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Inhibition of Vascular Endothelial Growth Factor Manipulates Follicles in Beef Females

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Summary

Vascular Endothelial Growth Factor (VEGF) is produced by cells surrounding the egg in the follicle. If VEGF is inhibited, ovulation does not occur. Understanding how VEGF regulates follicle development may allow for manipulation of estrous cycles. In previous studies in our laboratory, blocking the actions of VEGF decreased activation of early stage follicles in neonatal rat ovary cultures. Therefore, we hypothesized inhibition of VEGF actions would also inhibit follicle activation in bovine ovarian cortical cultures. Inhibition of VEGF did inhibit follicle progression, thus regulation of VEGF may be a way to manipulate follicle development and more accurately time ovulation.

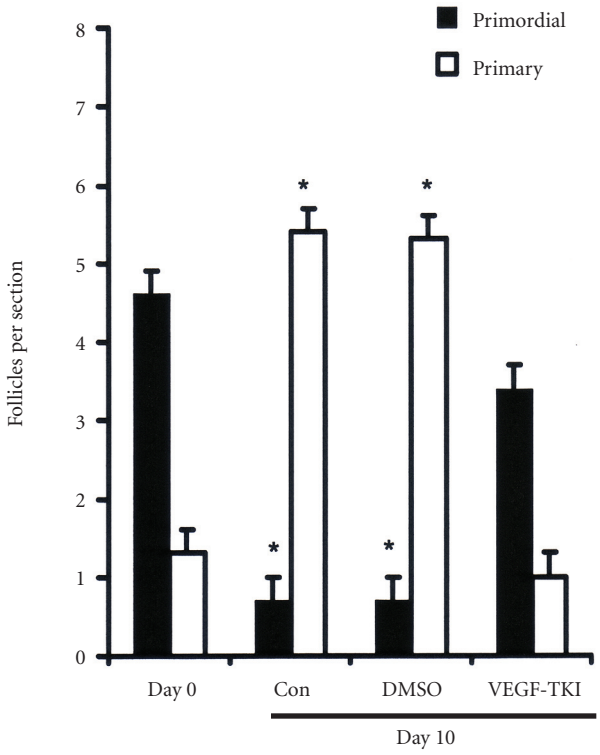

Figure 1. Numbers of follicles per section prior to treatment (day 0) in control, vehicle control (DMSO) and VEGF-TKI (VEGF inhibitor) after 10 days of treatment. There were greater later stage follicles (primary) in Control and DMSO treated cultures than those treated with VEGF-TKI (VEGF inhibitor; $P < 0.05$). The VEGF-TKI treatment had more primordial follicles (earliest stage of follicle development) and less had progressed to the later primary stage.

Introduction

Follicular development within the bovine ovary is a continuous process. It begins prior to birth and continues throughout the cow's reproductive lifespan. Regulatory mechanisms involved in primordial follicle pool development (earliest stage follicle development), progression or depletion in the ovary are poorly understood. Abnormal development or regulation of the primordial follicle pool can lead to ovarian dysfunction, including impairment of reproductive capacity or premature ovarian failure (depletion of follicles all at once at or near puberty). Manipulation of the primordial follicle pool may provide a means to increase reproductive efficiency in females. Specific growth factors must either stimulate or inhibit primordial follicle progression. VEGF has been demonstrated to be an important growth factor in the development of pre-ovulatory follicles, ovulation and formation of corpora lutea. No role has been identified for VEGF in the earlier stages of follicle development; however, research indicates VEGF expression is up-regulated during the primordial to primary follicle transition in postnatal rat ovaries. Therefore, we hypothesized that VEGF and its receptors regulate follicular progression in the bovine ovary.

Procedure

Ovaries were collected by mid-line laparotomy from neonatal calves (n = 5) at 44 ± 1.2 days of age, and the ovarian cortex was dissected from the medulla. *(The cortex of the ovary is the outside portions of the bovine ovary that contain the follicles. The middle portion of the ovary contains the medulla which has mainly vasculature and nerves.)* Ovarian cortical pieces (two pieces/well) were cultured in serum-free medium, with 0, 2, 4 or 8 μM of VEGF receptor tyrosine kinase inhibitor (VEGF-TKI) or DMSO (vehicle control) for 10 days in duplicate wells. VEGF-TKI was

replenished every day and medium changed every other day. On day 0 or day 10, cortical pieces (four pieces/calf/day/treatment) were collected and embedded in LR White resin for morphometric analysis.

Results

In control and DMSO-treated cultures, the number of primordial follicles per section decreased (day 0 = 4.6 ± 0.7 vs. day 10 = 0.7 ± 0.3; $P < 0.001$) and the number of primary follicles per section increased (day 0 = 1.3 ± 0.3 vs. day 10 = 5.4 ± 0.5; $P < 0.001$). Primordial follicle activation was not inhibited by 2 or 4 μM of VEGF-TKI; however, after 10 days in the presence of 8 μM VEGF-TKI, the number of primordial follicles per section was not different from day 0 controls (3.4 ± 0.4 follicles/section), indicating no activation had occurred when VEGF signaling was inhibited. Primordial follicles increased in diameter after 10 days in control and DMSO-treated cultures (day 0 = 16.0 ± 2.1 mm vs. day 10 = 26.0 ± 2.1 mm; $P < 0.05$). Ten days of treatment with 8 μM VEGF-TKI did not inhibit the growth of primordial follicles (30.3 ± 2.1 mM). Thus, VEGF-TKI (8 μM) inhibited primordial follicle activation in bovine ovarian cortical cultures. The current study supports previous results in the perinatal rat and indicates VEGF may be a regulator of primordial follicle activation.

Thus, regulation of VEGF early during bovine reproductive development may be important for inducing follicles to progress and develop. Regulation of VEGF may allow for manipulation of follicle development in the beef female.

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