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Granulosa Cell Gene Expression is Altered in Follicles from Cows with Differing Reproductive Longevity

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Summary

Heifers and cows that were culled from the herd due to failure to become pregnant were categorized into groups with low (≤ 2 year), moderate (>2 and < 6 year) or high (≥ 6 year) fertility. Antral follicle counts were numerically lower in the low group and increased in the moderate- and high-fertility group. Granulosa cells from dominant follicles in moderate- and high-fertility cows had a greater ratio of Vascular Endothelial Growth Factor 164 (VEGF164) to VEGF164B compared to the low-fertility cows. Furthermore, there was more CARTPT in granulosa cells from subordinate follicles in moderate- and high-fertility cows than low. Gene expression is altered in granulosa cells from cows differing in fertility, suggesting these are candidate genes that may be used as markers to assist in determining reproductive longevity in beef cows.

Introduction

Cows that stay in the herd longer and continue to produce a calf have greater reproductive longevity. While we may be able to predict reproductive longevity by a combination of number of antral follicles on the ovary, ovarian size, and reproductive tract score, there are no conclusive genetic or phenotypic markers of reproductive fertility in beef cattle. The long-term goal is to develop markers of reproductive longevity that may be implemented prior to selecting replacement heifers. A first step for this goal is to determine what genes are altered in granulosa cells (cells that communicate with and support egg maturation) to determine how ovarian follicle development (and development of the egg) may differ in cows culled from the herd at different ages due to pregnancy failure.

Procedure

All procedures were approved by the University of Nebraska–Lincoln Institutional Animal Care and Use Committee (IACUC).

Beef cows ranging in age from 1.5 to 11 years were synchronized with a modified Co-Synch protocol, and upon CIDR removal were injected with Lutalyse, and ovaries were removed by flank laparotomy (surgical incision) 36 hours later to obtain dominant and subordinate follicles prior to ovulation. Ovaries were weighed, measured for length and width, and all visible surface follicles were counted. Granulosa cells were collected and extracted for RNA. Quantitative Polymerase Chain Reaction (QPCR) was conducted to determine expression of genes known to influence follicle growth (VEGF164), follicle arrest (VEGF164B), and atresia (CARTPT). The expression of these genes was also correlated to follicle diameter (another potential biomarker of fertility).

Results

The number of antral follicles (follicles with fluid-filled cavities) present on the ovaries of cows from each fertility group is presented in Figure 1. While the numbers were numerically lower in the low-fertility group and increased to the high-fertility group, none of the groups were statistically different from each other. Figure 2 depicts the percentage of low (< 30), medium (30-60), and high (> 60) antral follicle counts (AFC) on the ovaries of cows for each

(Continued on next page)

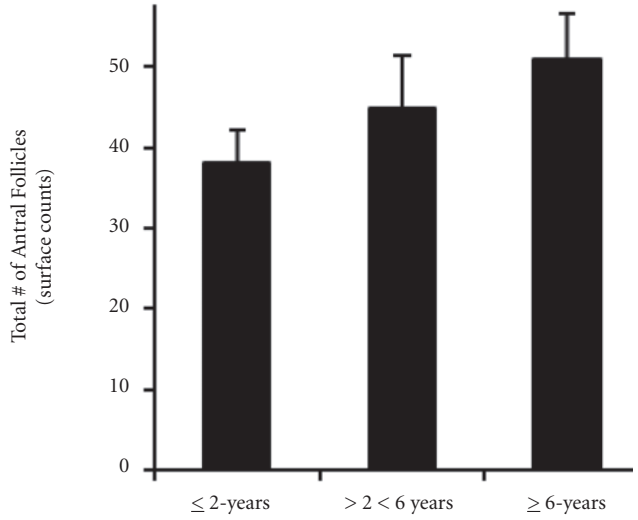

Figure 1. Total number of antral follicles in different fertility groups. These values were not significantly different from each other.

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fertility classification. Because there are still low antral follicle count cows present in the high-fertility group, we cannot use antral follicle counts as the only method to predict reproductive longevity. Low antral follicle count appears to be only a risk factor for infertility, not a sole determining factor. Furthermore, because our analysis of antral follicle count is retrospective, and antral follicle count decreases as animals get older, the low antral follicle count cows in the older cow groups may be due to them getting closer to the end of their reproductive lifespan. Thus, we decided to evaluate expression of genes that have been shown to influence follicle development to determine if we could detect any differences that may result in less than optimal follicle development and egg maturation.

Our laboratory has previously determined in rodents that treatment with VEGF164 stimulates follicle development, promoting early-stage follicles to later stages of development. Furthermore, treatment with VEGF165b inhibited follicle progression and arrested follicles at early stages of development. Therefore, our objective was to determine if the ratio of VEGF164 angiogenic to VEGF164B anti-angiogenic isoforms in granulosa cells from dominant follicles was greater in high-fertility cows and reduced in the low-fertility group. In Figure 3, the ratio of VEGF164 to VEGF164B is reduced in cows culled from the herd at less than or equal to 2 years of age when compared to either the moderate- or high-fertility group. Thus, the dominant follicles in the low-fertility group had less VEGF164 and more VEGF164B messenger RNA (mRNA) in their granulosa cells. Furthermore, the diameter of the dominant follicles in the low-fertility group was numerically less but not significantly different (data not shown). This suggests that the dominant follicles in the ≤ 2 years of age group are not growing optimally. This may affect egg maturation and development, resulting in less viable or fertile eggs.

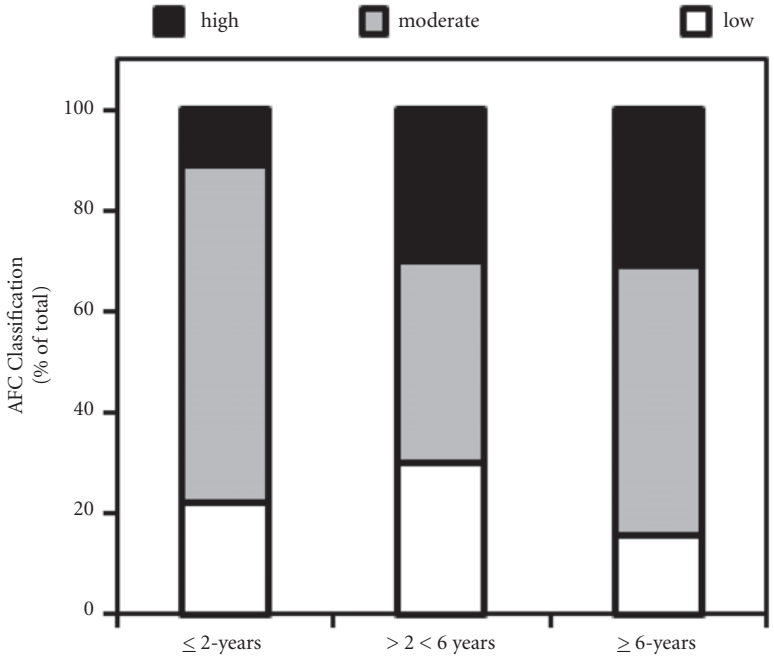

Figure 2. Percentage of cows that were categorized as high, medium or low antral follicle counts (AFC) in each fertility group.

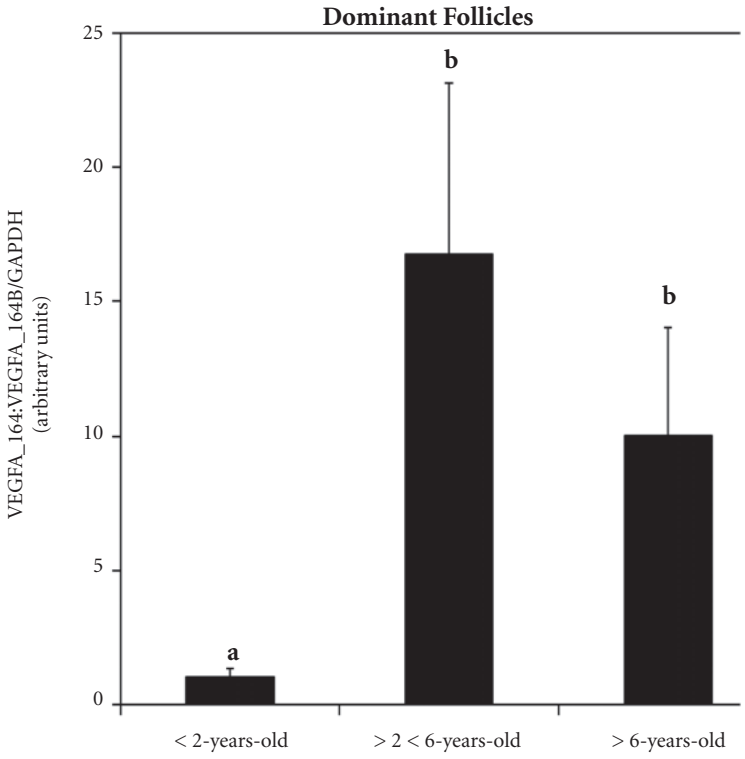

Figure 3. Quantitative PCR for VEGF164/VEGF164B ratio in granulosa cells from dominant follicles in cows with different fertility. Different subscripts indicate significance at $P < 0.05$.

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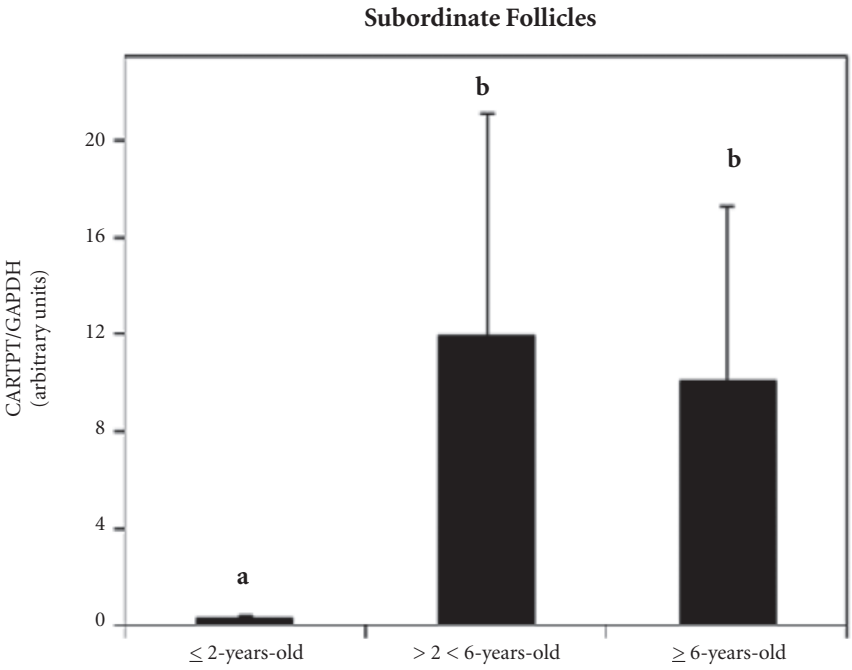

Figure 4. Quantitative PCR for CARTPT in granulosa cells from subordinate follicles taken from cows with different fertility. Different subscripts indicate significance at P < 0.05.

Interestingly, subordinate follicle diameters among the fertility groups were different, with the cows in low fertility being the smallest, the moderate were greater than the low, and the high having the largest follicle diameter (data not shown). The differences in subordinate follicle growth may be directly related to factors being produced by the dominant follicle. Also, when CARTPT, a gene expressed in subordinate and dominant follicles undergoing atresia, was measured, there was dramatically more expressed in subordinate follicles from the moderate- and high-fertility group than the low (Figure 4). This further supports our hypothesis that gene expression profiles are altered and may not be optimal in the low-fertility group, which possibly reflects why they failed to become pregnant.

Implications

Finding genes that predict fertility and reproductive longevity would allow for selection of heifers prior to their development to puberty and would increase profitability of cow/calf producers.

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