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Effect of injectable trace mineral complex supplementation on development of ovarian structures and serum copper and zinc concentrations in over-conditioned Holstein cows

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ABSTRACT

This study evaluated the effect of injecting trace minerals on reproductive performance in over-conditioned Holstein cows before synchronized estrus. Multiparous non-lactating, over-conditioned repeat breeder cows (n = 20) were assigned randomly to one of two treatments: 1) control (n = 10), and 2) supplementation with an injectable trace mineral complex 25 days before expected synchronized estrus (n = 10). Follicular waves were synchronized by intravaginal insertion of a CIDR for eight days and an intramuscular (i.m.) injection of a GnRH analogue. Estrus was induced at CIDR removal by an i.m. injection of $PGF_{2\alpha}$. Blood samples were collected before and after synchronized estrus. The response variables were follicle population (FP), diameter of the preovulatory follicle at CIDR removal (DFP0) and at estrus detection (DFP1), time of estrus after CIDR removal (TE), area of corpus luteum (ACL), pregnancy rate and copper and zinc serum concentrations. The statistical analysis of the variables was carried out with SAS. The FP, DFP0, DFP1, TE, ACL and serum concentrations of copper and zinc were not affected by the trace mineral injection ($P > 0.05$). Even though pregnancy rate at 40 (77.78 ± 13.46 *vs* 44.44 ± 16.56%) and 60 days after AI (66.67 ± 15.71 *vs* 33.33 ± 15.71%) was numerically higher for cows injected with trace minerals than for the control group, the differences were not significant ($P > 0.05$). In conclusion, while follicular and corpus luteum development were not affected by trace mineral injection, it may be a feasible way to increase the pregnancy rate in over-conditioned cows.

1. Introduction

Obesity alters the trace mineral status necessary for normal reproduction (Tungtrongchitr et al., 2003; Hidiroglou, 1979). Overweight and obese conditions reduce fertility as a result of poor oocyte competence, low embryo quality and pregnancy losses, probably by adversely affecting the hypothalamic-pituitary-ovary axis function (Brewer and Balen, 2010; Jungheim and Moley, 2010). Reduced fertility due to obesity is also well documented in humans (Pasquali et al., 2003), but little is known in dairy cattle.

The presence of over-conditioned (obese) cows is undesirable on dairy farms. Cows can be regarded as under- or over-conditioned based on their body condition score, on a scale of 1–5 where 1 is under- and 5 is over-conditioned (Wildman et al., 1982). In general, an over-conditioned or obese status is detrimental to health (Roche et al., 2009) and fertility in cattle (Stádník et al., 2002). Unfortunately, there is a lack of research attempting to improve fertility in over-conditioned cows. This is important because over-

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conditioned dairy cattle are often labeled as low producers and repeat breeders, and maintenance of these animals results in economic losses for the dairy farmer. In obese humans, correction of some of the hormonal imbalance (Chen et al., 1998), and in cattle, improvement in reproductive performance (Campbell et al., 1999), have been reported after trace mineral supplementation. To our knowledge, there is no available information regarding the effect of trace minerals on fertility in over-conditioned cattle. Therefore, this study evaluated injectable trace mineral supplementation before estrus as a strategy to improve fertility in over-conditioned cows.

2. Materials and methods

2.1. Location

The experiment was performed at the Universidad Autónoma Chapingo, México, dairy cattle research station farm. The farm is located at 19°29′N and 98°52′W, 2250 m above sea level. The climate is temperate sub-humid, with a mean annual temperature and rainfall of 15 °C and 645 mm, respectively (García, 1988). The experiment was conducted during May 2015. All the technical and management procedures for animals in the present research were performed using the guidelines set by the Canadian Council on Animal Care (2009).

2.2. Animals, treatments and experimental design

Multiparous non-lactating and repeat breeder (≥4 services) cows available in the herd were assessed by a standard body weight condition scoring system from 1 (under-conditioned) to 5 (over-conditioned). Cows with 4 (heavy) to 5 (over-conditioned) scores ($n = 20$), with an average score of 4.75 ± 0.55 and an average body weight of 749.6 ± 16.2 kg, were assigned randomly to one of two treatments: 1) the control group without mineral supplementation ($n = 10$) and 2) the treatment group supplemented with a subcutaneous injection (1 mL per 90.7 kg) of a trace mineral complex (Multimin 90, Nova-Tech, Inc., USA) containing 15, 10, 5 and 60 mg mL^{-1} of copper, manganese, selenium and zinc, respectively, 25 days before expected estrus ($n = 10$).

Cows were maintained as a single group and fed corn silage before, during and after the experiment. In addition, cows did not receive any mineral supplementation for at least two months before beginning the experiment. The experimental design was completely random with ten repetitions, and the experimental unit was one cow.

2.3. Estrus synchronization and breeding

Follicular waves were synchronized 14 days after the trace mineral injection was administered, by intravaginal insertion of a controlled internal drug release device (CIDR) containing 1.9 g of progesterone (CIDR 1900 CATTLE INSERT®, Zoetis, Mexico), and an i.m. injection of 250 μg of a GnRH analogue (GnRH®, Sanfer, Mexico) at CIDR insertion. The CIDR was removed after eight days, and estrus behavior was induced by intramuscular (i.m.) administration of 500 μg $PGF_{2\alpha}$ (Celosil®, MSD Animal Health, Mexico) at that time. After the CIDR was removed, cows were constantly monitored by direct observation for signs of standing estrus. Cows were artificially inseminated 12 h after estrus with a dose of semen (approximately 20×10^6 spermatozoa) from a single bull of proven fertility.

2.4. Response variables

The response variables measured were follicle population (FP), diameter of the preovulatory follicle at CIDR removal (DFP0) and at estrus detection (DFP1), time of estrus after CIDR removal (TE), area of the corpus luteum (ACL), pregnancy rate and serum copper and zinc concentrations.

The FP, DPF0, DFP1 and ACL were measured by real time ultrasonography (Aloka SSD-500, with 7.5 MHz linear transducer; Aloka Ltd, Tokyo, Japan). FP was measured by counting the number of antral follicles (≥1 mm) in both ovaries on days −25, −20, −15, −10 and −5 (day −25 was the day of trace mineral injection). The DFP0 and DFP1 were calculated by the average of horizontal and vertical measurements of the preovulatory follicle at CIDR removal and immediately after estrus detection. The preovulatory follicle at CIDR removal was that with the largest diameter, and its location was recorded and its diameter remeasured at estrus detection. The ACL was calculated directly by ultrasound nine days after AI. The TE was calculated as the time between CIDR removal and estrus appearance. Pregnancy to AI was determined with transrectal ultrasonography at 45 and 60 days after AI. The serum concentrations of copper and zinc were determined by means of atomic absorption spectrophotometry of serum collected on days −25, −20, −15, −10, −5, and −1, and nine days after AI, in five cows chosen at random from each group. Blood was collected using red cap BD vacutainer serum tubes, but care was taken to avoid any contact between blood and the tube cap during and after sample collection. In addition, cows were handled so as to minimize any distress that could compromise sample quality before and during sampling. If hemolysis was observed, the sample was discarded and another was taken as soon as possible on the same day. In general, sample management and the material needed for mineral determination was prepared according to the guidelines of Fick et al. (1979).

Table 1
Least squares means (± standard error) for the influence of injectable trace mineral complex supplementation on ovarian development and time of estrus of non-lactating, over-conditioned repeat breeder Holstein cows.

Variable	Group[a]		*P*-value
	Control	Injected with trace minerals complex	
Follicle population (≥1 mm)	8.38 ± 0.8	8.02 ± 0.8	0.77
Diameter of the preovulatory follicle at CIDR removal (mm)	13.15 ± 1.0	11.46 ± 1.0	0.25
Diameter of the preovulatory follicle at estrus detection (mm)	15.76 ± 0.7	16.13 ± 0.7	0.71
Time to estrus after CIDR removal (hours)	56.60 ± 17.8	90.52 ± 17.8	0.19
Area of the corpus luteum (mm^2)	50.40 ± 3.8	43.61 ± 3.8	0.23

[a] Within rows, means with different superscripts are statistically different ($P \leq 0.05$).

2.5. Statistical analysis

The response variables were analyzed using SAS 9.3 statistical software. The effects of mineral injection on FP, copper and zinc concentrations were analyzed by repeat measurements using PROC MIXED. The DFP0, DFP1, TE, ACL and pregnancy rate were analyzed by the Tukey test using PROC GLIMMIX, with the function link identity, but in the case of pregnancy rate, the function link used was logit. The experimental unit was one cow.

3. Results

The effect of injecting non-lactating, repeat-breeder, over-conditioned cows with a trace mineral complex on ovarian development and time to estrus presentation is shown in Table 1. The injections of trace minerals before a synchronized estrus had no effect ($P > 0.05$) on follicle population, preovulatory follicle size or corpus luteum development. However, time to estrus after CIDR removal in the control group tended ($P = 0.19$) to be shorter compared with cows that received trace mineral supplementation. Pregnancy rate at 40 and 60 days after AI was 33.3% higher in cows injected with trace minerals compared with control cows (Fig. 1). However, the effect of treatment was not significant ($P > 0.05$).

4. Discussion

This study evaluated the effect of injecting non-lactating, over-conditioned repeat breeder Holstein cows before a synchronized estrus with a trace mineral complex on reproductive traits and serum copper and zinc concentrations. Over-conditioned, obese cattle are often regarded as repeat breeders, which may result from a higher incidence of ovarian cysts (Stádník et al., 2002) and reduced oocyte quality (Kubovicova et al., 2012). Low quality embryo production has been reported in obese cattle (Velazquez et al., 2011) due to the high incidence of fragmented embryos (Makarevich et al., 2016). In addition, a greater clearance of progesterone has also been reported in obese cattle (Burke et al., 1998), compromising pregnancy maintenance.

Follicle population is associated with fertility in cattle. A low follicle population (five antral follicles) has been previously reported in obese cattle (Kubovicova et al., 2012). According to Ireland et al. (2007, 2008), cows with a high follicle population (≥25 antral follicles) produce more oocytes of better quality and yield more transferable embryos than those with a low population (≤15 antral follicles). This may explain the higher fertility observed by Mossa et al. (2012) in dairy cattle with high follicle populations. Considering the follicle population obtained in our results, and those of Ireland et al. (2007, 2008) and Mossa et al. (2012), the animals in the present study can be regarded as cows with low fertility.

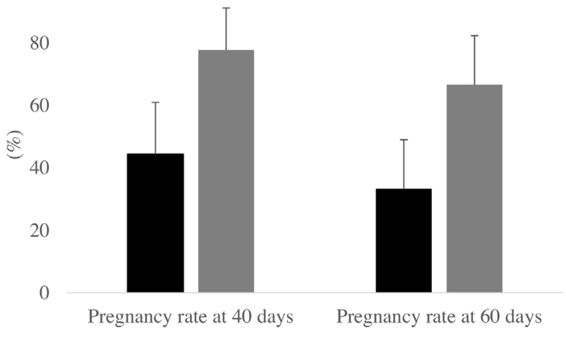

Fig. 1. Pregnancy rate at 40 and 60 days after artificial insemination in non-lactating, over-conditioned, repeat breeder Holstein cows injected with a trace mineral complex (gray bars) and control group (black bars).The mean serum concentrations of copper (0.42 ± 0.02 *vs* 0.39 ± 0.02 $mg\ L^{-1}$) and zinc (0.19 ± 0.007 *vs* 0.20 ± 0.007 $mg\ L^{-1}$) for the control and treatment groups, respectively, were not affected ($P > 0.05$) by treatment. The concentration patterns of these minerals over the sampling period are depicted in Figs. 2 and 3.

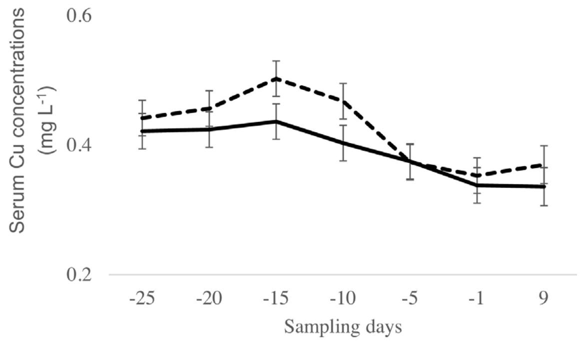

Fig. 2. Copper (Cu) serum concentration in non-lactating, over-conditioned, repeat breeder Holstein cows injected with a trace mineral complex (solid line) and in the control group (dotted line).

Pregnancy success in cattle has been related to the size of the preovulatory follicle (Machatkova et al., 2004) and corpus luteum (Nogueira et al., 2012). However, the growth of the preovulatory follicle and the corpus luteum were not affected ($P > 0.05$) by trace mineral supplementation in this study. Even though significant differences in pregnancy rate were not achieve between experimental groups, most likely due to small sample size, one can suggest that the numerically higher pregnancy rate obtained in obese cows injected with trace minerals could have resulted from affecting other variables not measured in this study, such as improvement in oocyte quality, embryo survival or both.

Copper and zinc serum concentrations of cows injected with trace minerals were deficient, as in the control group (Puls, 1988; Kincaid, 1999). It is likely that the injected dose of trace minerals was not enough to induce an increase in serum concentrations of copper and zinc. According to Gambling et al. (2008) and Tian and Diaz (2013), copper and zinc deficiencies disrupt preimplantation development leading to early embryo death. However, the pregnancy rate was numerically higher in cows injected with trace minerals than in the control group. One can speculate that these minerals were absorbed and stored in selected tissues such as liver in the case of copper (Balemi et al., 2010), and in fat tissue and the small intestines in the case of zinc, as reported by Kennedy and Failla (1987) in obese individuals, allowing them to have a source of these minerals to fulfill their reproductive task.

Trace mineral supplementation has been shown to improve fertility in cattle (Campbell et al., 1999; Griffiths et al., 2007), probably by reducing embryo death (Lamb et al., 2008). Sales et al. (2011) suggested that the increased pregnancy rate in recipient heifers may be due to an increase in embryo quality in donor heifers supplemented with trace minerals. However, to our knowledge there is no information regarding the influence of supplementing trace minerals on fertility in obese cattle. This is relevant, since micronutrient supplementation may be important for body weight regulation through normalizing insulin metabolism (Astrup and Bügel, 2010). Among the micronutrients, zinc concentrations are reduced in obese individuals (Marreiro et al., 2002), while cooper concentrations increase as body mass index increases (Song et al., 2007). Therefore, fertility could be boosted by strategic supplementation of trace minerals in obese cattle.

Low concentrations of zinc have been found in repeat breeder cows (Barui et al., 2015). The results of Marreiro et al. (2006) demonstrated that zinc supplementation reduces plasma insulin in obese women, probably by attenuating pancreatic secretion or by enhancing the peripheral response or insulin utilization (Chen et al., 1998). Interestingly, Adamiak et al. (2005) reported that cows with a moderate to high body condition have decreased fertility due to high concentrations of insulin. Insulin increases the concentrations of IGF-I (Mashek et al., 2001; Rhoads et al., 2004), which is detrimental to oocyte quality (Thomas et al., 2007) and embryo survival (Katagiri et al., 1996). On the other hand, oocyte competence and embryo development have been enhanced by insulin sensitizers in obese mice (Minge et al., 2008), suggesting that zinc supplementation may improve fertility indirectly in obese cattle by altering insulin availability. However, since insulin concentrations were not measured in this study, we cannot assume that

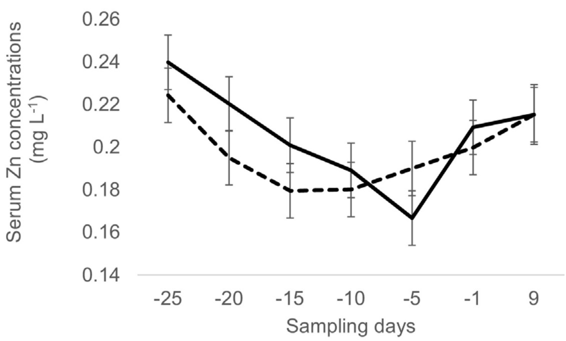

Fig. 3. Zinc (Zn) serum concentration in non-lactating, over-conditioned, repeat breeder Holstein cows injected with a trace mineral complex (solid line) and in the control group (dotted line).

the numerically increased pregnancy rate in obese cattle supplemented with trace minerals was accompanied by a reduction in blood insulin concentrations.

In conclusion, the injected trace mineral supplementation before synchronized estrus did not affect follicular or corpus luteum development, but it may be a feasible way to improve reproductive performance in over-conditioned cattle.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Adamiak, S.J., Mackie, K., Watt, R.G., Webb, R., Sinclair, K.D., 2005. Impact of nutrition on oocyte quality: cumulative effects of body composition and diet leading to hyperinsulinemia in cattle. Biol. Reprod. 73, 918–926.
Astrup, A., Bügel, S., 2010. Micronutrient deficiency in the etiology of obesity. Int. J. Obes. 34, 947–948.
Balemi, S.C., Grace, N.D., West, D.M., Smith, S.L., Knowles, S.O., 2010. Accumulation and depletion of liver copper stores in dairy cows challenged with a Cu-deficient diet and oral and injectable forms of Cu supplementation. N. Z. Vet. J. 58, 137–141.
Barui, A., Batabyal, S., Ghosh, S., Saha, D., Chattopadhyay, S., 2015. Plasma mineral profiles and hormonal activities of normal cycling and repeat breeding crossbred cows: a comparative study. Vet. World 8, 42–45.
Brewer, C.J., Balen, A.H., 2010. The adverse effects of obesity on conception and implantation. Reproduction 140, 347–364.
Burke, J.M., Hampton, J.H., Staples, C.R., Thatcher, W.W., 1998. Body condition influences maintenance of a persistent first wave dominant follicle in dairy cattle. Theriogenology 49, 751–760.
Campbell, M.H., Miller, J.K., Schrick, F.N., 1999. Effect of additional cobalt, copper, manganese, and zinc on reproduction and milk yield of lactating dairy cows receiving bovine somatotropin. J. Dairy Sci. 82, 1019–1025.
Canadian council on animal care in science, 2009. CCAC Guidelines On: the Care and Use of Farm Animals in Research, Teaching and Testing. Canadian Council on Animal Care, Ottawa, CA.
Chen, M.D., Liou, S.J., Lin, P.Y., Yang, V.C., Alexander, P.S., Lin, W.H., 1998. Effects of zinc supplementation on the plasma glucose level and insulin activity in genetically obese (ob/ob) mice. Biol. Trace Elem. Res. 61, 303–311.
Fick, R.K., McDowell, L.R., Miles, H.P., Wilkinson, S.N., Funk, J.D., Conrad, H.J., Valdivia, R., 1979. Methods of Mineral Analysis for Plant and Animal Tissues. University of Florida, Department of Animal Science. Gainesville, Florida, EE. UU. Universidad Mayor de San Marcos, Department of Animal Production and Food Inspection, Lima, Peru.
Gambling, L., Andersen, H.S., McArdle, H.J., 2008. Iron and copper and their interactions during development. Biochem. Soc. Trans. 36, 1258–1261.
García, E., 1988. Modificaciones del sistema de clasificación climática de Köppen. Instituto de Geografía, .Universidad Nacional Autónoma de México, México.
Griffiths, L.M., Loeffler, S.H., Socha, M.T., Tomlinson, D.J., Johnson, A.B., 2007. Effects of supplementing complexed zinc, manganese, copper and cobalt on lactation and reproductive performance of intensively grazed lactating dairy cattle on the South Island of New Zealand. Anim. Feed Sci. Technol. 137, 69–83.
Hidiroglou, M., 1979. Trace element deficiencies and fertility in ruminants: a review. J. Dairy Sci. 62, 1195–1206.
Ireland, J.J., Ward, F., Jimenez-Krassel, F., Ireland, J.L., Smith, G.W., Lonergan, P., Evans, A.C., 2007. Follicle numbers are highly repeatable within individual animals but are inversely correlated with FSH concentrations and the proportion of good-quality embryos after ovarian stimulation in cattle. Hum. Reprod. 22, 1687–1695.
Ireland, J.L., Scheetz, D., Jimenez-Krassel, F., Themmen, A.P., Ward, F., Lonergan, P., Smith, G.W., Perez, G.I., Evans, A.C., Ireland, J.J., 2008. Antral follicle count reliably predicts number of morphologically healthy oocytes and follicles in ovaries of young adult cattle. Biol. Reprod. 79, 1219–1225.
Jungheim, E.S., Moley, K.H., 2010. Current knowledge of obesity's effects in the pre- and periconceptional periods and avenues for future research. Am. J. Obstet. Gynecol. 203, 525–530.
Katagiri, S., Moon, Y.S., Yuen, B.H., 1996. The role for the uterine insulin like growth factor I in early embryonic loss after superovulation in the rat. Fertil. Steril. 65, 426–436.
Kennedy, M.L., Failla, M.L., 1987. Zinc metabolism in genetically obese (ob/ob) mice. J. Nutr. 117, 886–893.
Kincaid, R.L., 1999. Assessment of trace mineral status of ruminants: a review. Proc. Am. Soc. Anim. Sci. 77, 1–10.
Kubovicova, E., Makarevic, A.V., Hegeduesova, Z., Slezakova, M., Bezdicek, J., 2012. Effect of body condition score on oocyte yield and in vitro embryo development. Výzkum v Chovu Skotu 54.
Lamb, G.C., Brown, D.R., Larson, J.E., Dahlen, C.R., DiLorenzo, N., Arthington, J.D., DiCostanzo, A., 2008. Effect of organic or inorganic trace mineral supplementation on follicular response, ovulation, and embryo production in superovulated Angus heifers. Anim. Reprod. Sci. 106, 221–231.
Machatkova, M., Krausova, K., Jokesova, E., Tomanek, M., 2004. Developmental competence of bovine oocytes: effects of follicle size and the phase of follicular wave on *in vitro* embryo production. Theriogenology 61, 329–335.
Makarevich, A.V., Stádník, L., Kubovičová, E., Hegedüšová, Z., Holásek, R., Louda, F., Beran, J., Nejdlová, M., 2016. Quality of preimplantation embryos recovered in vivo from dairy cows in relation to their body condition. Zygote 24, 378–388.
Marreiro, D.N., Fisberg, M., Cozzolino, S.M., 2002. Zinc nutritional status in obese children and adolescents. Biol. Trace Elem. Res. 86, 107–122.
Marreiro, D.N., Geloneze, B., Tambascia, M.A., Lerário, A.C., Halpern, A., Cozzolino, S.M., 2006. Effect of zinc supplementation on serum leptin levels and insulin resistance of obese women. Biol. Trace Elem. Res. 112, 109–118.
Mashek, D.G., Ingvartsen, K.L., Andersen, J.B., Vestergaard, M., Larsen, T., 2001. Effects of a four-day hyperinsulinemic-euglycemic clamp in early and mid-lactation dairy cows on plasma concentrations of metabolites hormones, and binding proteins. Domest. Anim. Endocrinol. 21, 169–185.
Minge, C.E., Bennett, B.D., Norman, R.J., Robker, R.L., 2008. Peroxisome proliferator-activated receptor-gamma agonist rosiglitazone reverses the adverse effects of diet-induced obesity on oocyte quality. Endocrinology 149, 2646–2656.
Mossa, F., Walsh, S.W., Butler, S.T., Berry, D.P., Carter, F., Lonergan, P., Smith, G.W., Ireland, J.J., Evans, A.C., 2012. Low numbers of ovarian follicles ≥3 mm in diameter are associated with low fertility in dairy cows. J. Dairy Sci. 95, 2355–2361.
Nogueira, É., Saravi-Cardoso, G., Romero-Marques, H., Menezes-Dias, A., Vinhas-Ítavo, L.C., Corrêa-Borges, J., 2012. Effect of breed and corpus luteum on pregnancy rate of bovine embryo recipients. Rev. Bras. Zootec. 41, 2129–2133.
Pasquali, R., Pelusi, C., Genghini, S., Cacciari, M., Gambineri, A., 2003. Obesity and reproductive disorders in women. Hum. Reprod. Update 9, 359–372.
Puls, R., 1988. Mineral Levels in Animal Health: Diagnostic Data. Sherpa International, Canada.
Rhoads, R.P., Kim, J.W., Leury, B.J., Baumgard, L.H., Segoale, N., Frank, S.J., Bauman, D.E., Boisclair, Y.R., 2004. Insulin increases the abundance of the growth hormone receptor in liver and adipose tissue of periparturient dairy cows. J. Nutr. 134, 1020–1027.
Roche, J.R., Friggens, N.C., Kay, J.K., Fisher, M.W., Stafford, K.J., Berry, D.P., 2009. Invited review: body condition score and its association with dairy cow productivity, health, and welfare. J. Dairy Sci. 92, 5769–5801.
Sales, J.N.S., Pereira, R.V.V., Bicalho, R.C., Baruselli, P.S., 2011. Effect of injectable copper, selenium, zinc and manganese on the pregnancy rate of crossbred heifers

(*Bos indicus* X *Bos taurus*) synchronized for timed embryo transfer. Livest. Sci. 142, 59–62.

Song, C.H., Choi, W.S., Oh, H.J., Kim, K.S., 2007. Associations of serum minerals with body mass index in adult women. Eur. J. Clin. Nutr. 61, 682–685.

Stádník, L., Louda, F., Ježková, A., 2002. The effect of selected factors at insemination on reproduction of Holstein cows. Czech J. Anim. Sci. 47, 169–175.

Thomas, F.H., Campbell, B.K., Armstrong, D.G., Telfer, E.E., 2007. Effects of IGF-I bioavailability on bovine preantral follicular development *in vitro*. Reproduction 133, 1121–1128.

Tian, X., Diaz, F.J., 2013. Acute dietary zinc deficiency before conception compromises oocyte epigenetic programming and disrupts embryonic development. Dev. Biol. 376, 51–61.

Tungtrongchitr, R., Pongpaew, P., Phonrat, B., Tungtrongchitr, A., Viroonudomphol, D., Vudhivai, N., Schelp, F.P., 2003. Serum copper, zinc, ceruloplasmin and superoxide dismutase in Thai overweight and obese. J. Med. Assoc. Thai. 86, 543–551.

Velazquez, M.A., Hadeler, K.G., Herrmann, D., Kues, W.A., Ulbrich, S., Meyer, H.H.D., Remy, B., Beckers, J.F., Sauerwein, H., Niemann, H., 2011. In vivo oocyte developmental competence is reduced in lean but not in obese superovulated dairy cows after intraovarian administration of IGF1. Reproduction 142, 41–52.

Wildman, E.E., Jonesa, G.M., Wagner, P.E., Boman, R.L., Troutt Jr., H.F., Leschb, T.N., 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65, 495–501.