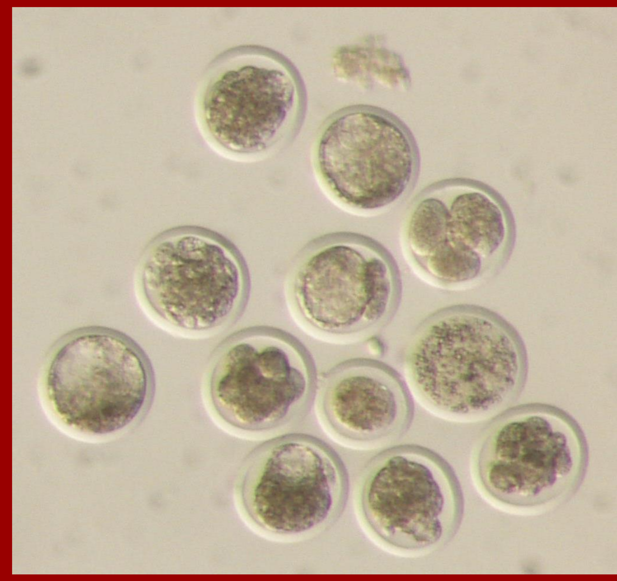
Effects of injectable trace mineral supplementation on embryo development and quality in superovulated dairy heifers



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ABSTRACT

Supplementation of injectable trace minerals is often used to combat the malabsorption of microminerals due to antagonists in feed and water. One such supplement, Multimin90™, containing copper, zinc, manganese, and selenium, has recently been shown to have a positive impact on reproductive efficiency and pregnancy rates. The objective of this study was to determine how the supplementation of MultiMin90™ impacts embryo quality. We hypothesized that MultiMin90™ would improve embryo quality, as determined by the assessment of embryos recovered from ovarian superstimulated donor heifers seven days post-artificial insemination. Seventeen dairy heifers from the Iowa State University Dairy Teaching Farm were randomly assigned to receive a saline control (CON) or Multimin90™ (MM), given at label dose of 1 mL/100 lbs of body weight. All heifers were then put on an industry standard ovarian superovulation protocol, with embryos recovered on day 7 after artificial insemination. Embryos were graded for quality and developmental stage, and data were analyzed using SAS. On average, the proportion of nonfertilized embryos tended to be greater in CON heifers (P = 0.15), and the average proportion of Stage 4 (P = 0.11) and Stage 5 (P = 0.11) embryos tended to be greater in MM heifers. No differences were noted in total embryos recovered as a result of total treatment (P = 0.51). This concludes that our hypothesis was partially correct. Though MM did decrease the overall average proportion of nonfertilized embryos, there was not a significant trend towards increased embryo quality across the board. However, the results do point towards a biologically relevant trend towards fewer total nonfertilized embryos and more total transferrable embryos produced when treated with MM..

INTRODUCTION

- MultiMin90™ is an injectable trace mineral supplement that contains four minerals: copper, zinc, manganese, and selenium. It is routinely used with the purpose of balancing critical nutrients in a cow's diet ("MultiMin90: How Does It Work?", 2015). Recently, this supplement has been shown to have a positive impact on reproductive efficiency and pregnancy rates in beef cattle (Mundell et al., 2012).
- Dietary deficiency of minerals contained in MultiMin90™ can result in irregular or suppressed estrus cycles, trouble producing a live calf, and difficulties during and after calving ("Minerals," 1996). Moreover, as a combined injection, these minerals have been shown to increase conception rates and help the immune system ("Building Your Beef Herd," 2015). Improved pregnancy rates could be accomplished through a number of avenues, including reduced embryonic loss, hormone production, less loss of pregnancy, or embryo quality; though the exact pathway is still unclear.
- This study focused on the effects of MultiMin90™ on embryo quality through a superovulation and embryo flushing protocol.

OBJECTIVE & HYPOTHESIS

The objective of this study was to determine if supplementation of an injectable trace mineral product would improve embryo quality. We hypothesized that MultiMin90™ would improve embryo quality, as determined by the assessment of embryos recovered from ovarian superstimulated dairy heifers seven days post-artificial insemination.

Table 1. Standard superstimulation protocol used.

Day	Time	Administrations
0	am	CIDR™ insertion + 2.5 cc PGF2α + 7.0 cc Saline or Multimin
4	am	3.0 cc GnRH
5	pm	2.0 cc FSH
6	am	2.0 cc FSH
6	pm	1.5 cc FSH
7	am	1.5 cc FSH
7	pm	1.5 cc FSH
8	am	1.5 cc FSH
8	pm	1.0 cc FSH + 2.5 cc PGF2α
9	am	1.0 cc FSH + 2.5 cc PGF2α + CIDR™ removal
10	pm	AI + 3.0 cc GnRH
11	am	AI
17	am	Embryo recovery

Table 3. The 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 5. 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 2. The 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 4. 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 6. 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

MATERIALS AND METHODS

- The superovulation protocol was as diagrammed:

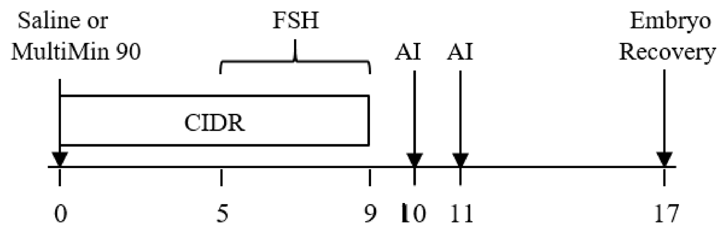

Figure 1. Standard superstimulation protocol diagram.

- 18 heifers of similar age and body weight from the Iowa State University Dairy Teaching Farm were used for this study from March 2016 to October 2016.
- Heifers were randomly assigned to receive a 7.0 mL injection of the saline (CON) or MultiMin90™ (MM) treatment per label dose (1 mL/100 lbs of body weight), followed by an industry standard superstimulation protocol (Figure 1, Table 1).
- Embryos were recovered on d 17 (7 d after AI). All recovered embryos were collected via non-surgical uterine flush and were evaluated according to International Embryo Transfer Society standards. Each embryo was given a developmental stage (Table 2) and quality grade (Table 3).
- 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 0.05 < P ≤ 0.15 were considered a tendency.

RESULTS AND DISCUSSION

- There was no effect of treatment on total number of embryos, number of transferrable embryos, or number of freezable embryos (P ≥ 0.14; Table 4).
- MM heifers had no differences in the number of Quality Grade 1 embryos (P = 0.32), but did have greater numbers of Quality Grade 2 (P = 0.08) and Quality Grade 3 (P = 0.15) embryos compared to CON (Table 5).
- On average, the proportion of nonfertilized embryos tended to be greater in CON heifers than MM heifers (P = 0.15; Table 2).
- MM heifers tended to have an increased number of Stage 4 embryos (P = 0.09; Table 6). On average, the proportion of Stage 4 (P = 0.11) and Stage 5 (P = 0.11) embryos tended to be greater in MM. This could indicate a synchronous ovulation pattern and embryo development pattern.

CONCLUSIONS

- Our hypothesis was partially correct. Though MM treatment did decrease the average proportion of nonfertilized embryos, there is not a significant trend towards increased embryo quality overall. However, embryos were more uniform in their development.
- Although not statistically significant, the numerical increase in transferrable embryos likely has major economic benefit at the producer level.

FUTURE IMPLICATIONS

- More heifers need to be added to the dataset to strengthen the conclusions and to better understand the impact of MM treatment on embryos.
- Once the impact of MM on embryos is understood, further research should study which of the four micronutrients in MultiMin90™ is the cause of this impact.
- Future research should also study the timing of the injections to determine if there is an appropriate time frame for treatment with MM in a superovulation program.