Effects of Injectable Trace Mineral Supplementation on Embryo Development and Quality in Superovulated Dairy Heifers – First Year Progress Report

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Summary and Implications

Injectable supplementation of trace minerals is often used to combat the malabsorption of microminerals due to antagonists in feed and water. Previous research has shown that Multimin90, a commercial supplement containing copper, zinc, manganese, and selenium, has been shown to have a positive impact on reproductive efficiency and pregnancy rates in beef cattle. The objective of this study was to determine the effects of supplementation of MultiMin90 on embryo quality in superovulated heifers. This report includes the preliminary results of the first year of an ongoing study. Though there was no difference in overall embryo quality, supplementation of MultiMin90 tended to reduce the proportion of nonfertilized embryos per flush and resulted in one more embryo per flush when compared to a saline-treated control.

Introduction

MultiMin90 is an injectable trace mineral supplement that contains 15 mg copper/mL, 60 mg zinc/mL, 10 mg manganese/mL, and 5 mg selenium/mL. Recently, this supplement has been shown to have a positive impact on reproductive efficiency and pregnancy rates in beef cattle.

In cattle, a deficiency of trace minerals can result in irregular or suppressed estrus cycles, reduced pregnancy rates, and difficulties during parturition. Moreover, as a combined injection, these minerals have been shown to increase conception rates and provide benefit to the immune system. Improved pregnancy rates could be accomplished through a number of avenues, including reduced embryonic loss, altered hormone production or clearance, decreased pregnancy losses, or improved embryo quality, though the exact pathway is still unclear.

The objective of this study was to determine how the supplementation of this product impacts embryo quality. This was accomplished by using superovulation and embryo flushing to study embryo development and quality. It was hypothesized that MultiMin90 would improve quality of embryos recovered from ovarian superstimulated donor heifers seven days post-artificial insemination (AI).

Materials and Methods

Eighteen non-pregnant, yearling dairy heifers from the Iowa State University Dairy Farm were used for this study. The heifers received a common diet that met or exceeded NRC requirements. Each heifer was randomly assigned to receive an injection of saline (CON) or MultiMin90 (MM) per label dose (1 mL/100 pounds of body weight), with a total nine receiving treatment and nine as controls.

Heifers were treated and superovulated in pairs, for a total of nine reps. On day 0 of each rep, heifers were given a Controlled Internal Drug Release (CIDR) device and concurrently administered either their CON or MM treatment. This was paired with an industry standard, 17-day superstimulation protocol with GnRH, PGF2α, and decreasing doses of FSH administered twice daily (Figure 1). Heifers were artificially inseminated on the evening of day 10 and morning of day 11 approximately 12 hours apart. An additional dose of GnRH was given concurrently with the first AI.

Embryos were recovered on day 17 (7 days after artificial insemination) via non-surgical uterine flush. Embryos were evaluated according to International Embryo Transfer Society standards. Each embryo was given a developmental stage (Table 1) and quality grade (Table 2).

Data were analyzed using the MIXED procedures of SAS with animal as the experimental unit. P-values ≤ 0.05 were considered to be significant; whereas $0.06 < P \leq 0.15$ were considered a tendency. One heifer of the CON treatment did not respond to the superstimulation protocol and was therefore excluded from the dataset.

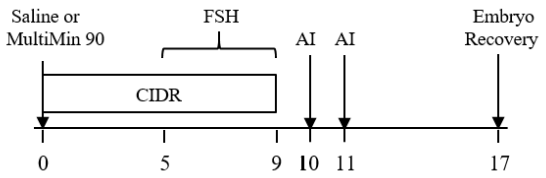

Figure 1. Standard superstimulation protocol used.

Results and Discussion

There was no effect of the treatment on total number of embryos, number of transferrable embryos, or number of freezable embryos ($P \geq 0.21$; Table 3). On average, the proportion of nonfertilized embryos per flush tended to be greater in CON heifers than in MM heifers ($P = 0.15$; Table 4). MM heifers tended to have an increase in the number of Quality 2 ($P = 0.08$) and Quality 3 ($P = 0.15$) embryos compared to the CON heifers (Table 4). Though MM did not impact embryo quality, these data suggest that MM does increase the average proportion of viable embryos per flush due to the lower average proportion of nonfertilized embryos.

MM tended to increase the number of Stage 4 embryos ($P = 0.09$; Table 5). Moreover, on average, the proportion of Stage 4 ($P = 0.11$) and Stage 5 ($P = 0.11$) embryos per flush tended to be greater in the MM heifers. It might be suggested that such trends indicate a more synchronous ovulation and embryo development pattern.

In conclusion, these preliminary data indicate that while MM treatment does decrease the average proportion of nonfertilized embryos, there is not a significant trend towards increased embryo quality overall. Although not statistically significant, the numerical increase in transferrable embryos likely has economic benefit for the producer. Additional replications of this work are planned for the coming year.

Table 1. International Embryo Transfer Society standards for embryo staging**.**

Stage	Characteristics
1	Unfertilized
2	2—12 cell mass
3	Early morula
4	Morula
5	Early blastocyst
6	Blastocyst
7	Expanded blastocyst
8	Hatched blastocyst
9	Expanded hatched blastocyst

Table 2. International Embryo Transfer Society standards for embryo quality grade.

Quality Grade	Characteristics
1: Excellent or Good	Uniform, spherical in appearance. Minor irregularities in structure. At least 85% of cell mass intact and viable.
2: Fair	Moderate irregularities in structure. At least 50% of cell mass intact and viable.
3: Poor	Many irregularities in structure. At least 25% of cell mass intact and viable.
4: Dead or Degenerating	Oocytes, 1 cell embryos, or degenerating embryos.

Table 3. Effects of an injectable trace mineral supplement at initiation of superovulation protocol on embryo production in yearling dairy heifers.

Total Embryos	Con[1]	MM[2]	SEM[3]	*P*-value
Total Embryos, no.	4.78	6.00	1.28	0.51
Transferrable Embryos, no.	2.44	4.13	0.88	0.21
Freezable Embryos, no.	2.44	3.88	0.92	0.30

[1]Control (Saline)

[2] Multimin90 containing 60 mg/mL Zn, 10 mg/mL Mn, 5 mg/mL Se, and 15 mg/mL Cu

[3] n = 9 for MM; n = 8 for CON

Table 4. Effects of an injectable trace mineral supplement at initiation of superovulation protocol on embryo quality in yearling dairy heifers.

Embryo Quality	CON[1]	MM[2]	SEM[3]	*P*-value
Quality 1, no.	2.11	3.38	0.85	0.32
Average Proportion of Flush[4], %	38.66	56.98	12.56	0.30
Average Proportion of Transferrable[5], %	89.29	76.96	7.64	0.31
Average Proportion of Freezable[6], %	88.48	78.70	7.02	0.23
Quality 2, no.	0.24	0.74	0.23	0.08
Average Proportion of Flush[4], %	2.87	8.76	2.92	0.17
Average Proportion of Transferrable[5], %	11.52	21.30	7.02	0.23
Average Proportion of Freezable[6], %	11.52	21.30	7.02	0.23
Quality 3, no.	0.00	0.25	0.11	0.15
Average Proportion of Flush[4], %	0.00	8.75	4.37	0.20
Average Proportion of Transferrable[5], %	0.00	10.42	6.17	0.29
Quality 4, no.	2.22	1.88	0.92	0.79
Degenerate, no.	0.67	1.25	0.44	0.37
Average Proportion of Flush[4], %	17.12	16.66	9.75	0.97
Nonfertilized, no.	1.56	0.63	0.65	0.34
Average Proportion of Flush, %	29.17	6.97	9.65	0.15
Average Proportion of Flush[4], %	46.30	23.63	12.18	0.22

[1] Control (Saline)

[2] Multimin90 containing 60 mg/mL Zn, 10 mg/mL Mn, 5 mg/mL Se, and 15 mg/mL Cu

[3] n = 9 for MM; n = 8 for CON

[4] of total structures collected, the number of embryos in a given stage

[5] of total embryos graded Quality 1-3, proportion that were of given stage

[6] of total embryos graded Quality 1-2, proportion that were of given stage

Table 5. Effects of an injectable trace mineral supplement at initiation of superovulation protocol on embryo development in yearling dairy heifers.

Developmental Stage	CON[1]	MM[2]	SEM[3]	*P*-value
Stage 3, no.	0.00	0.13	0.09	0.29
Average Proportion of Flush[4], %	-0.10	2.54	1.73	0.29
Stage 4, no.	0.55	1.76	0.45	0.09
Average Proportion of Flush[4], %	11.57	34.87	8.88	0.11
Average Proportion of Transferrable[5], %	43.73	44.05	17.04	0.99
Average Proportion of Freezable[6], %	44.05	46.13	16.97	0.93
Stage 5, no.	0.56	1.13	0.33	0.26
Average Proportion of Flush[4], %	6.48	17.97	4.50	0.11
Average Proportion of Transferrable[5], %	13.67	24.05	6.39	0.31
Average Proportion of Freezable[6], %	12.95	27.77	6.95	0.18
Stage 6, no.	0.78	0.63	0.44	0.81
Average Proportion of Flush[4], %	13.43	6.61	6.39	0.47
Average Proportion of Transferrable[5], %	24.40	10.36	8.82	0.31
Average Proportion of Freezable[6], %	24.40	10.36	8.82	0.31
Stage 7, no.	0.78	0.63	0.44	0.81
Average Proportion of Flush[4], %	9.72	1.92	4.69	0.27
Average Proportion of Transferrable[5], %	15.93	4.80	6.98	0.30
Average Proportion of Freezable[6], %	15.93	4.80	6.98	0.30
Stage 8, no.	0.11	-0.01	0.09	0.34
Average Proportion of Flush[4], %	1.41	-0.09	1.09	0.34
Average Proportion of Transferrable[5], %	2.39	-0.04	1.54	0.31
Average Proportion of Freezable[6], %	2.39	-0.04	1.54	0.31

[1] Control (Saline)

[2] Multimin90 containing 60 mg/mL Zn, 10 mg/mL Mn, 5 mg/mL Se, and 15 mg/mL Cu

[3] n = 9 for MM; n = 8 for CON

[4] of total structures collected, the number of embryos in a given stage

[5] of total embryos graded Quality 1-3, proportion that were of given stage

[6] of total embryos graded Quality 1-2, proportion that were of given stage