from the concentrations on d 0. Since GLM revealed a significant (P<0.05) group-by-day interaction for each of the parameters, main effects were not considered; the actual p-values were 0.000 for glucose, total protein, magnesium, and phosphorus, 0.002 for calcium, 0.001 for zinc, 0.040 for copper and 0.025 for iron. Therefore, further comparisons were made to analyze group within day and day within group differences for each of the above parameters.

Glucose (mg/dL)

Glucose concentrations were greater (P<0.05) in pregnant than in non-pregnant cows on each of the sampling days beginning d 9 until d 27. Within the pregnant group, there was a progressive increase in glucose concentration from d 0 with the first significant percent change recorded on d 18. In contrast, the concentration in non-pregnant cows did not show a significant change from d 0 between different days postinsemination (Figure 1).

Total trotein (mg/dL)

The concentration of total protein was greater (P<0.05) in pregnant than in non-pregnant cows on each of the sampling days beginning d 3 until d 27. Within the pregnant group, concentration varied between days with the first significant percent change from d 0 recorded on d 9. In non-pregnant cows, the percent change on d 12 to d27 was lesser than on d 3 and d 6 (Figure 2).

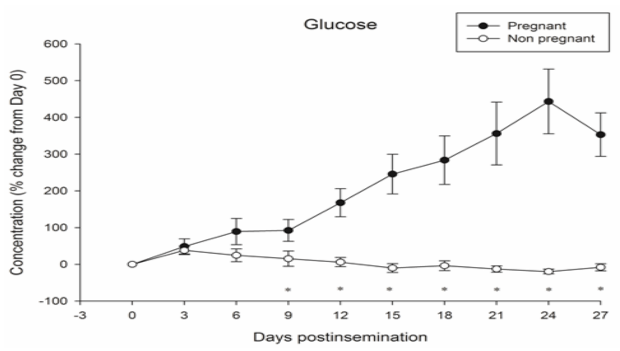

Figure 1 Urinary concentrations (Mean±SEM) of glucose in pregnant and non-pregnant dairy cows. Asterisks indicate significant difference between the groups

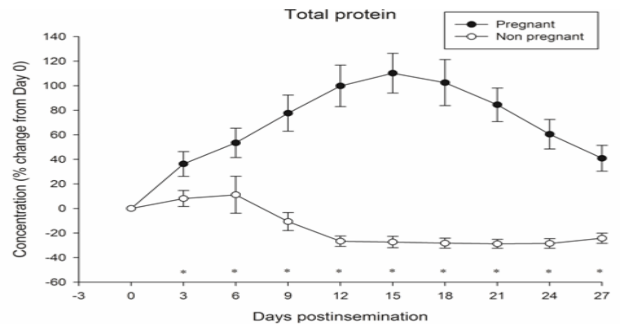

Figure 2 Urinary concentrations (Mean±SEM) of total protein in pregnant and non-pregnant dairy cows. Asterisks indicate significant difference between the groups

Minerals

Pregnant cows had lesser (P<0.05) concentrations of calcium than in non-pregnant cows on each of the days from d 3 to d 21. In pregnant cows, concentration varied between days with a significant percent increase from d 0 recorded on d 15 to d 24. Non-pregnant cows showed a significant percent increase from d 0 on d 3, d 6, d 9, d 15 and d 21 (Figure 3).

Magnesium concentrations were greater (P<0.05) in pregnant than in non-pregnant cows on each of the sampling days beginning d 12 until d 27. Within the pregnant group, magnesium concentration showed a rapid increase from d 9 to d 12 followed by a gradual decrease thereafter. In contrast, there was no difference between days within the non-pregnant group (Figure 4).

Phosphorus concentrations were greater (P<0.05) in pregnant than in non-pregnant cows on each of the sampling days beginning d 6 until d 27. In pregnant cows, phosphorus concentration showed an increase from d 0 beginning d 6 and reached a peak on d 15 followed by a gradual decrease until d 24. In non-pregnant cows, concentrations were greater on d 6, d 12, d 15 and d 18 than on d 0, d 24 and d 27 (Figure 5). Iron concentrations were lesser (P<0.05) in pregnant than in non-pregnant cows on d 9, d 24 and d 27.

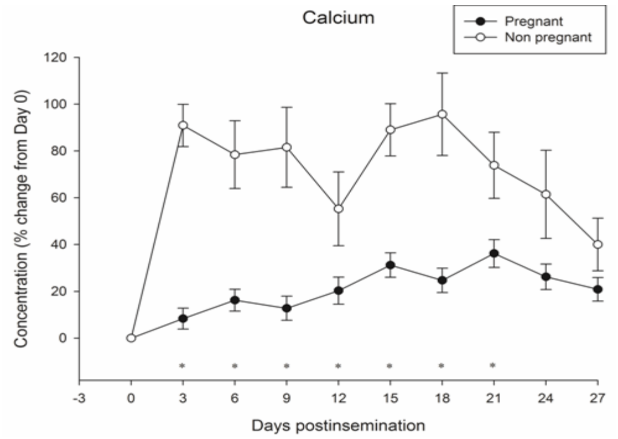

Figure 3 Urinary concentrations (Mean±SEM) of calcium in pregnant and non-pregnant dairy cows. Asterisks indicate significant difference between the groups

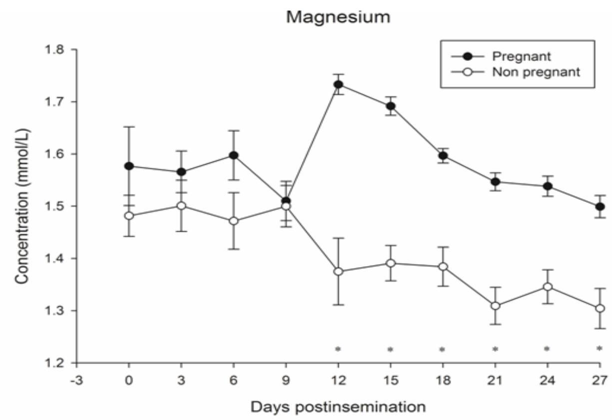

Figure 4 Urinary concentrations (Mean±SEM) of magnesium in pregnant and non-pregnant dairy cows. Asterisks indicate significant difference between the groups

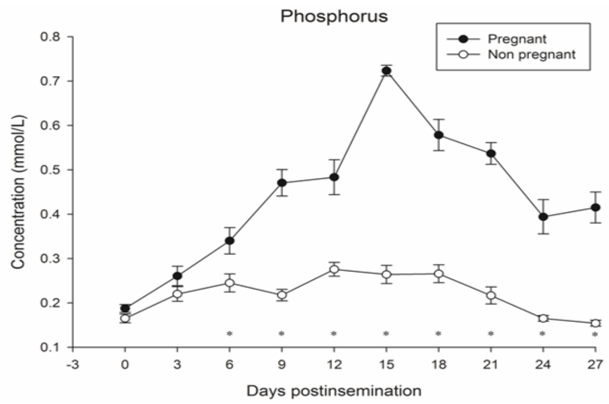

Figure 5 Urinary concentrations (Mean±SEM) of phosphorus in pregnant and non-pregnant dairy cows. Asterisks indicate significant difference between the groups

In pregnant cows, percent change in iron concentration was greater on d 15 than on d 3, d 6, d 9, d 24 and d 27. On the contrary, in non-pregnant cows, percent change in concentration did not differ between days (Figure 6).

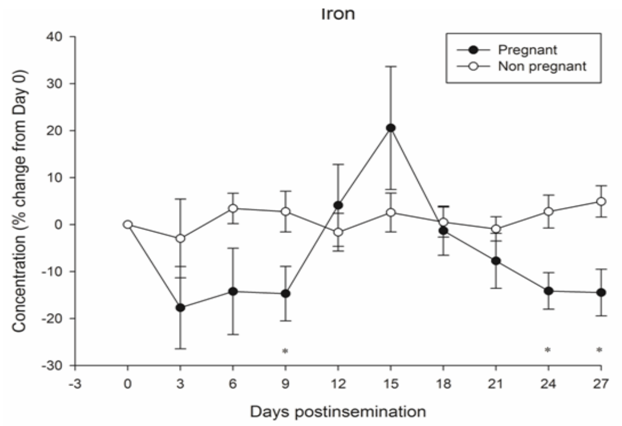

Figure 6 Urinary concentrations (Mean±SEM) of iron in pregnant and non-pregnant dairy cows. Asterisks indicate significant difference between the groups

Zinc concentrations were greater (P<0.05) in pregnant than in non-pregnant cows on d 15 and d 18. In pregnant cows, concentration showed an increase to reach a peak on d 15 followed by a gradual decrease. On the contrary, in non-pregnant cows, concentration did not show a significant difference in concentration between days (Figure 7).

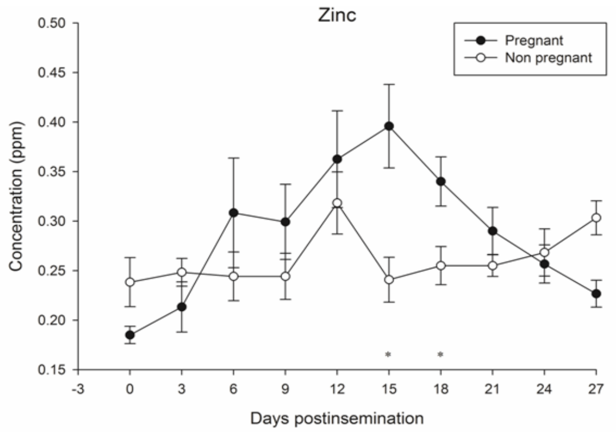

Figure 7 Urinary concentrations (Mean±SEM) of zinc in pregnant and non-pregnant dairy cows. Asterisks indicate significant difference between the groups

Copper concentrations were greater (P<0.05) in pregnant than in non-pregnant cows on d 15 and d 21. In pregnant cows, concentration did not differ between different days. In non-pregnant cows, concentration on d 18 was greater than that on d 21. There was no difference in concentration between the other days (Figure 8).

There were notable differences in the biochemical and mineral composition of urine between pregnant and non-pregnant dairy cattle. More interestingly, differences in some of the components were evident very early in gestation, which opens up the possibility of using them in future as markers for early diagnosis of pregnancy in cattle.

Using currently available, methods, bovine pregnancy can be diagnosed reliably at the earliest on d 28 by PAG assay (Green *et al.* 2005) or on d 29 by transrectal ultrasonography (Romano *et al.* 2006). With the combined attributes of possibly allowing pregnancy diagnosis much earlier than the currently used methods, the simple and non-invasive sample collection, and relatively simple and economical tests that can be used to detect the changes, urine based biochemical and mineral tests could be the method that the bovine industry has been longing for since a long time.

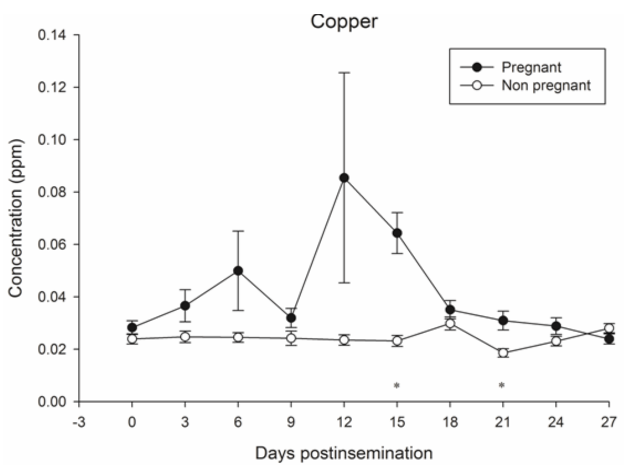

Figure 8 Urinary concentrations (Mean±SEM) of copper in pregnant and non-pregnant dairy cows. Asterisks indicate significant difference between the groups

The effect of pregnancy on urinary glucose concentrations has not received much attention in farm animals so far. In women, however, it has been an area of intense research and there is sufficient evidence to indicate that glycosuria occurs during human pregnancy (Davison and Dunlop, 1980). Pregnant women excreted ten or more times the amount of glucose excreted by non-pregnant women (Renschler *et al.* 1967; Lind and Hytten, 1972) and the increased excretion began very soon after conception (Davison, 1974).

Similar to glucose, differences in urinary total protein concentration between pregnant and non-pregnant farm animals have not been evaluated. However, research in humans has shown that protein concentration in urine is significantly higher during pregnancy (Ibeh *et al.* 2006). A recent study investigating the use of acute phase protein ceruloplasmin as a marker of pregnancy in giant panda (Willis *et al.* 2011) showed elevated levels of active urinary ceruloplasmin during the first week of pregnancy and the levels remain elevated until 20-24 days prior to parturition. It would be interesting to investigate if the difference in urinary protein concentration between pregnant and non-pregnant cows resulted from changes in excretion pattern of proteins in general or that of a particular protein or a set of proteins. Results of this study indicated significant differences in urinary concentrations of various minerals between pregnant and non-pregnant cows during the first month after breeding. This information was previously not known in cattle. Pregnant cows had reduced urinary calcium concentration than non-pregnant cows, which contrasts with the observations during pregnancy in humans (Ritchie *et al.* 1998). Increased urinary phosphorus concentrations in pregnant cows are in line with the increased phosphorus excretion reported during pregnancy in humans (Mozdzien *et al.* 1995). Similarly, results on urinary magnesium concentration were in agreement with those of Salinas *et al.* (1987) who reported that urinary magnesium loss was higher in each trimester of pregnancy in humans compared to their non-pregnant counterparts. In contrast to the consistent differences in the above parameters, iron, zinc and copper concentrations in urine showed sporadic differences between pregnant and non-pregnant cows on just a few days across the sampling period. Results on zinc and copper excretion were different from those of Vierboom *et al.* (2002) who reported that there were no differences in urinary zinc and copper concentrations between pregnant and open cows.

The physiological basis for the differences in urine composition between pregnant and non-pregnant cows is not known. However, it can be speculated that the differences are either a result of changes in the plasma concentrations of the components or changes in their excretion through the urinary system or a combination of both. The differences in urinary excretion could result from changes in glomerular filtration or absorption from the tubular portion of the nephrons. In humans, it has been well established that renal function is altered during pregnancy due to changes in effective renal plasma flow, glomerular filtration rate and renal tubular function (Davison and Dunlop, 1980). However, information on changes in renal function during pregnancy is lacking in farm animals and thus warrants investigation in the future.

A limitation of this study was that the retrospective allocation of animals to the two groups based on pregnancy diagnosis did not allow control over potential confounding factors such as age and parity. However, data on these variables were included in the initial model and there was no effect of these factors on the urinary composition, therefore, ruling out potential confounding from these factors. Another limitation of this study was the timing of pregnancy diagnosis at d 60 post breeding. It could be argued that some of the animals diagnosed non-pregnant at d 60 could have actually been pregnant for a portion or whole of the sampling period. However, from a more practical perspective, this could be an advantage rather than a limitation because with this model, only those animals were grouped as pregnant that actually maintained the pregnancy until d 60. This is relevant because it is well established that majority of the pregnancy losses in dairy cows occur before d 60 of gestation (Santos *et al*. 2004; Diskin and Morris, 2008).

CONCLUSION

The results of this study provide evidence that there are notable differences in urinary concentrations of certain biochemical and mineral components between pregnant and non-pregnant crossbred dairy cows. From an applied standpoint, these differences can be exploited to devise simple and economical methods of pregnancy diagnosis for use in dairy cattle.

ACKNOWLEDGEMENT

The authors would like to thank the Dean, College of Veterinary and Animal Science and Director, Experiment Station, G.B. Pant University of Agriculture and Technology, Pantnagar for providing facilities during the study.

REFERENCES

Davison J.M. and Dunlop W. (1980). Renal hemodynamics and tubular function in normal human pregnancy. *Kidney Int.* **18,** 152-161.

Davison J.M. (1974). Changes in renal function and other aspects of homeostasis in early pregnancy. *J. Obstet. Gynaecol. Br. Cwlth.* **81(12),** 1003.

Diskin M.G. and Morris D.G. (2008). Embryonic and early foetal losses in cattle and other ruminants. *Reprod. Domest. Anim.* **43(2),** 260-267.

Fricke P.M. and Lamb G.C. (2005). Potential applications and pitfalls of reproductive ultrasonography in bovine practice. *Vet. Clin. North Am. Food Anim. Pract.* **21,** 419-436.

Fricke P.M. (2002). Scanning the future-ultrasonography as a reproductive management tool for dairy cattle. *J. Dairy Sci.* **85,** 1918-1926.

Green J.A., Parks T.E., Avalle M.P., Telugu B.P., McLain A.L., Peterson A.J., McMillan W., Mathialagan N., Hook R.R., Xie S. and Roberts R.M. (2005). The establishment of an ELISA for the detection of pregnancy-associated glycoproteins (PAGs) in the serum of pregnant cows and heifers. *Theriogenology*. **63,** 1481-1503.

Ibeh G., Onyeike E.N. and Isodikari A.G. (2006). Protein levels in urine of pregnant women in Rivers State, Nigeria. *J. Appl. Sci. Environ. Mgt.* **10(3),** 171-173.

Jensen K.L., English B.C., Menard R.J. and Holland R.E. (2009). Livestock producers' views on accessing food-animal veterinary services: implications for student recruitment, training, and practice management. *J. Vet. Med. Educ.* **36,** 30-38.

Kolmer J.A., Spaulding E.H. and Robinson H.W. (1951). Approved Laboratory Techniques. Appleton Century Craft, New York, USA.

Lind T. and Hytten F.E. (1972). The excretion of glucose during normal pregnancy. *J. Obstet. Gynaecol. Br. Cwlth.* **79,** 961-965.

Lucy M., Green J. and Poock S. (2011). Pregnancy determination in cattle: a review of available alternatives. Pp. 367-376 in Proc. Appl. Reprod. Strategies Beef Cattle, Joplin, MO, USA.

Mozdzien G., Schinninger M. and Zazgornik J. (1995). Kidney function and electrolyte metabolism in healthy pregnant women. *Wien. Med. Wochenschr.* **145(1),** 12-17.

Purohit G. (2010). Methods of pregnancy diagnosis in domestic animals: the current status. *Web. Med. Central Reprod.* **1(12),** 1305.

Renschler H.E., Bach H.G. and Baeyer H. (1967). The urinary excretion of glucose in normal pregnancy. *German Med. Monthly*. **12,** 24-26.

Ritchie L.D., Fung E.B., Halloran B.P., Turnlund J.R., Van Loan M.D., Cann C.E. and King J.C. (1998). A longitudinal study of calcium homeostasis during human pregnancy and lactation and after resumption of menses. *Am. J. Clin. Nutr.* **67(4),** 693-701.

Romano J.E., Thompson J.A., Forrest D.W., Westhusin M.E., Tomaszweski M.A. and Kraemer D.C. (2006). Early pregnancy diagnosis by transrectal ultrasonography in dairy cattle. *Theriogenology*. **66(4),** 1034-1041.

Salinas M., Martínez M.E., Catalán P., Sánchez Casas E., Herrero E. and Navarro P. (1987). Serum levels and urinary excretion of magnesium in pregnancy. Effect of milk intake. *Arch. Latinoam. Nutr.* **37(3),** 444-453.

Santos J.E., Thatcher W.W., Chebel R.C., Cerri R.L. and Galvão K.N. (2004). The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. *Anim. Reprod. Sci.* **83,** 513-535.

SPSS Inc. (2007). Statistical Package for Social Sciences Study. SPSS for Windows, Version 16.0. Chicago SPSS Inc.

Stevenson J.S. (2005). Breeding strategies to optimize reproductive efficiency in dairy herds. *Vet. Clin. North Am. Food Anim. Pract.* **21,** 349-365.

Vierboom M.M., Engle T.E., Siciliano P.D. and Kimberling C.V. (2002). Effects of gestational status on apparent absorption and retention of copper and zinc in cows and ewes. *Proc. West. Sec. Am. Soc. Anim. Sci.* **53,** 519–522.

Willis E.L., Kersey D.C., Durrant B.S. and Kouba A.J. (2011). The acute phase protein ceruloplasmin as a non-invasive marker of pseudopregnancy, pregnancy and pregnancy loss in the giant panda. *PLoS One*. **6(7),** 21159.