Theriogenology 81 (2014) 428–436

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Expression and localization of locally produced growth factors regulating lymphangiogenesis during different stages of the estrous cycle in corpus luteum of buffalo (*Bubalus bubalis*)

Ibne Ali [a,1], Vikrant Singh Chouhan [a,1], Satyaveer Singh Dangi [a], Mahesh Gupta [a], Ujjwala Tandiya [a], Iqbal Hyder [a], Vijay Pratap Yadav [a], Rudra Prasanna Panda [a], Vazhoor Babitha [a], Vimla Nagar [a], Arvind Sonwane [b], Firdous Ahmad Khan [c], Bikash Chandra Das [a], Gyanendra Singh [a], Sadhan Bag [a], Mihir Sarkar [a,*]

[a] *Physiology and Climatology, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India*
[b] *Animal Genetics, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India*
[c] *Department of Animal Sciences and D.H. Barron Reproductive and Perinatal Biology Research Program, University of Florida, Gainesville, Florida, USA*

ARTICLE INFO

Article history:
Received 27 June 2013
Received in revised form 21 October 2013
Accepted 21 October 2013

Keywords:
Buffalo
Corpus luteum
Estrous cycle
Growth factors
Lymphangiogenesis

ABSTRACT

Recent experiments using expression, immunolocalization, and cell culture approaches have provided leading insights into regulation of luteal angiogenesis by different growth factor systems and its role in the function of corpus luteum (CL) in buffalo. On the contrary, lymphangiogenesis and its regulation in the CL are still poorly understood. The aim of this study was to evaluate the expression and localization of lymphangiogenic factors (vascular endothelial growth factor [VEGF]-C and VEGFD), their receptor (VEGFR3), and lymphatic endothelial marker (LYVE1) in bubaline CL during different stages of the estrous cycle and to investigate functional role of VEGFC and VEGFD in luteal lymphangeogenesis. The mRNA and protein expression of VEGFC, VEGFD, and VEGFR3 was significantly greater in mid and late luteal phases, which correlated well with the expression of LYVE1. The lymphangiogenic factors were localized in luteal cells, exclusively in the cytoplasm. Immunoreactivity of VEGFC was greater during midluteal phase and that of VEGFD was greater during the mid and late luteal phases. Luteal cells were cultured *in vitro* and treated for different time duration (24, 48, and 72 hours) with VEGFC and VEGFD each at 50, 100, and 150 ng/mL concentration and VEGFC with VEGFD at 100 ng/mL concentration. The temporal increase in LYVE1 mRNA expression was significant ($P < 0.05$) in VEGFC and VEGFC with VEGFD treatment and no significant change was seen in VEGFD treatment. Thus, it seems likely that VEGFD itself has little role in lymphangiogenesis but along with VEGFC it might have a synergistic effect on VEGFR3 receptors for inducing lymphangiogenesis. In summary, the present study provided evidence that VEGFC and VEGFD, and their receptor VEGFR3, are expressed in bubaline CL and are localized exclusively in the cell cytoplasm, suggesting that these factors have a functional role in lymphangiogenesis of CL in buffalo.
© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Buffalo (*Bubalus bubalis*) is an important livestock species, concentrated mostly in the tropical and subtropical regions of the world. India alone has 112.9 million buffaloes, which is approximately 57.8% of the world buffalo

* Corresponding author. Tel.: +91 0 581 2310455; fax: +91 0 581 2301327.
E-mail address: msarkar24@gmail.com (M. Sarkar).
[1] These authors contributed equally to the work.

0093-691X/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2013.10.017

population [1]. Indian farmers encounter an estimated loss of 19 to 20 MT of milk each year because of subestrus or silent estrus with a hormonal etiology, leading to production loss and poor reproductive efficiency in buffaloes [2–4]. Infertility might result from poor development and incomplete vascularization of CL, leading to a deranged luteal function. A thorough understanding of the endocrine, autocrine, and paracrine factors that regulate luteal development and function would be helpful in elucidating the mechanisms underlying luteal insufficiency thereby facilitating development of approaches for alleviation of infertility resulting from luteal dysfunction.

The reproductive cycle in mammals is a process of chronological proliferation, differentiation, and transformation of ovarian follicular cells followed by formation and regression of the CL in a cyclic manner [5]. It is well established that gonadotropins and growth hormone are the primary regulators of this process [6]. In addition, recent reports provided evidence on the essential modulatory role of locally produced factors such as steroid hormones, peptides, and growth factors in the development of the follicle, CL, and regulation of the reproductive cycle. These locally produced factors constitute a complex intraovarian autocrine/paracrine regulatory system to regulate the reproductive functions [7].

Regulation of luteal angiogenesis in buffalo has been the focus of our recent studies in which we demonstrated the expression and localization of vascular endothelial growth factor (VEGF) system across the estrous cycle [8]. Angiogenesis of the CL has been studied extensively in various animal species but the development of lymphatic vessels, or lymphangiogenesis, has attracted relatively little attention. Early morphological studies revealed profuse networks of lymphatic vessels in the CL of the sheep, swine, dog, and rabbit [9–13]. Moreover, leukocytes comprising monocytes/macrophages, granulocytes, and T lymphocytes have been localized to the CL in several species [14–16]. In contrast, luteal lymphangiogenesis and its regulation by growth factors in buffalo remain largely unknown.

Lymphatic vessel endothelial hyaluronan receptor, one of the most specific and widely used lymphatic endothelial markers, is expressed in a subset of endothelial cells in the large central veins and currently provides the first indicator of lymphatic endothelial competence [17,18]. Study of the lymphatic system is now accelerating because of great progress in identifying specific markers of lymphatic endothelial cells (LyECs), including lymphatic endothelial marker (LYVE1) [19–21]. Moreover, recent studies have suggested that the VEGFC/VEGFD-VEGFR3 system regulates lymphangiogenesis in CL of primates [22] and cattle [23]. The receptor tyrosine kinase VEGFR3 is activated by VEGFC and VEGFD, both members of the VEGF family of growth factors [24,25]. Endothelial cells committed to the lymphatic lineage express high levels of VEGFR3, and as the lymphatic vascular system begins to develop, VEGFR3 expression becomes restricted exclusively to LyECs with the exception of the fenestrated blood vessels found in endocrine organs such as thyroid, adrenal glands, and pancreas [26].

To date, there is no information on the presence of a lymphatic system in the CL of buffalo and its regulation by growth factors. This study was, therefore, conducted to test the hypothesis that a lymphatic network exists in the buffalo CL and that occurrence of lymphangiogenesis changes in the buffalo CL during the estrous cycle. Specific objectives included evaluation of expression and localization of lymphangiogenic factors (VEGFC and VEGFD), their receptor (VEGFR3), and LYVE1 in bubaline CL during different stages of estrous cycle and investigation of the functional role of VEGFC and VEGFD in luteal lymphangeogenesis.

2. Materials and methods

2.1. Collection of CL during the estrous cycle

Entire reproductive tracts from buffalo cows were collected at a local slaughterhouse within 10 to 20 minutes after slaughter and were transported on ice to the laboratory. The stage of the estrous cycle was defined using macroscopic observation of the ovaries (color, consistency, CL stage, and number and size of follicles) and the uterus (color, consistency, and mucus) as described previously [27]. Forty ovaries, each with CL, were used to extract 10 CL per group for RNA extraction, Western blot analysis, and immunohistochemistry studies. The CL was assigned to the following stages: early luteal phase (Days 1–4), midluteal phase (Days 5–10), late luteal phase (Days 11–16), and regressed CL (Days >16) of the estrous cycle. Luteal tissue was frozen in liquid nitrogen and stored at −80 °C until RNA and protein isolation.

2.2. Collection of follicles during final follicular growth

Only follicles that appeared healthy (i.e., well vascularized and having transparent follicular wall and fluid) and with diameter greater than 14 mm were used. Large follicles (>14 mm) were collected only after CL regression, with signs of mucus production in the uterus and cervix and were assumed to be preovulatory. For the RNA extraction, the follicles were dissected from the ovary. The surrounding tissue (theca externa) was removed with forceps using a stereomicroscope as previously described [27]. After aspiration, the follicular fluid (FF) was stored at −20 °C until determination of progesterone (P4) concentration. Because healthy follicles have relatively constant P4 levels in FF, only follicles with P4 less than 100 ng/mL FF were used for the evaluation, to exclude atretic follicles [27]. The follicles were snap frozen in liquid nitrogen and stored at −80 °C until RNA and protein isolation.

2.3. Hormone determination

Concentrations of P4 were determined directly in the FF with an enzyme immunoassay using the second antibody technique [28]. We used as enzyme solution P4-6b-hydroxy-hemisuccinate-horseradish peroxidase (HRP). Primary polyclonal antibody was raised in a rabbit against P4-7a-carboxyethylthioether-BSA and goat anti-rabbit secondary antibody was used. The effective dose for 50% inhibition of the assay was 8 ng/mL. The FF was diluted 1:5 with PBS. The minimal detectable limit was 0.4 ng/mL. The intra- and interassay coefficients of variation were 6.5% and 8.3%, respectively.

Download English Version:

https://daneshyari.com/en/article/2097556

Download Persian Version:

https://daneshyari.com/article/2097556

Daneshyari.com