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MGA and Growth Promotants Administered to Beef Feedlot Heifers Have No Effect on Subsequent Oocyte Quality or *in vitro* Embryo Production

N. Miller, D. Grieger, and K. Fike

Introduction

Beef feedlot heifers have the potential to serve as viable donors of oocytes post-slaughter for *in vitro* embryo production. Oocyte quality is a critical factor affecting the success of *in vitro* embryo production and can be influenced by factors such as age and reproductive status, ovarian follicle size, and nutritional status of the donor female. In a conventional feedlot setting, heifers are typically administered steroid-based growth promotants and fed melengestrol acetate (MGA) for suppression of estrus, which increases circulating concentrations of reproductive steroids, particularly estradiol. The effects of these management practices on oocyte quality and numbers are unknown. The purpose of this study was to compare oocytes harvested from traditionally managed beef feedlot heifers implanted with growth promotants and fed MGA with oocytes from heifers given neither MGA nor growth promotants, and to evaluate potential effects of these feedlot management practices on early embryo development.

Experimental Procedures

Beef heifers ($n = 172$) were fed a finishing diet at the Kansas State University Beef Research Center feedlot for 120 days. Heifers were divided into 2 treatments: (1) conventionally managed heifers (MGA-Implant) were fed MGA (0.5 mg/head/day) for 120 days and implanted with a single growth promotant 120 days prior to harvest (Revalor IH; 80 mg trenbolone acetate and 8 mg estradiol; Merck Animal Health, Summit, NJ); and (2) control heifers did not receive either MGA or growth promotants during the finishing period.

Heifers were harvested and ovaries were collected within 30 minutes of harvest, then grouped within treatment based on time from harvest to ovary collection. Oocytes were aspirated from collected ovaries using a vacuum pump system and maintained in groups based on time of heifer harvest. All media were provided by Sexing Technologies, Inc. (Navasota, TX). Oocytes were washed twice in TL-Hepes media then evaluated. Oocytes that were denuded, had discolored cytoplasm, or were starting to degenerate were recorded and subsequently removed from the study. Remaining oocytes were placed in M199 holding media until all oocytes were collected. Four to five hours post-slaughter, all oocytes were placed in maturation media and shipped overnight to begin the *in vitro* fertilization (IVF) process at Sexing Technologies, Inc. laboratory facilities.

After 23.5 hours in maturation media, oocytes were placed in fertilization media with semen from a Holstein bull with proven IVF quality (per Sexing Technologies, Inc.) at 1.0×10^6 sperm/mL. After 18 hours in fertilization media, the presumptive zygotes were washed twice in TL-Hepes media. All cumulus cells were then removed from the

zygotes. Any zygotes with cracked zona pellucidas, shrinking cytoplasm, or lacking clear polar bodies were recorded and subsequently removed from the study. Remaining zygotes were placed in culture media for 7 days.

On day 2 post-IVF, developmental stages were assessed, including the number that achieved 8-cell, 2- to 4-cell, and 1-cell stages of early embryo development. Any cells that had not divided by day 2 were removed from the study. On day 7, embryo grades were assigned. Morula, early blastocyst, and blastocyst stages of development were classified as C2 embryos but considered to be of insufficient quality for freezing; C1⁻ embryos were those that were blastocyst or expanded blastocyst stages and freezable; and C1 embryos were those with a very compact inner cell mass and were beginning to hatch and were freezable.

Results and Discussion

A total of 1,820 oocytes were harvested from 152 ovaries in the MGA-Implant group. The control group yielded 1,272 oocytes from 145 ovaries. A tendency for a time × treatment interaction was observed for the number of oocytes per ovary ($P = 0.07$). Fertilization rate (zygotes produced per the number of oocytes with opportunity to be fertilized) was similar for both treatments (MGA-Implant: 79.9%, Control: 82.3%; Figure 1), indicating no effect of treatment on the ability of oocytes to be fertilized. A similar percentage of zygotes (successfully fertilized oocytes) per ovary cleaved by day 2 post-IVF (MGA-Implant: 46.8%, Control: 47.9%; Figure 1). Cleavage rates were determined by evaluating the total number of zygotes that had achieved the 2- to 8-cell stage of early embryo development by day 2 post-IVF per number of zygotes produced and are indicative of early embryonic growth. A similar number of embryos per ovary were produced for both the MGA-Implant and control groups (Figure 2). Freezable embryos (C1 and C1⁻) produced were also similar for both treatments (Figure 2). Across both groups, the majority of embryos produced were assigned a grade of C2, which are those embryos that are of sufficient quality for fresh transfer, but not freezable.

Beef feedlot heifers fed MGA and administered steroid-based growth promotants can serve as a viable source of oocytes for *in vitro* embryo production. Feeding MGA and administering growth promotants to heifers does not affect oocyte fertilization rate, early embryo development, or number of *in vitro* embryos produced compared with heifers not fed MGA or administered growth promotants.

Implications

Administration of MGA and growth promotants to feedlot heifers has no subsequent effects on number of oocytes harvested or *in vitro* embryos produced. Beef feedlot heifers have the potential to serve as a source of viable oocytes for large-scale *in vitro* embryo production.

REPRODUCTION

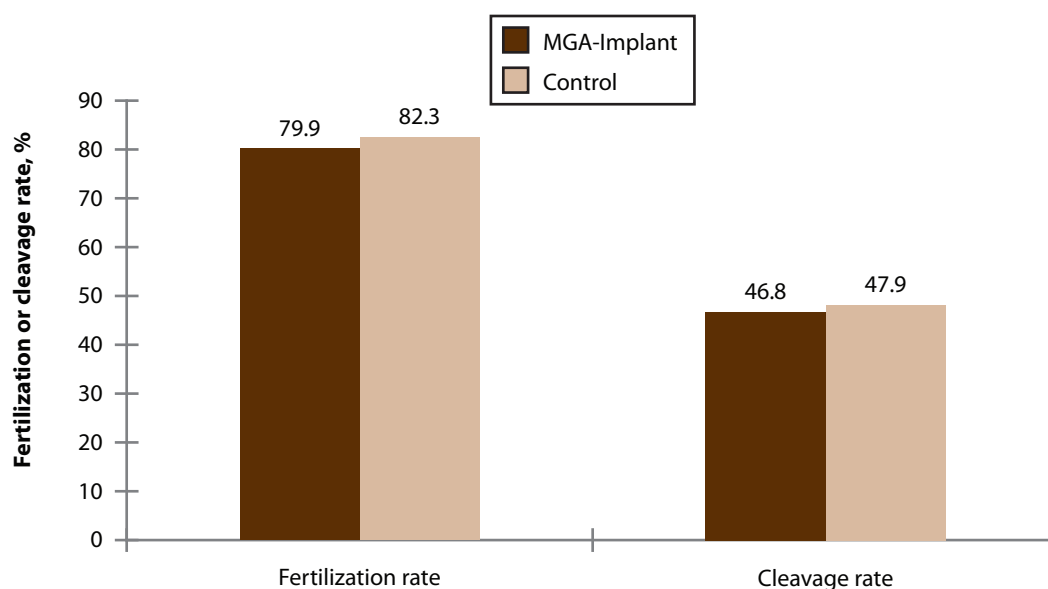


Figure 1. Fertilization (zygotes produced per oocytes with opportunity to be fertilized) and cleavage rates (2- to 8-cell embryos produced per number of zygotes) of oocytes and zygotes, respectively. Oocytes were harvested from beef feedlot heifers fed melengestrol acetate (MGA) and implanted with growth promotants (MGA-Implant) or untreated (Control) and subjected to *in vitro* fertilization.

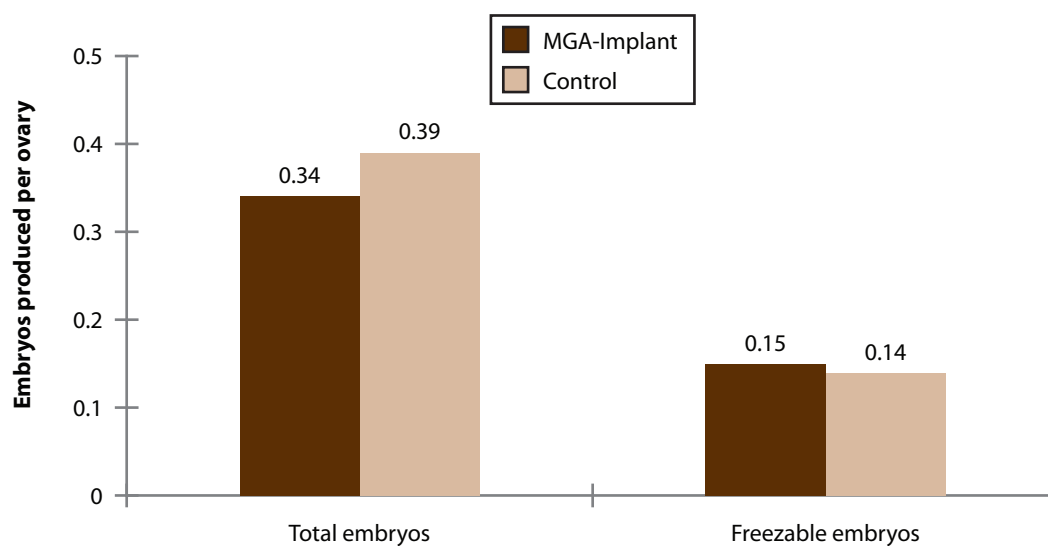


Figure 2. Total and freezable embryos produced per ovary from *in vitro* fertilization of oocytes harvested from beef feedlot heifers fed melengestrol acetate (MGA) and implanted with growth promotants (MGA-Implant) or untreated (Control).