Livestock Science ▮ (▮▮▮▮) ▮▮▮–▮▮▮

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Vascular endothelial growth factor expression correlated with microvessel density in the antral follicle of sheep ovary

Linlin Hao [a,b], Dian Wang [a], Guishan Zhang [a], Wei Ma [a], Changhong Li [c], Huaizhi Jiang [a,*]

[a] College of Animal Science and Technology, Jilin Agricultural University, Changchun 130118, China
[b] College of Animal Science, Jilin University, Changchun 130062, China
[c] BaiCheng Normal University, Baicheng 137000, China

ARTICLE INFO

Article history:
Received 18 June 2013
Received in revised form
10 April 2014
Accepted 14 April 2014

Keywords:
Estrous cycle
Vascular regeneration
Ovarian follicle

ABSTRACT

Vascular Endothelial Growth Factor (VEGF) promotes the proliferation and angiogenesis of endothelial cells and plays important physiological roles in the follicular development and oocyte maturation. However, the role of VEGF in endothelial cells is only partly understood in the angiogenesis of the sheep ovary. Our study analyzed the protein expression of VEGF and its correlation with microvessel density (MVD) in the sheep ovary from 0 to 15 d after the synchronization of estrus. The results showed that VEGF was expressed at increasingly higher levels from the primordial follicle to the secondary follicle ($P<0.05$). In Graafian follicles VEGF expression was increased from 0 to 5 d followed by a significant decline ($P<0.05$), and it was markedly increased again at 15 d ($P<0.05$). Capillaries began to appear around the follicular theca of the primary follicle. MVD significantly increased around the secondary follicle and in Graafian follicles from 0 to 5 d and 12 to 15 d ($P<0.05$). Pearson correlation analysis showed a highly positive correlation between VEGF expression level and MVD in the primordial follicle, primary follicle and secondary follicle ($r=0.81$, $p=0.01$). These data indicate that VEGF has a close relationship with vascular regeneration of sheep ovary.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

As is true in all placental mammals, the ovarian follicles of female sheep develop to a certain stage and become dormant, with a subset of follicles differentiating to become dominant follicles that will ultimately be used in ovulation. Previous studies showed that the selective development of follicles is related to their level of vascular contact (Miyamoto et al., 1996). It is known that angiogenesis is regulated by many vascular factors, in particular vascular endothelial growth factor (VEGF), which signals endothelial cells through paracrine or autocrine mechanisms to promote mitosis of endothelial cells as well as proliferation and metastasis. VEGF plays a necessary role in stimulating the formation and growth of new blood vessels and maintaining the integrality and permeability of the blood vessel wall. Studies on the expression of VEGF in human and rat ovaries have shown that VEGF promotes follicular development by facilitating ovarian angiogenesis (Neulen et al., 1999; Geva and Jaffe 2000). It has long been known that VEGF plays an important role in the cyclical changes of ovaries; however, VEGF-induced angiogenesis in the sheep ovary has been understood partly (Redmer and Reynolds, 1996). Our current study aimed to describe

* Corresponding author. Tel.: +86 13756293858.
E-mail address: jianghz6806@126.com (H. Jiang).

http://dx.doi.org/10.1016/j.livsci.2014.04.013
1871-1413/© 2014 Elsevier B.V. All rights reserved.

Please cite this article as: Hao, L., et al., Vascular endothelial growth factor expression correlated with microvessel density in the antral follicle of sheep ovary. Livestock Science (2014), http://dx.doi.org/10.1016/j.livsci.2014.04.013

the pattern of VEGF expression throughout the different periods of estrous cycle by determining the expression of VEGF in sheep ovaries with immunohistochemistry, and monitoring microvessel density (MVD) by CD34 in epithelial cells to explore the correlation between VEGF and MVD.

2. Materials and methods

2.1. Experimental animals

Ten 1.5-year old healthy ewes were selected from the key herd of Ujumuqin sheep at the Jilin Province Yaheng Agricultural Science and Technology Development Co., Ltd. The experimental sheep were pre-fed for 7 d. Two sheep were harvested on days 0, 5, 9, 12 and 15 after synchronization of estrus. Intravaginal suppositories containing 5 mg Norelegestromin (Beijing Yinuo Biotechnology Co., Ltd.) were inserted at 08:00 on the days of estrus, to synchronize estrus. After 7 d (on day 0 of the estrus cycle), the suppositories were removed, and 2 ml PGF2α and 1.5 ml PMSG (300 IU) were injected intramuscularly at 08:00. Sheep ovaries were collected on days 0, 5, 9, 12 and 15 of the estrus cycle. Animal experiments were performed in accordance with the guidelines on animal care and use established by the Jilin University Animal Care and Use Committee. Collected ovaries were washed with precooled PBS and then fixed in neutral formaldehyde solution prior to testing.

2.2. Measurement of the distribution of VEGF in the sheep ovaries with immunohistochemistry.

Paraffin-embedded wax blocks were made from fixed ovaries, and 3 μm serial sections were sliced. Rabbit Anti VEGF Polyclonal Antibodies (Beijing Bioss Co., Ltd.) were used as the primary antibody. UltraSensitiveTM SP Kit-9710 (Fuzhou, Maixin Co., Ltd.) for streptavidin–perosidase (S–P) immunohistochemistry containing the goat anti-rabbit IgG antibody was used. Data were taken from three selective visual fields for the primordial follicle, primary follicle, secondary follicle and antral follicles after color developing by DAB (Fuzhou, Maixin Co., Ltd.). The integrated optical densities (IOD) were measured to reflect the expression level of VEGF protein.

2.3. Measurement of MVD of sheep ovaries with immunohistochemistry

Vascular endothelial cells labeled for CD34 were observed under the 40× power field, and three areas with the richest numbers of capillaries near the dark brown area were selected. Then, under the 400× power field, three visual fields with clear staining of microvascular epithelial cells were chosen, and the microvascular cells in an area were counted and averaged.

2.4. Data analysis

After immunohistochemical staining, Image-Pro Plus 5.2 software (Media Cybernetics) was used to capture and analyze images. Data are presented as mean ± SEM in the text. SPSS 13.0 (IBM) was used for single factor analysis of variance and correlation analysis.

3. Results

3.1. Changes in VEGF expression in the primordial follicle, primary follicle and secondary follicle of sheep

Ovarian follicles were classified into the primordial follicle, primary follicle, secondary follicle which were

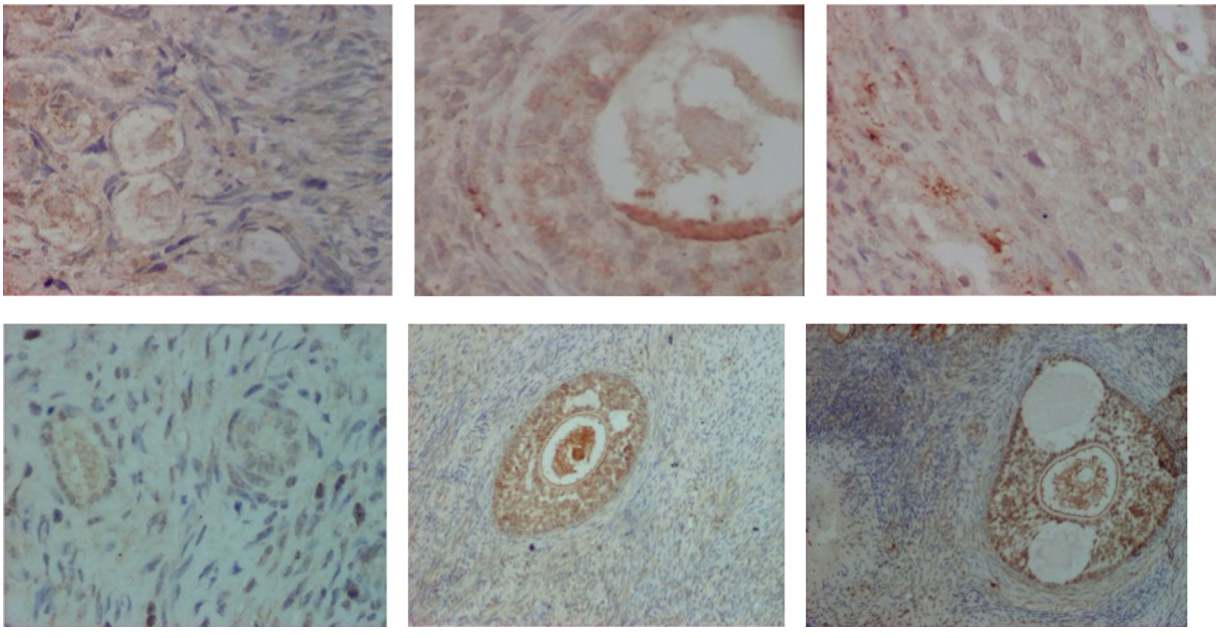

Fig. 1. VEGF (upper) and CD34 (lower) expression in primordial (left), primary (middle) and secondary (right) ovary follicles (400×).

Please cite this article as: Hao, L., et al., Vascular endothelial growth factor expression correlated with microvessel density in the antral follicle of sheep ovary. Livestock Science (2014), http://dx.doi.org/10.1016/j.livsci.2014.04.013

Download English Version:

https://daneshyari.com/en/article/8502235

Download Persian Version:

https://daneshyari.com/article/8502235

Daneshyari.com