PEER REVIEWED

Clinical Observation on Differential Effects of the Trace Elements Selenium and Copper during Bovine Embryo Transfer

Jakob Scherzer[1]*, *MagMedVet, DrMedVet, DiplECAR;* **Manu Sebastian**[2], *DVM, MS, PhD, DiplACVP, DiplABT;* **Catherine Davis**[1], *BS,* **Douglas C Ensley**[3,4], *DVM, MS;* **David J Hurley**[1,3], *BA, PhD*
[1]*Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA 30602*
[2]*Department of Pathology, College of Veterinary Medicine, University of Georgia, Tifton, GA 31793*
[3]*Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA 30602*
[4]*Fort Dodge Animal Health, Overland Park, KS 66210*
**Corresponding author: Jakob Scherzer, Department of Large Animal Medicine, University of Georgia, 501 D. W. Brooks Dr., Athens, GA 30602, Tel. (706) 542-6319, Fax. (706) 542-8833, e-mail: jakobs@uga.edu*

Abstract

This field study was undertaken because previous studies indicated that deficiencies in selenium (Se) and/or copper (Cu) could negatively affect reproductive performance in cattle. Blood (EDTA) and serum were collected from 43 cows four weeks after embryo transfer at the time of pregnancy examination, and Se and Cu levels were measured. Mean blood Se and serum Cu levels were 0.1 ± 0.07 ppm and 0.66 ± 0.12 µg/ml, respectively. Observed rate of successful pregnancy in recipient cows (termed pregnancy rate in this paper) was decreased with low or marginal blood Se concentrations (P=0.02), but serum Cu concentrations had no influence on pregnancy rate. While embryos of quality 1 or 2 had an equivalent pregnancy rate and the two sires used had no effect on pregnancy rate, a significant effect of donor cow on the pregnancy rate in recipients was observed (P=0.03). If the donor effect was included in the analysis, recipient cows with low or marginal blood Se concentration tended to have reduced embryo transfer efficacy (P=0.08).

Keywords: bovine, embryo transfer, copper, selenium

Résumé

Cette étude sur le terrain a été menée suite à l'observation dans des travaux antérieurs que des carences en sélénium et/ou en cuivre pouvaient avoir un effet néfaste sur la reproduction chez les bovins. Les niveaux de sélénium et de cuivre ont été mesurés à partir d'échantillons de sang et de sérum prélevés chez 43 vaches quatre semaines après le transfert embryonnaire lors de l'examen de gestation. La concentration sanguine moyenne de sélénium était de 0.1 ± 0.07 ppm et de 0.66 ± 0.12 µg/ml pour le cuivre. Le taux de succès de la gestation chez les vaches receveuses (le taux de gestation dans cette article) diminuait lorsque la concentration sanguine de sélénium était basse ou négligeable (P=0.02) mais n'était pas relié à la concentration sérique de cuivre. Bien que le taux de gestation était similaire pour les embryons de qualité 1 ou 2 et ne différait pas selon l'ascendance paternelle, il y avait un effet significatif de l'identité de la vache donneuse sur le taux de gestation des vaches receveuses (P= 0.03). Lorsque l'effet de la vache donneuse était pris en compte dans l'analyse, le transfert embryonnaire était marginalement moins efficace chez les vaches receveuses ayant des concentrations sanguines de sélénium basses ou négligeables.

Introduction

The effect of trace elements on reproductive efficiency in cattle has been the focus of research for many years. However, there is still debate about the mode of action of trace elements in the reproductive process. Selenium (Se) is an essential micronutrient for many biological functions. The enzyme glutathione peroxidase contains Se and is involved in the antioxidant defense of the body, and functions by neutralizing peroxide radicals.[11] It has been proposed that radicals affect the formation of prostaglandins, which are involved in inflammatory and reproductive processes, when the level of Se is too low to allow optimal glutathione peroxidase function. Several selenoproteins, e.g. selenoprotein P and W, have also been identified which have similar antioxidant effects.[4] Through the enzyme iodothyronine deiodinase, Se is involved in the regulation of pituitary hormones, steroid and cholesterol metabolism, and in the immune response.[15] Selenium supplementation has been shown to have beneficial effects on fertility in cattle.[10] There is strong evidence that Se deficiency reduces embryo survival during implantation.[18]

Copper (Cu) deficiency appears to be the most common micronutrient deficiency in cattle worldwide.[23] Primary Cu deficiency results from low Cu in the diet, while secondary deficiency is attributed to a reduction in Cu absorption or utilization due to the antagonistic effects of molybdenum (Mo), sulfur, or iron.[3] In beef cows, Cu supplementation is shown to have a beneficial effect on fertility.[14] In beef heifers, secondary Cu deficiency retards embryo development and leads to early embryonic death due to toxic effects of Mo.[16]

Satisfactory intake of trace elements by ruminants depends on their presence in soil and feed plants. According to Oldfield,[17] Se levels vary in the eastern United States. In Georgia, areas of adequate and low Se soil have been documented, and the Se level shows considerable heterogeneity even within the major geographical regions of the state. This farm-based study was undertaken to determine the relationship between the Se and Cu concentrations in cattle within the area serviced by the Theriogenology Service of the College of Veterinary Medicine, University of Georgia, and to evaluate if there was a relationship between the levels of these trace elements and successful embryo transfer. The importance of adequate supplementation of recipient animals with Se is well accepted among embryo transfer practitioners, but to our knowledge, has not been studied previously.

Materials and Methods

Animals

A farm-based trial was conducted March through April of 2007 on a commercial beef cattle farm in northeastern Georgia. Fifty Angus crossbred cows (three to seven years of age, body condition score [BCS] 5-7 using a scale of 10) were initially selected for the trial, but only 43 met all criteria to be utilized as embryo transfer recipients. All study cows calved at least six weeks prior to the start of the estrus synchronization protocol. The cows were kept on pasture and had access to a trace element and vitamin premix.[a] This study was approved by the Clinical Research Committee of the College of Veterinary Medicine, University of Georgia.

Treatments and sample collections

Fifty cows were synchronized using a combination of Ovsynch and a controlled internal drug release device (CIDR).[b] The protocol was initiated with an intramuscular (IM) injection of 100 µg of a gonadotropin-releasing hormone (GnRH) analog (gonadorelin diacetate tetrahydrate)[c] on day -9 relative to estrus, and at the same time the CIDR (containing 1.9 g of progesterone) was inserted intravaginally and maintained for seven days. Recipients subsequently received an IM injection of 25 mg dinoprost tromethamine[d] on day -2 relative to estrus. On this day, the CIDR implants were removed. On the day of estrus, 100 µg of GnRH was injected IM. Seven days later, an embryo was transferred to the uterine horn ipsilateral to the demonstrated corpus luteum (CL) in each of 43 cows. Seven of the cows originally placed on the study did not develop a CL that could be identified by rectal palpation, therefore, these cows were excluded from the study.

Embryos were collected from five superovulated cows that had been subjected to artificial insemination (AI) with frozen semen on the day of estrus. Semen from two bulls was used for AI, but only one bull was assigned to each donor cow. The embryos from each cow were flushed using a standard protocol.[5] Recovered embryos were graded by microscopic examination according to International Embryo Transfer Guidelines, and quality 1 and 2 embryos from the five donor cows were cryopreserved for transfer to the recipient cows. The embryos were frozen using 1.5 M ethylene glycol as a cryopreservative to permit direct transfer of embryos to recipients after thawing.[6] Embryos were stored frozen for a period of six weeks in liquid nitrogen. The frozen embryos were thawed for five seconds in air, followed by 15 seconds in a water bath at 86°F (30°C). The embryo transfer was completed within five minutes of thawing. All transfers were performed by the same veterinary clinician.

Pregnancy was assessed 28 days after transfer by transrectal ultrasonography, using a real-time scanner with a 5-MHz linear array transducer.[e] Blood samples were collected at this time by coccygeal venipuncture from all 43 cows that received embryos. Blood was collected into EDTA-containing glass tubes and similar tubes without anti-coagulant. EDTA blood samples were stored at 39°F (4°C) until analyzed. The blood, collected into tubes without anti-coagulant, was allowed to clot and centrifuged at 800xg for 20 minutes and the serum was collected and stored at -22°F (-30°C) until utilized in the assays.

Trace element analysis

Selenium and Cu were measured using a Perkin-Elmer Analyst 600 Graphite Furnace Atomic Absorption Spectrophotometer at the Tifton Clinical Veterinary Laboratory. The analysis was performed under the standard clinical protocol, and all samples were treated blindly as direct clinical submissions.

Statistical analysis

Analyses were performed using SAS V 9.1.[f] The correlation between Cu and Se levels was analyzed using the nonparametric Spearman rank correlation analysis.

Pregnancy rate was defined as the observed rate of successful establishment of pregnancy in cows receiving an embryo. The pregnancy rate between embryo quality groups (1 and 2) was compared using chi-square analysis. The effect of Se and Cu on the frequency of pregnancy was tested using a logistic model with Se and Cu levels assigned a scalar value (0=low; 1=marginal or 2=adequate) and used as a continuous factor. To allow consideration of the differential fertility among donor cows and sires in the assessment, the differential frequency of successful pregnancy among donor cows (cows 1, 2, 3 and a pool of the results from cows 4 and 5) and sires (sires 1 and 2) were then compared using chi-square analysis. The effect of Se and Cu levels on the frequency of successful pregnancy was then calculated using a logistic model which included a donor cow classification factor and the Se and Cu level factor (0, 1 or 2).

Results

The levels of Se and Cu in the cows are shown in Table 1. Mean blood Se and serum Cu levels were 0.1 ± 0.07 ppm and 0.66 ± 0.12 µg/ml, respectively. A significant negative correlation was observed between Se and Cu levels among cows (r= -0.38, P=0.0117), however, the correlation analysis of Cu and Se separately for each donor cow (except five which did not have enough data) produced a non-significant negative correlation. Only five of 43 cows had low blood Se levels, and five had low serum Cu levels; none had both. Among the 43 cows, 19 had marginal blood Se and 22 had marginal serum Cu. Examination of Table 1 indicates there was a subpopulation of 13 cows that had either low or marginal levels of both Se and Cu.

The overall pregnancy rate for the recipient cows was 41.9% (18/43). There was no effect of embryo quality on pregnancy rate (P=0.29); both embryos quality 1 and 2 performed equally. Blood Se level (low <0.05 ppm, marginal 0.05-0.07 ppm, and adequate >0.07 ppm) significantly influenced the frequency of successful pregnancies (P=0.0244, odds ratio=3.5), but no significant effect of serum Cu (low <0.5 µg/ml; marginal 0.5-0.7 µg/ml; and adequate >0.7 µg/ml) (P=0.77) on pregnancy rate was observed. Pregnancy rate for each of the blood Se levels was: low Se (0%, 0/5); marginal Se (36.8%, 7/19); and adequate Se (57.9%, 11/19). Similarly, the rate of successful pregnancies relative to serum Cu levels were: low Cu (20%, 1/5); marginal Cu (54.6%, 12/22); and adequate Cu (31.3%, 5/16). The pregnancy rate was 30.8% (4/13) in cows with low or marginal levels of both Se and Cu, similar to that observed for those with low or marginal Se alone (29.2%, 7/24).

Numbers of embryos recovered/transferred differed between donor cows; 11/11 in cow 1, 23/9 in cow 2, 17/10 in cow 3, 11/11 in cow 4, and 2/2 in cow 5. When analysis of success of embryos transferred from different cows was conducted, there was a significant effect of donor cow. The frequency of successful pregnancy based on donor cow ranged from as high as 100% (2/2) to as low as 10% (1/10, P=0.03), with a mean of 51.3%. There was no effect of sire used for AI on pregnancy rate (48.8% vs 51.2%, P=0.17). When the donor was included as a factor in the analysis, the effect of blood Se level on the odds of a successful pregnancy tended toward significance (P=0.08; Figure 1). No effect of serum Cu level on odds of successful pregnancy was observed (P=0.81).

Discussion

When embryos of quality 1 or 2 were transferred to recipient cows in this farm-based study, the pregnancy rate was decreased in cows with marginal or low levels of

Table 1. Selenium and copper levels in recipient cows in relation to the establishment of pregnancy.

Recipient cow no.	Donor cow no.	Sire No.	Blood Se	Serum Cu	Pregnancy Outcome
1	2	2			Open
2	2	2			Pregnant
3	2	2			Pregnant
4	1	1			Open
5	1	1			Open
6	2	2			Open
7	2	2			Open
8	2	2			Open
9	2	2			Open
10	2	2			Pregnant
11	1	1			Pregnant
12	4	1			Pregnant
13	3	2			Open
14	3	2			Open
15	3	2			Open
16	4	1			Pregnant
17	3	2			Open
18	4	1			Pregnant
19	4	1			Pregnant
20	3	2			Open
21	4	1			Pregnant
22	3	2			Pregnant
23	3	2			Open
24	3	2			Open
25	4	1			Pregnant
26	3	2			Open
27	3	2			Open
28	4	1			Open
29	4	1			Open
30	1	1			Open
31	1	1			Pregnant
32	1	1			Open
33	1	1			Open
34	1	1			Open
35	4	1			Open
36	1	1			Pregnant
37	4	1			Pregnant
38	1	1			Open
39	1	1			Pregnant
40	5	2			Pregnant
41	2	2			Open
42	2	2			Pregnant
43	5	2			Pregnant

Color key

low	< 0.05 ppm	< 0.5 µg/ml
marginal	0.05 - 0.07 ppm	0.5 - 0.7 µg/ml
adequate	> 0.07 (-1) ppm	> 0.7 (- 1.2) µg/ml

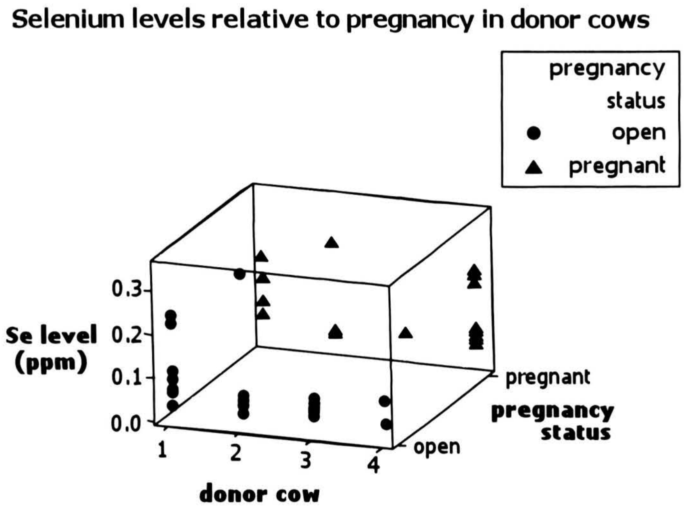

Figure 1. Selenium levels and pregnancy status in different donor cows. This 3D-graph displays each donor animal on the X-axis (labeled donor cow 1, 2, 3) or a pool of the results for cows 4 and 5 (shown as "4"). The Se level for each recipient cow is displayed on the Y-axis, and the pregnancy status for the recipient animals is displayed on the Z-axis. Pregnant recipient cows tended to have higher Se levels (*P*=0.08).

blood Se in the recipient. This effect was not observed in recipients with marginal or low serum Cu levels. Although the two sires used had no effect on pregnancy rate, the donor cow had a significant influence on the establishment of pregnancy in recipient animals, with the pregnancy rate ranging from 10 to 100%. This study was principally designed to monitor the effect of trace minerals in recipients, and not to assess parameters associated with the donors, so there is insufficient data to further explore this finding in this study.

Measurement of Se in EDTA blood adequately represents the nutritional Se status in cattle, while serum Se concentration does not appear an appropriate measurement.[13] Liver biopsy is a good sample for diagnosing Cu deficiency in cattle; however, this was not feasible in these client-owned animals. It is recommended to test 10% of a herd, or a minimum of 10 to 15 animals, to have a reliable probability of diagnosing Cu deficiency using serum analysis.[9] The entire herd was tested in this study.

Selenium and Cu levels differed markedly between animals, despite the fact that all animals in this herd received the same trace element and vitamin premix. This can only be explained by a difference in actual intake of premix between animals, possibly due to hierarchy within the herd which limited access to the feed bunks.

It has been postulated that Se has an effect on successful implantation of the embryo during the fourth week after estrus.[8,18] In one of the authors' (JS) experience, cows with sub-optimal blood Se concentration may not return to estrus 14 days after embryo transfer, but many embryos still fail to survive due to unsuccessful implantation. As a consequence, embryonic resorption takes place. In the present study, there was ultrasonographic evidence of resorption in one of the recipients with low Se. After AI, a negative effect on fertility is only observed in animals with low Se levels. In contrast, pregnancy rate can be reduced after embryo transfer in cows with marginal blood Se concentrations, as observed in this field study. An embryo transferred into the uterus of a recipient is essentially an allograft, therefore an immune response may be stimulated in the recipient that could lead to rejection of the embryo. In an earlier study, researchers reported that prostaglandin E2 (PGE2) concentrations increased in recipient cows after transfer of embryos to recipients with subsequent increases in body and milk temperatures (0.72°F or 0.4°C), which was likely due to the pyrogenic effect of PGE2.[7] It was postulated that the PGE2 response plays an important role in preventing rejection of the embryo by the recipient.[7] It was further suggested that PGE2 has a luteotrophic effect, which contrasts the luteolytic effect of PGF2α.[20]

It is well accepted that sub-optimal blood Se concentration impairs the inflammatory response, its regulation, and also alters PGE2 synthesis.[2] However, the role of Se on a successful pregnancy after embryo transfer is not understood. Many herds have cows with widely differing levels of Se, as demonstrated in this herd. As a result, the role of less than optimal Se in the establishment of pregnancy may be much more important than is currently realized.

While Cu did not appear to have a direct impact on successful pregnancy in this study, there may be confounding factors. The most common cause of Cu deficiency in Georgia is sulfur antagonism.[19] It was previously reported that early embryonic death due to secondary Cu deficiency is normally associated with the toxic effects of Mo.[16,22] Thus, it is not surprising that low or marginal Cu concentration did not affect pregnancy rate in recipient cattle in this study, as sulfur was the most likely cause of lower than optimal Cu levels in these cows.

The pregnancy rate in the cows with both low or marginal Se or Cu levels (30.8%) was similar to the pregnancy rate in cows with low or marginal Se alone (29.2%), while animals with low or marginal Cu levels had higher pregnancy rates (48.1%, 13/27). This suggests that in this study low or marginal Se levels in recipient cows were responsible for the lower pregnancy rates. Furthermore, secondary Cu deficiency in Georgia is caused by excess sulfur, which does not exert a toxic effect on the embryo.

These findings support those of a previous study that indicated transferring quality 1 or 2 embryos to recipient cows did not result in differential pregnancy rates.[21] There was a significant effect of the donor cow on the pregnancy outcome in this study. It is possible that the trace elements status of the donors influenced pregnancy outcome. However, Se and Cu levels of donor cows were not evaluated in this trial, as it was not specifically designed to assess parameters associated with the biochemical or biological properties of embryos from different donors. Also, the morphological quality of embryos was only assessed at the time of freezing using an established grading system. This donor-associated effect on embryo transfer has not been observed before, or at least it has not been reported. There is anecdotal evidence that pregnancy success differs between donor animals; however, it has not been systematically studied. Only standard light microscopic evaluation of embryo morphology to grade embryos prior to cryopreservation was utilized in this study. A study that utilized electron microscopy to examine embryos concluded that using a stereomicroscope was not fully adequate to accurately determine embryo quality.[1] Consequently, differences among embryos used in this study could reflect the degree of error in determining embryo quality graded based on assessment of embryo morphology utilizing optical microscopy only. Addition of another assessment of embryo quality by a non-invasive technique would potentially help to more accurately grade embryos of similar morphological grade. Recently, an embryo respirometer, which measures oxygen consumption of the embryo, has shown promising results *in vitro*, and may potentially help to more accurately determine embryo quality.[12]

Conclusions

In this field study, the pregnancy rate after embryo transfer decreased in recipient cows with marginal or low blood Se levels. In contrast, marginal or low serum Cu levels had no effect on successful embryo transfer rates.

No differences in pregnancy rates were observed when embryo quality 1 or 2 were utilized. Likewise, the two sires used produced similar pregnancy rates. However, the source of the embryo (i.e. the donor cow) had a significant influence on establishment of pregnancy in recipient animals. Future studies should more thoroughly investigate the differential effect of the donor cow on pregnancy outcome.

Endnotes

[a]Accel Fortifier, Accelerated Genetics, Baraboo, WI
[b]EASY-BREED™ CIDR®, Pfizer Animal Health, New York, NY
[c]Cystorelin®, Merial Limited, Duluth, GA
[d]Lutalyse®, Pfizer Animal Health, New York, NY
[e]Aloka 500V, Choice Medical Systems Inc., St. Petersburg, FL
[f]SAS V 9.1, Cary, NC

Acknowledgements

The authors wish to thank Gretsch Angus Farms, Georgia, USA, for participating in this study. Statistical analysis was performed by Dr. Deborah Keys.

References

1. Aguilar MM, Galina CS, Merchant H, Montiel F, Canseco R, Marquez YC: Comparison of stereoscopy, light microscopy and ultrastructural methods for evaluation of bovine embryos. *Reprod in Dom Anim* 37:341-346, 2002.
2. Arosh JA, Banu SK, Kimmins S, Chapdelaine P, Maclaren LA, Fortier MA: Effect of interferon-{tau} on prostaglandin biosynthesis, transport, and signaling at the time of maternal recognition of pregnancy in cattle: Evidence of polycrine actions of prostaglandin E2. *Endocrinology* 145:5280-5293, 2004.
3. Black DH, French NP: Effects of three types of trace element supplementation on the fertility of three commercial dairy herds. *Vet Rec* 154:652-658, 2004.
4. Brown KM, Arthur JR: Selenium, selenoproteins and human health: a review. *Public Health Nutrition* 4:593-599, 2001.
5. Dawson J: Bovine embryo transfer. *In Pract* 22:80-89, 2000.
6. Dochi O, Yamamoto Y, Saga H, Yoshiba N, Kano N, Maeda J, Miyata K, Yamauchi A, Tominaga K, Oda Y, Nakashima T, Inohae S: Direct transfer of bovine embryos frozen-thawed in the presence of propylene glycol or ethylene glycol under on-farm conditions in an integrated embryo transfer program. *Therio* 49:1051-1058, 1998.
7. Gil Z, Kural J, Szarek J, Wierzchos E: Increase in milk and body temperature of cows as a sign of embryo entry into the uterus. *Therio* 56:685-697, 2001.
8. Hafez ESE, Hafez B: Fertilization and cleavage, in Hafez B, Hafez ESE (eds): *Reproduction in Farm Animals*. Philadelphia, Lippincott Williams & Wilkins, 2000.
9. Hall JO: Appropriate methods of diagnosing mineral deficiencies in cattle. *Proc Tri-State Dairy Nutrition Conf*, Fort Wayne, Indiana, April 25-26, 2006.
10. Harrison JH, Hancock DD, Conrad HR: Vitamin E and selenium for reproduction of the dairy cow. *J Dairy Sci* 67:123-132, 1984.
11. Kommisrud E, Osteras O, Vatn T: Blood selenium associated with health and fertility in Norwegian dairy herds. *Acta veterinaria Scandinavica* 46:229-240, 2005.
12. Lopes AS, Greve T, Callesen H: Quantification of embryo quality by respirometry. *Therio* 67:21-31, 2007.
13. Maas J, Galey FD, Peauroi JR, Case JT, Littlefield ES, Gay CC, Koller LD, Crisman RO, Weber DW, Warner DW: The correlation between serum selenium and blood selenium in cattle. *J Vet Diagn Invest* 4:48-52, 1992.
14. Muehlenbein EL, Brink DR, Deutscher GH, Carlson MP, Johnson AB: Effects of inorganic and organic copper supplemented to first-calf cows on cow reproduction and calf health and performance. *J Anim Sci* 79:1650-1659, 2001.
15. Nohl H: Biochemische Grundlagen Vitamin-E-und Selen-Mangel-bedingter Erkrankungen. *Wien tierärztl Monatsschrift* 71:217-223, 1984.
16. O'Gorman J, Smith FH, Poole DBR, Boland MP, Roche JF: The effect of molybdenum-induced copper deficiency on reproduction in beef heifers. *Therio* 27:265, 1987.
17. Oldfield JE: Selenium world atlas. Association S-TD, editor, Grimbergen, Belgium, 2000.

18. Robinson JJ: Nutrition in the reproduction of farm animals. *Nutr Res Rev* 3:253-276, 1990.
19. Rossi J: Mineral supplements for beef cattle. The University of Georgia, Cooperative Extension, Bulletin 895, 2006.
20. Shelton K, Parkinson TJ, Hunter MG, Kelly RW, Lamming GE: Prostaglandin E-2 as a potential luteotrophic agent during early pregnancy in cattle. *J Reprod Fertil* 90:11-17, 1990.
21. Spell AR, Beal WE, Corah LR, Lamb GC: Evaluating recipient and embryo factors that affect pregnancy rates of embryo transfer in beef cattle. *Therio* 56:287-297, 2001.
22. Ward GM: Molybdenum toxicity and hypocuprosis in ruminants: a review. *J Anim Sci* 46:1078-1085, 1978.
23. Wikse SE, Herd D, Field R, Holland P: Diagnosis of copper deficiency in cattle. *J Am Vet Med Assoc* 200:1625-1629, 1992.