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Summary

Cows were treated with two progestin concentrations to develop ovulatory follicles exposed to different hormone environments. Cows were assigned to Control Group receiving a CIDR for 7 days (4–6 ng/ml of progestin), or to MGA-14 Group receiving 5mg/head/day of MGA for 14 days (< 1 ng of progestin). Our hypothesis was that the MGA-14 treatment would develop larger, persistent follicles with less granulosa cells per follicle volume and may have altered gene expression profiles in oocytes and granulosa cells. Cows in the MGA-14 treatment had larger follicles and less granulosa cells per volume than controls, suggesting that their development mimicked persistent follicles and may be of poorer quality.

Introduction

Melengestrosterol acetate (MGA) is commonly used in the beef industry to suppress estrus in feedlot heifers or to synchronize estrus to increase reproductive efficiency. The estrus directly after MGA treatment withdrawal has been demonstrated to have reduced conception rates, potentially, through the development of a persistent follicle. A persistent follicle is a follicle that remains dominant on the ovary for an extended time period but will regress on its own. If the MGA treatment produces a persistent follicle, the oocyte within that follicle may be compromised, incapable of fertilization, or unable to develop a viable embryo.

Currently, we do not have any genetic markers of oocyte quality nor do we understand what makes a “good” oocyte versus a “compromised” oocyte. We also do not understand how the granulosa cells surrounding the oocyte may aid in oocyte development. Therefore, the objective of the current study was to produce ovulatory follicles exposed to different hormonal environments to determine differences in gene expression profiles of oocytes and granulosa cells developed under different levels of progesterone. We plan to examine the gene expression profiles in oocytes and granulosa cells from both treatments to develop markers of follicle and oocyte “quality.”

Procedure

Cows used for this trial were from the physiology herd located at the Agricultural Research and Development Center at Ithaca, Neb. The physiology cow herd is composed of ¾ MARC III (¼ Pinzgauer, ¼ Red Polled, ¼ Hereford, ¼ Angus) and ¼ Red Angus-European Cross cows. Approximately 194 were used in the experiment with 95 in the control group and 99 in the MGA-14 group.

Control treatment

The 95 control cows were administered an injection of GnRH to ovulate any dominant follicles and a CIDR was inserted for 7 days. At the end of the seven days the CIDR was removed and the cows were administered PGF (5 mg/head; PG.) Follicles were then aspirated at 18 hour (n=19), 36 hour (n=18), and 60 hour (n=48) time points after the PG injection. The level of progestin in this treatment group was at least 4–6 ng/ml. With this higher level of progestin normal follicular waves would occur without the development of persistent follicles.

MGA-14 treatment

In the MGA-14 treatment, approximately 99 cows were injected with PG and fed MGA-14 at a rate of 5mg/head/day for 14 days. At the end of the treatment, MGA-14 was removed from the cows’ diets and they were given an injection of PG. The expected level of progestin administered to the cows was to be <1 ng/ml. Follicles were aspirated similar to the control group at 18 hour (n=24), 36 hour (n=25), and 60 hour (n=48) time points after the PG injection. The lower level of progestin in these cows would allow for increased LH which would develop a larger, persistent follicle.

Of the 194 cows, approximately 12 did not have samples due to lack of a dominant follicle or the follicle being lost prior to collection. Therefore, these were removed from the study.

Once all the collections were complete the samples were returned to the lab. The oocytes and granulosa cells were separated from the follicular fluid and either collected for RNA or protein. The follicular fluid was frozen for analysis of progesterone and estradiol. Follicles with a greater E₂ to P₄ ratio (i.e. >1) are considered to be estrogenic dominant follicles. RNA extraction is currently being conducted on the granulosa cell pellets and RIA analysis is being conducted on the follicular fluid.

(Continued on next page)
Results

Follicle diameter

The MGA-14 treatment group had follicle diameters that were statistically larger than the control group at each aspiration time point \((P<0.05;\) Figure 1). As the follicles were aspirated at later time points, the differences between the two treatment groups became larger (Figure 1). The increase in follicle diameter in the MGA-14 treatment group is consistent with the formation of a persistent follicle. Persistent follicles have been demonstrated to develop when cows are exposed to levels of progesterone that are less than 1 ng/ml. Greater concentrations of progesterone (4-6 ng/ml) allow for follicle turnover and follicular waves to occur similar to our control treatment.

Granulosa pellet size

There was an effect on the size of the granulosa cell pellet due to the treatment at the 6 hour aspiration time point \((P<0.05;\) Figure 2) with the MGA-14 treatment being larger (Control: 24.0 \(\mu\)l vs. MGA: 46.2 \(\mu\)l). However, there was no different in size of the granulosa pellet at the 18 and 60 hour aspiration time points.

Follicular fluid contained in dominant follicle

There was an effect on the amount of follicular fluid contained in the dominant follicle due to treatment with the greatest amounts collected from the MGA-14 group at the 6 \((P<0.01;\) Figure 3) and 60 hours \((P<0.05;\) Figure 3) aspirations. Again, the MGA-14 treatment group exhibited larger volumes of follicular fluid at all time points. Further analysis will be conducted on the follicular fluid to determine steroid profiles in follicles from each treatment group.

Granulosa cell pellet size to follicular fluid ratio

There was an effect on the granulosa cell pellet size to follicular fluid
ratio (which is indicative of total number of granulosa cells per follicle volume) at the 60 hour aspiration point with the control group having a higher ratio ($P<0.05$; Figure 4). Numerically, the Control group had a higher pellet to follicular fluid ratio at all aspiration points than the MGA-14 treatment group. These data support our hypothesis that there would be less granulosa cells per follicle volume in the MGA-14 treatment versus the Control. In persistent follicles the granulosa cell layer diminishes and may be the reason that the oocyte is less viable. Thus, by 60 h there was less granulosa cells in the MGA-14 follicles per volume than the control.

From this experiment thus far, we can conclude that feeding (5mg/head/day) for 14 days of MGA caused a larger diameter follicle to develop which resembles a persistent follicle. We are now evaluating the mRNA of granulosa cells and oocyte RNA to determine differences in gene expression between the two treatment groups. We speculate that the differential gene expression in these two treatments may help us identify markers of potential “oocyte and follicle quality.” These markers would allow us to develop more objective assays to determine oocyte quality prior to fertilization and embryo transfer in beef females.

Figure 4. The ratio of granulosa cell size to the amount of follicular fluid aspirated (Ratio) from cows from Control and MGA-14 treatments at 18, 36, and 60 hours after PG. The * represents a difference of ($P<0.05$).

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