Follicle stimulating hormone receptors are expressed in ewe's reproductive and nonreproductive tissues

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Abstract

Follicle stimulating hormone receptors (FSHR) are present in granulosa cells, Sertoli cells, and other sites within the reproductive tract (uterine tube, uterus, placenta, and cervix). They are also present in many nonreproductive tissues (skin and adipose tissue, bone and cartilage, thyroid tissue, bladder and urethra, monocytes and endothelial cells within colon, pancreas, kidney, lung, liver, and in stomach cancer tissues of various species). However, the expression of FSHR outside of the reproductive tract in sheep, has not been reported. Objective was to determine FSHR expression in ovine ovary, uterus, liver, kidney, heart, and skeletal muscle. Tissue samples were collected at slaughter and FSHR expression was determined via immunohistochemistry in formalin-fixed and paraffin-embedded tissue sections. Within the reproductive tract, FSHR were expressed in granulosa cells, luteal cells, and endometrial glandular epithelial cells. In liver, FSHR were expressed within hepatocytes and vascular endothelial cells. Expression of FSHR in cardiac myocytes was limited to cell membranes. Kidneys had FSHR expression on the renal tubular epithelial cells and the capillary endothelial cells. Lastly, in the skeletal muscle, FSHR were expressed in the endomysium. In human cancers, FSHR activation in nonreproductive tissues is involved in vascular remodeling, angiogenesis, cell proliferation, migration, and invasion. Effects of FSHR activation on ovine nonreproductive tissues remains unknown and warrants investigation into risks of frequent use of FSH agonists in sheep for nonseasonal estrous cycle manipulation.

Keywords: Heart, kidney, liver, skeletal muscle, ovary, uterus

Introduction

Follicle stimulating hormone is secreted from the anterior pituitary gland and its secretion is regulated by estradiol, inhibin, and activin.[1-3] Receptors for follicle stimulating hormone are present in granulosa cells and Sertoli cells.[4,5] In addition, follicle stimulating hormone receptors (FSHR) are present in the bovine uterine tube and ovine uterus, placenta, and cervix.[6-8] Besides the reproductive tract, FSHR are present in the skin and adipose tissue, bone and cartilage, thyroid tissue, bladder and urethra, monocytes and endothelial cells within colon, pancreas, kidney, lung, liver, and stomach cancer.[9-17] Expression of FSHR is well documented outside of the reproductive tract in other species, but not in sheep. Follicle stimulating hormone agonists are routinely used for nonseasonal estrous cycle manipulation in ewes. If FSHR are expressed outside the reproductive tissues, this could result in nonspecific side effects (e.g. vascular remodeling, angiogenesis).[17] Objective was to determine whether FSHR are expressed in tissues outside of the reproductive tract in sheep. We hypothesized that in addition to ovary and uterus, FSHR is expressed in liver, skeletal muscle, heart, and kidney.

Materials and methods

Tissues (ovarian follicle, ovarian corpus luteum, endometrium, heart, liver, thigh muscle, and kidney) were collected from a single crossbred ewe at slaughter. This ewe had not received any treatment prior to slaughter. All procedures were approved by the Institutional Animal Care and Use Committee. Tissues were fixed in 10% formalin, embedded in paraffin, and sectioned (5 μm) onto charged slides. Sections were incubated at 60ºC for 45 minutes, deparaffinized in xylene, and rehydrated in a graded ethanol series (100, 75, and 50%). Sections were washed in a diluted buffer (Wash buffer 10X #S3006, Dako, Carpinteria, CA) and then exposed to heat-induced antigen retrieval with diluted sodium citrate buffer (Target Retrieval Solution 10X concentration #S1699, Dako) in a pressure cooker (Nordicware® tender cooker, Minneapolis, MN) boiled for 10 minutes and cooled for 20 minutes. Slides were washed

with deionized water and tissue-specific endogenous peroxidase activity was blocked with 3% hydrogen peroxide (diluted from 30% hydrogen peroxide solution #5240-05, Macron, PA). Nonspecific binding was inhibited using a serum-free protein block for 20 minutes at room temperature (Protein Block Serum-Free ready to use #X0909, Dako). For FSHR, a polyclonal antirabbit FSH receptor (F3929, Sigma-Aldrich, St. Louis, MO) was applied at a 1:200 dilution and incubated for 105 minutes at room temperature. Universal negative control rabbit IgG (NC495H, Biocare Medical, Pacheco, CA) was applied to all adjacent sections to serve as negative controls. All slides were thoroughly washed in wash buffer prior to applying a secondary antibody (IH-8064 ImmunoBiosCience, Mukilteo, WA) for 30 minutes at room temperature. Slides were then thoroughly washed in wash buffer and then incubated with a chromogen (NovaRED, Vector Laboratories, Burlingame, CA) for 7.5 minutes at room temperature to detect the peroxidase activity. Slides were then washed in wash buffer, counterstained with hematoxylin, washed in tap water, dehydrated in a graded ethanol series (50, 75, and 100%), passed through 3 successive xylene baths, and coverslipped.

Slides were evaluated at 400 x magnification with a Leica DM4000B microscope using bright field microscopy. Representative images from each tissue were digitally captured using a QImaging camera (QICAM 12-bit, #QIC-F-M-12-C, QImaging, Surrey, BC, Canada) and QCapturePro image capturing software (QImaging). Evidence of FSHR expression in the tissues was determined by the presence of red staining specific to individual cells.

Results

Within the reproductive tract, FSHR were expressed in ovarian granulosa cells and endothelial cells (Figure 1a), ovarian luteal cells (Figure 1b), and endometrial glandular epithelial cells and endothelial cells (Figure 1c). Within liver, FSHR were expressed in hepatocytes (liver epithelial cells) and endothelial cells (Figure 1d). Follicle stimulating hormone receptors were expressed on the cell membrane of cardiac myocytes (Figure 1e). In the kidney, FSHR were expressed on the renal tubular epithelial cells and the endothelial cells (Figure 1f). Lastly, in the skeletal muscle, FSHR were expressed in the endomysium (Figure 1g). Negative controls showed no positive staining (data not shown).

Discussion

Similar to findings in humans and mice,[17,18] in our study, FSHR were expressed in vascular endothelial cells within the liver and kidney in sheep. In human cancer, endothelial cell FSHR respond to FSH stimulation by vascular remodeling and angiogenesis.[17,19] Stimulation of vascular endothelial cell FSHR in menopausal women results in vascular cell adhesion molecule 1 (VCAM-1) synthesis that recruits monocytes in the pathogenesis of atherosclerosis.[20,21] In addition, epithelial cell FSHR stimulation in humans induces proliferation, migration, and cancer cell invasion.[22-24] Effect of FSHR activation on ovine nonreproductive tissues remains unknown and warrants investigation into risks of frequent use of FSH agonists in sheep for nonseasonal estrous cycle manipulation.

In addition to FSHR expression in the epithelium, they are also expressed in muscle. Similar to what was observed in the present study, Atlantic salmon also expressed FSHR in cardiac myocytes.[25] With respect to skeletal muscle, FSHR expression in the current study was limited to the endomysium. In human adipocytes, FSHR activation opposes β3 adrenergic signaling by interacting with Gαi-coupled receptor and the cAMP response element-binding protein (CREB)-mediated pathway.[26,27] Beta-3 adrenergic receptor stimulation of skeletal muscle induced hypertrophy in mice.[28] Beta-3 adrenergic receptor stimulation of cardiac muscle induced vasodilation and relaxation to contractility.[29,30] It is not known if stimulation of FSHR in skeletal and/or cardiac muscle would alter β3 adrenergic signaling. However, this could substantially impact overall animal health.

In conclusion this is the first report of FSHR expression outside the reproductive tract in sheep. However, replication is necessary involving more animals. Frequent use of FSH agonists in sheep in nonseasonal estrous cycle manipulation may be accompanied by health risks associated with FSHR activation (e.g. stimulation of vascular remodeling, angiogenesis, VCAM-1 synthesis,

epithelial cell proliferation, and alterations in cardiac and/or skeletal β3 adrenergic signaling).

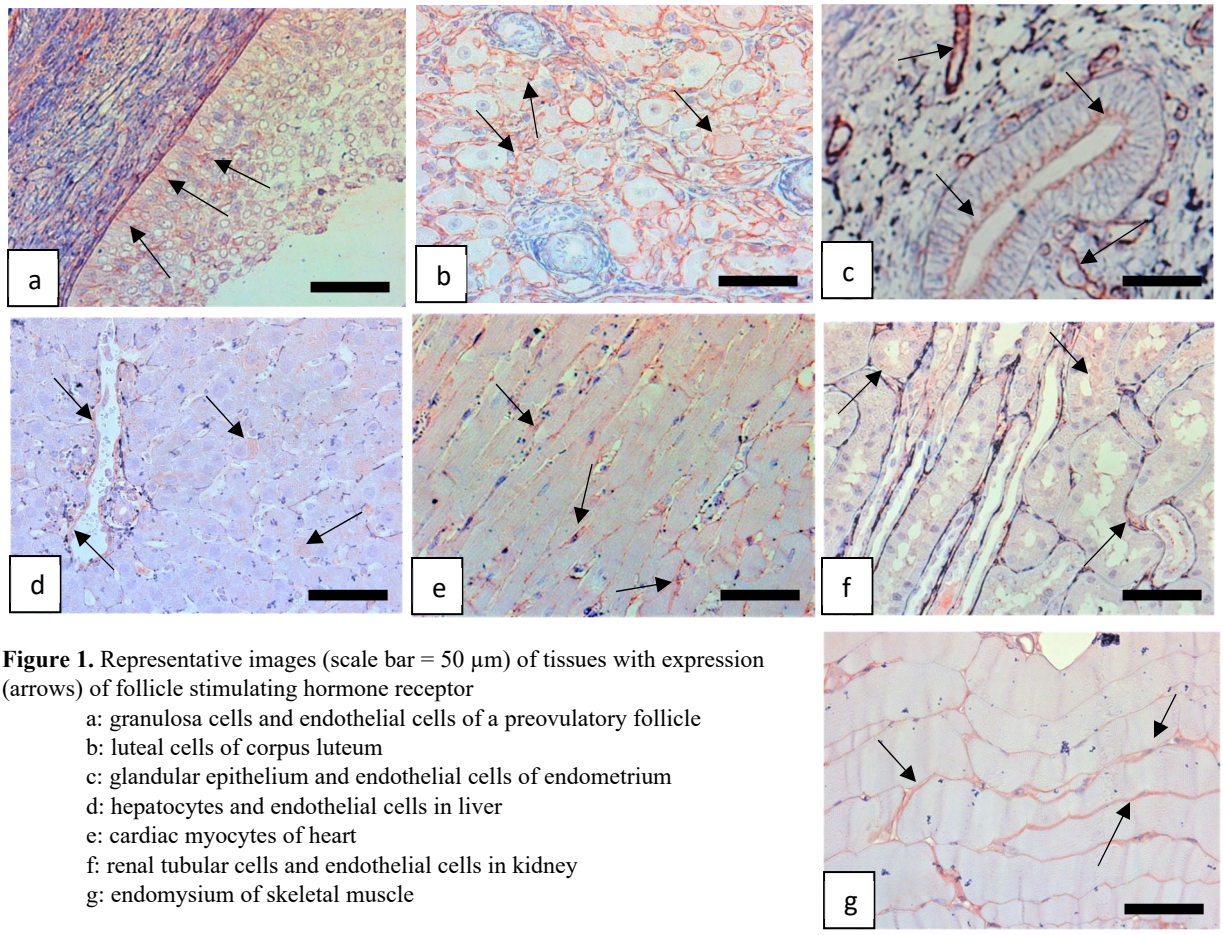

Figure 1. Representative images (scale bar = 50 µm) of tissues with expression (arrows) of follicle stimulating hormone receptor
a: granulosa cells and endothelial cells of a preovulatory follicle
b: luteal cells of corpus luteum
c: glandular epithelium and endothelial cells of endometrium
d: hepatocytes and endothelial cells in liver
e: cardiac myocytes of heart
f: renal tubular cells and endothelial cells in kidney
g: endomysium of skeletal muscle

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Conflict of interest

Authors disclose that there were no actual or potential conflicts of interest regarding the research that affected their ability to objectively present or review the research or data.

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