The Effects of Injectable Trace Mineral Supplements in Donor Cows at the Initiation of a Superovulation Protocol on Embryo Production and Pregnancy Rates in Recipient Females

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Objectives of this study were to determine the effects of an injectable trace mineral supplement (Multimin® 90) administered to donor beef cows at the initiation of a superovulation protocol on embryo production (i.e. quality and quantity) and pregnancy rate in recipient females. The injectable trace mineral increased the concentration of selenium in the liver; however, in this study no effects were observed on embryo production or pregnancy rates of recipients receiving those embryos.

Summary

We evaluated the effects of administering injectable trace mineral supplements at the initiation of a superovulation protocol on embryo outcomes (Exp 1) and pregnancy rates (Exp 2). In Exp 1, 35 crossbred Angus cows were randomly assigned to one of two treatments at initiation of a 16 d superovulation protocol; 1) cows received 90 mg Cu, 60 mg Mn, 30 mg Se, and 360 mg Zn as an injectable TM supplement (6 ml Multimin 90 s.q.; ITM); or 2) were untreated (CON). All donors were exposed to a common superovulation protocol, embryos were recovered 7 d after AI, graded for quality and developmental stage, and frozen until transfer. A replicated crossover design was used and each cow was flushed 4 times, with 120 d between flushes (total n = 70 for CON and 69 for ITM). In Exp 2, crossbred beef females were synchronized to receive embryos, and females with a CL 7 d after induced ovulation randomly assigned to receive an embryo originating from; 1) donors exposed to ITM (n =196); or 2) donors exposed to CON (n = 212). Embryo recovery data were analyzed using PROC MIXED of SAS for main effects of treatment, period, and their interaction, whereas the GLIMMIX procedure of SAS was used to analyze recipient pregnancy rates. In Exp 1 number of viable embryos recovered from cows responding to superovulation was similar ($P = 0.94$) among ITM (4.53 ± 0.63) and CON cows (4.46 ± 0.63). Moreover, number of degenerate, unfertilized, and total ova collected ($P \geq 0.67$), and number of embryos in respective developmental stages or quality grades ($P \geq 0.24$) were not affected by treatment. In Exp 2 pregnancy rates to embryo transfer were similar ($P = 0.52$) among CON (49.1%) and ITM (45.9 %) treatments. In this study, ITM administration failed to enhance embryo outcomes or pregnancy rates.

Introduction

Embryo transfer (ET) is an exceptional method of improving genetics in cattle herds. After superovulating and flushing, genetically superior calves can be gestated and raised by recipient females of different genetics. However, cost of ET can be prohibitive. Technologies and techniques that improve success rates and efficiencies in ET (i.e. reduce cost) have the potential to be widely adopted by producers who currently use ET, and also have the potential to expand the utilization of ET to a new audience of producers.

Proper nutritional status is paramount to optimal reproductive rate in cattle, and this concept extends to proper nutrition of embryo donor females (Lamb, 2010). Inclusive in recommendations for donor female nutrition are discussions about trace mineral status. In scenarios of artificial insemination (Mundell et al, 2012) and timed embryo transfer of recipient females (Sales et al., 2011) administration of injectable trace mineral supplements improved overall pregnancy rates. Though free choice trace mineral source did not impact embryo production in donor females (Lamb et al., 2008), a small preliminary study by Boas et al. (2017), showed that donors treated with ITM had a tendency (P = 0.15) to have fewer unfertilized oocytes per collection. Though promising, these preliminary results need to be tested on a larger scale.

Therefore, the objectives of this study were to evaluate the impact of administering injectable trace mineral supplements in embryo transfer programs on:

1) Number of embryos recovered, embryo quality, and developmental stage

2) Pregnancy rates of recipients receiving embryos originating from donors either receiving or not receiving injectable trace mineral supplements

Experimental Procedures

All procedures for this experiment were approved by the North Dakota State University Animal Care and Use Committee.

In Exp 1, 35 multiparous crossbred Angus cows (BW = 1274 ± 20 lbs, BCS 5.75 ± 0.11) were selected from Central Grassland Research and Extension Center (CGREC), Streeter, ND to serve as embryo donors. Donors were randomly assigned to one of two treatments at initiation of a 16-day superovulation protocol; 1) cows received 6 ml Multimin®90 s.c.; ITM; or 2) were untreated (CON).

All females were exposed to a common superovulation protocol and a single sire was used for all inseminations. Cows were kept in pens at CGREC and were fed to meet NRC requirements. In addition, all donors were offered 4 ounces/head daily of Purina® Wind and Rain® Storm® 7.5 CP Avalia® 4 Original XPC Altosid®. Liver biopsies were collected at the beginning of the superovulation protocol and at embryo recovery. Embryos were recovered via uterine flush and graded prior to being frozen and stored in liquid nitrogen.

In Exp 2, 380 crossbred Angus cows (BCS = 6.03 ± 0.06, DPP = 85.6 ± 0.94 days) and 117 crossbred Angus heifers (BCS = 5.74 ± 0.03) were selected from the CGREC herd to serve as embryo recipients. Recipients were randomly assigned to receive an embryo originating from 1) donors exposed to ITM (n = 196); or 2) donors exposed to CON (n = 212).

Females had equal access to native range pastures and free choice access to Purina® Wind and Rain® Storm® 7.5 CP Avalia® 4 Original XPC Altosid® in mineral feeders located near waterers in the pastures for at least 30 days before transfer protocol started. Females were synchronized for fixed-time embryo transfer (FTET) using a 7-day Co-Synch + CIDR estrus synchronization protocol. Seven days after the final working event of synchronization females were presented for ovarian evaluation and those females having a corpus luteum received an embryo transferred into the uterine horn on the side of the CL. Liver (n = 21) and blood (n = 336) samples were collected from a subsample of cows at embryo transfer. Pregnancy rate to embryo transfer was determined by transrectal ultrasonography 36 days after transfer in cow recipients and 48 days after transfer in heifer recipients.

Results and Discussion

Administering ITM increased ($P < 0.0001$) concentrations of Se in the liver compared with CON cows (Table 1), but did not impact ($P \geq 0.17$) concentrations of Cu or Mn. In contrast, change in concentrations of Zn was greater ($P = 0.02$) in CON cows compared with ITM cows. Pogge et al. (2012) observed increases in Se, Mn, and Zn after ITM administration, and perhaps some of the disparity among reports is related to shipment of our donor females from CGREC to Fargo for embryo collection.

There was no treatment effect ($P = 0.96$) on the number of viable embryos produced per cow when respective analysis compared all cows in the experiment (Table 2), or when comparing only those cows responding to superovulation (CON = 4.46 ± 0.63 and ITM = 4.53 ± 0.63). In addition, treatment with ITM did not influence the number of degenerate ($P = 0.75$) or unfertilized ($P = 0.54$) embryos. Moreover, number of embryos in each respective grade and developmental stage were similar ($P \geq 0.19$) among treatments (Table 2). It is noteworthy that donors were offered minerals to meet NRC requirements and were not in a deficient status. Thus, an increase in TM status given trough ITM seemed to not influence superovulatory response and embryo development. Similarly, Lamb et al. (2008) observed that free choice mineral source (either organic or inorganic) did not impact embryo production in donor females. A recent report with limited numbers; however, showed that 9 dairy heifers given ITM at the beginning of superovulation tended (P = 0.15) to have fewer unfertilized embryos compared with the 9 control heifers (Boas et al., 2017).

Literature has shown conflicting results regarding the effects of ITM supplementation in reproductive processes. Though several reports have shown enhanced pregnancy rates when ITM was administered to cows receiving AI or to females receiving embryos (Sales et al., 2011; Mundell at al., 2012; Brasche et al.,

Table 1. Change in liver trace mineral concentration at day 16 in cows receiving control (CON) or injectable trace mineral (ITM) at d 0 of superovulation.

		Period			
	Treatment	**d 0**	**d 16**	**Change[1]**	***P* – value**
		mean ± SE , ug/g DM			
Selenium	CON	2.72 ± 0.20	2.77 ± 0.17	0.04 ± 0.15^{a}	< 0.0001
	ITM	2.79 ± 0.18	3.89 ± 0.30	1.09 ± 0.15^{b}	
Copper	CON	193.86 ± 18.84	187.62 ± 17.44	- 6.2 ± 18.9^{a}	0.17
	ITM	187.55 ± 17.15	224.66 ± 24.69	37.1 ± 18.9^{a}	
Manganese	CON	12.03 ± 0.40	12.03 ± 0.77	0.002 ± 0.71^{a}	0.97
	ITM	12.53 ± 0.49	12.43 ± 0.34	-0.10 ± 0.71^{a}	
Zinc	CON	109.31 ± 3.92	139.59 ± 7.66	30.27 ± 7.4^{a}	0.02
	ITM	127.99 ± 9.37	132.65 ± 6.07	4.66 ± 7.4^{b}	

[1] Change reflects the increase or decrease in mineral concentration from d 0 to d 16.

Table 2. Embryo production in all treated donor cows receiving control (CON) or injectable trace mineral (ITM) at d 0 of superovulation.

	Treatment		***P* - value**
	Control (CON)	**Multimin (ITM)**	
No. cows	70	69	
	(mean ± SE)		
Viable embryos, no.	3.37± 0.52	3.39 ± 0.52	0.96
Degenerate embryos, no.	0.75 ± 0.22	0.65 ± 0.22	0.75
Unfertilized oocytes, no.	2.60 ± 0.60	3.07 ± 0.60	0.54
Total ova/embryo, no.	6.79 ± 1.13	7.04 ± 1.13	0.80
Grade 1	3.12 ± 0.50	3.10 ± 0.50	0.96
Grade 2	0.11 ± 0.04	0.11 ± 0.04	0.97
Grade 3	0.12 ± 0.07	0.17 ± 0.07	0.67
Morula[1]	1.92 ± 0.32	1.66 ± 0.32	0.52
Compact morula[2]	1.20 ± 0.26	1.15 ± 0.26	0.91
Blastocyst[3]	0.18 ± 0.14	0.31 ± 0.14	0.50
Expanded blastocyst[4]	0.01 ± 0.07	0.15 ± 0.07	0.19

[1] Stage 4, [2] Stage 5, [3] Stage 6, [4] Stage 7.

Table 3. Pregnancy rates in beef females after transfer of embryos from dams receiving control (CON) or injectable trace mineral (ITM) treatments at the initiation of superovulation protocol.

	Treatment		
	CON	ITM	*P* - value
	--------% (no. of no.)--------		
Heifer recipients	43.4 (23 of 53)	49.01 (25 of 51)	0.56
Cow recipients	50.9 (81 of 159)	44.8 (65 of 145)	0.28
All recipients	49.1 (104 of 212)	45.9 (90 of 196)	0.52

2014; Kirchhoff, 2015; Stokes et al., 2017), pregnancy rates in Exp 2 were similar ($P = 0.52$) among recipient females that received embryos from donors in CON (49.1 %) and ITM (45.9 %) treatments, respectively (Table 3). A major difference between the current report and previous research was that recipients in our study did not receive ITM directly. We were essentially testing whether there was a lasting effect of ITM before superovulation which could enhance pregnancy rates, not whether administration of ITM directly to a recipient would improve pregnancy outcomes. We failed to detect an improvement in pregnancy outcomes due to the enhanced TM status from ITM that occurred before and during the first 7 days of embryo development.

In summary, Multimin® 90 increased concentrations of selenium in the liver; however, in this experiment no effects were observed on embryo production or pregnancy rates of recipients receiving those embryos.

Literature Cited

Boas, K., P. Gunn, S. Hansen, T. Dohlman, M. Jahnke. 2017. Effects of injectable trace mineral supplementation on embryo development and quality in superovulated dairy heifers–first year progress report. Animal Industry Report: AS 663, ASL R3144. https://lib.dr.iastate.edu/ans_air/vol663/iss1/17.

Brasche, C. J., J. B. Hall, M. E. Drewnoski. 2014. Effect of an injectable trace mineral on pregnancy rate of virgin heifers when synchronized using the 5 day Co-Synch plus CIDR or 14 day CIDR-PG protocol. J. Anim. Sci. 92(Suppl. 2):462 (Abstr.).

Kirchhoff, A. A. 2015. The effect of a supplemental trace mineral injection on developing beef bull and heifer reproduction. Master Thesis. Kansas State Univ., Manhattan.

Lamb, C. 2010. Donor and recipient factors affecting an embryo transfer program. North Central Research and Outreach Center, University of Minnesota, Grand Rapids. 1–11.

Lamb, G. C., D. R. Brown, J. E. Larson, C. R. Dahlen, N. DiLorenzo, J. D. Arthington, A. DiCostanzo.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.

Mundell, L. R., J. R. Jaeger, J. W. Waggoner, J. S. Stevenson, D. M. Grieger, L. A. Pacheco, J. W. Bolte, N. A. Aubel, G. J. Eckerle, M. J. Macek, S. M. Ensley, L. J. Havenga, K. C. Olson. 2012. Effects of prepartum and postpartum bolus injections of trace minerals on performance of beef cows and calves grazing native range. Prof. Anim. Sci. 28:82–88.

Pogge, D. J., E. L. Richter, M. E. Drewnoski, S. L. Hansen. 2012. Mineral concentrations of plasma and liver after injection with a trace mineral complex differ among Angus and Simmental cattle. J. Anim. Sci. 90:2692–2698.

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 Bos taurus) synchronized for timed embryo transfer. Livestock Sci. 142:59–62.

Stokes, R. S., A. R. Ralph, A. J. Mickna, W. P. Chapple, A. R. Schroeder, F. A. Ireland, D. W. Shike. 2017. Effect of an injectable trace mineral at the initiation of a 14 day CIDR protocol on heifer performance and reproduction. Translational Animal Science. 4:458-466.