

# **Effect of Plasma from Cyclic versus Nutritionally Induced Anovulatory Beef Heifers on Proliferation of Granulosa and Theca Cells in vitro**

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## **Story in Brief**

The effects of plasma from cyclic versus nutritionally induced anovulatory beef heifers was evaluated on proliferation of bovine granulosa and theca cells in vitro. Granulosa and theca cells were obtained from small (1 to 5 mm) and large (>7.9) follicles, respectively, of cattle and cultured for 4 d. During the last 2 d of culture, cells were exposed to 0, 1% or 10% of plasma from cyclic or anovulatory heifers. Cell numbers were determined. Regardless of source, increasing percentage of plasma to culture medium increased cell numbers. However, the plasma-induced increase was greater in cells exposed to cyclic heifer plasma versus anovulatory heifer plasma. These results indicate that plasma from anovulatory heifers contains factor(s) that support a weaker mitotic stimulus toward bovine granulosa and theca cells. Systemic factors may play a role in directly regulating granulosa and theca cell proliferation in cattle.

Key Words: Theca Cells, Granulosa Cells, In Vitro, Proliferation, Cattle

## **Introduction**

Nutritionally induced reduction in growth of dominant ovulatory and non-ovulatory follicles of cattle is associated with a systemic decrease in insulin-like growth factor-I (IGF-I) concentrations (Bossis et al., 2000), but whether the reduction in follicle growth is due to direct ovarian effects of plasma factors such as IGF-I or due to indirect changes in other ovarian specific factors is unknown. The IGFs are thought to play an important role in regulating the development of dominant follicles including stimulation of ovarian cell mitosis and estradiol production (Spicer and Echterkamp, 1995; Spicer, 2004). Increased estradiol secretion by the selected dominant ovulatory follicle causes estrus and induces an ovulatory surge of luteinizing hormone (LH) which subsequently induces ovulation and release of the oocyte (Ginther et al., 1997; Mihm and Bleach, 2003). Therefore, we set out to compare the effect of plasma collected from cyclic versus anovulatory heifers on proliferation of granulosa and theca cells in vitro.

## **Materials and Methods**

Ovaries were obtained from cattle at a local commercial slaughterhouse. Granulosa cells from small (1 to 5 mm) follicles and theca cells from large (>7.9 mm) follicles were collected and cultured as previously described (Langhout et al., 1991; Spicer and Chamberlain, 1998). Isolated granulosa and theca cells were cultured in medium containing 10% fetal calf serum for 48 h, washed with serum-free medium, and cultured for an additional 48 h in serum-free medium containing 0, 1 or 10% of plasma from either cyclic or nutritionally induced anovulatory beef heifers (Bossis et al., 2000). Plasma for each type of heifer was pooled from five animals and sterilized via filtration

through 0.22  $\mu\text{m}$  filters. At the termination of each experiment, numbers of cells were determined using a Coulter counter as previously described (Langhout et al., 1991).

Experimental data are expressed as fold of 0% plasma values. Data are presented as the least squares means  $\pm$  SEM of measurements from three replicated experiments. Data were analyzed as a 2 x 2 factorial ANOVA with “plasma type” and “plasma dose” as main effects. Mean differences were determined by Fisher’s protected least significant difference test.

## Results

Increasing percentage of plasma to medium increased ( $P < 0.05$ ) both granulosa (Figure 1) and theca (Figure 2) cell numbers by severalfold. However, at both 1% and 10%, nutritionally induced anovulatory heifer plasma was less ( $P < 0.05$ ) effective at stimulating granulosa (Figure 1) and theca (Figure 2) cell proliferation.

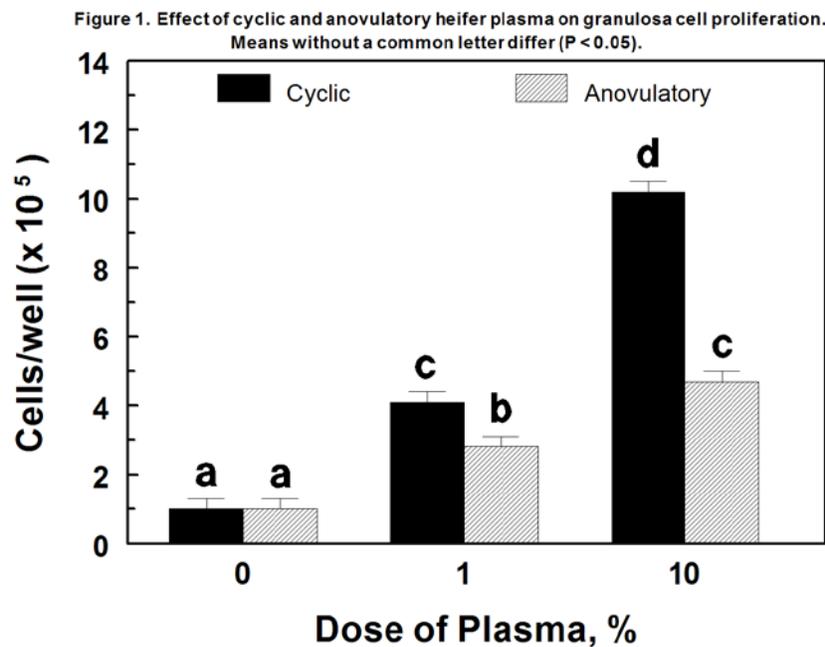
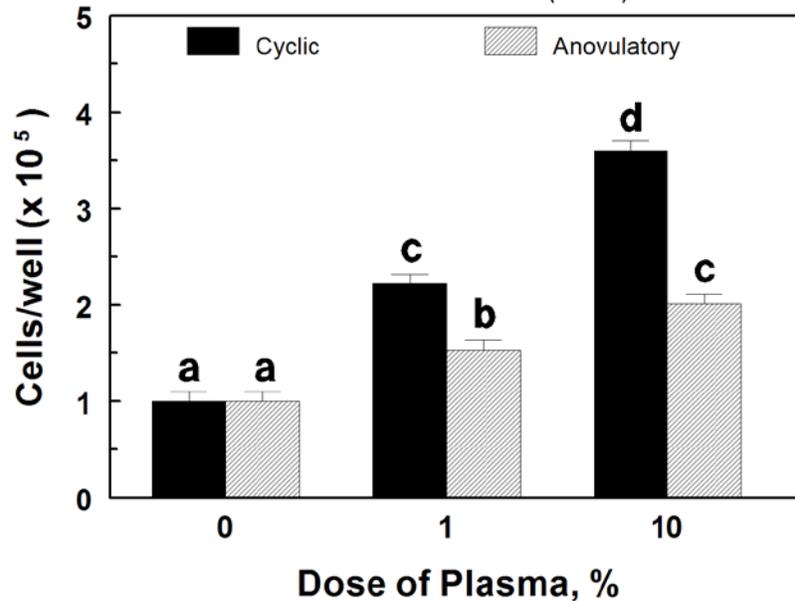


Figure 2. Effect of cyclic and anovulatory heifer plasma on theca cell proliferation. Means without a common letter differ ( $P < 0.05$ ).



### Discussion

Nutritionally induced anestrus is associated with reduced growth of the dominant follicle and a reduction in systemic IGF-I concentrations. Results of the present study indicate that plasma from nutritionally induced anovulatory heifers is less stimulatory to granulosa and theca cell proliferation than is plasma from cyclic heifers. Whether a lower concentration of IGF-I is the only factor responsible for the reduced proliferative stimulus of the nutritionally induced anovulatory heifer plasma will require further study. Consistent with this suggestion, IGF-I dramatically stimulates proliferation of bovine granulosa and theca cells (Langhout et al., 1991; Spicer and Chamberlain, 1998). Both systemic and locally produced IGF-I may play a role in regulating granulosa and theca cell function during ovarian follicular growth in cattle (Spicer and Echterkamp, 1995). If further research identifies the factor(s) responsible for increased mitogenic activity of the cyclic heifer plasma, then potential products for increasing reproductive efficiency can be targeted for future development and potential on-farm use.

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### **Acknowledgements**

The authors thank Mikkelson Beef Inc. (Oklahoma City, OK) for their generous donations of bovine ovaries, and C. C. Chamberlain for technical assistance.

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