Expression of progesterone receptor protein in the ovine uterus during the estrous cycle: Effects of nutrition, arginine and FSH

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ABSTRACT

To evaluate expression of progesterone receptor (PGR) AB in follicle stimulating hormone (FSH)-treated or non-treated sheep administered with arginine (Arg) or saline (Sal) fed a control (C), excess (O) or restricted (U) diet, uterine tissues were collected at the early, mid and/or late luteal phases. In exp. 1, ewes from each diet were randomly assigned to one of two treatments, Arg or Sal administration three times daily from day 0 of the first estrous cycle until uterine tissue collection. In exp. 2, ewes were injected twice daily with FSH on days 13—15 of the first estrous cycle. Uterine tissues were immuno-stained to detect PGR followed by image analysis. PGR were detected in luminal epithelium (LE), endometrial glands (EG), endometrial stroma (ES), myometrium (Myo), and endometrial and myometrial blood vessels. The percentage of PR-positive cells and/or intensity of staining were affected by phase of the estrous cycle, plane of nutrition, and/or FSH but not by Arg. In exp. 1, percentage of PGR-positive cells in LE and EG but not in ES and Myo was greater at the early and mid than late luteal phase, was not affected by plane of nutrition, and was similar in LE and EG. Intensity of staining was affected by phase of the estrous cycle and plane of nutrition in LE, EG and Myo, and was the greatest in LE, less in EG, and least in ES and Myo. In exp. 2, percentage of PGR-positive cells in LE, EG, ES and Myo was affected by phase of the estrous cycle, but not by plane of nutrition; was greater at the early than mid luteal phase; and was greatest in LE and EG, less in luminal (superficial) ES and Myo and least in deep ES. Intensity of staining was affected by phase of the estrous cycle and plane of nutrition in all compartments but ES, and was the greatest in LE and luminal EG, less in deep EG, and least in ES and Myo. Comparison of data for FSH (superovulated) and Sal-treated (non-superovulated) ewes demonstrated that FSH affected PR expression in all evaluated uterine compartments depending on plane of nutrition and phase of the estrous cycle. Thus, PGR are differentially distributed in uterine compartments, and PGR expression is affected by nutritional plane and FSH, but not Arg depending on phase of the estrous cycle. Such changes in dynamics of PGR expression indicate that diet plays a regulatory role and that FSH-treatment may alter uterine functions.

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1. Introduction

During the estrous cycle, uterus and ovaries are in constant interactions that are mediated by the endocrine system [1,2]. Through secretion of several factors including steroid hormones (e.g., estradiol-17β [E2] and progesterone [P4]), the ovaries control uterine functions, and through the production of prostaglandins, the uterus affects ovarian functions [3,4]. Both, E2 and P4 regulate uterine functions, including cell proliferation and apoptosis, angiogenesis and secretory activities, and these effects depend on the stage of the estrous cycle or pregnancy [1,2,5,6]. The steroid hormones exert their direct effects through nuclear and membrane receptors in uterine tissues [3,4]. Uterine expression of estrogen and progesterone receptors (ESR and PGR, respectively) has been well described for numerous mammalian species [3,4]. Steroid receptors are expressed in several uterine compartments and their

expression is regulated by E2, P4 and other regulatory factors in several species [4]. Consequently, expression of steroid receptors changes during the estrous cycle in several species including ruminants and humans [7—13].

Nuclear PGR appears in two isoforms (PGRA and PGRB) that differ in molecular size (94 kDa vs. 116 kDa, respectively), and both are encoded by the same gene in several species [3,4]. Both PGR isoforms are involved in the regulation of uterine functions, but their roles may differ [3,4]. In ewes and cows, PGR are expressed in all uterine compartments, and expression levels are greatest at the early luteal phase and then decline as the estrous cycle progresses [8—10,12,13]. PGR are critical for preparation of uterus for implantation through a complex paracrine signaling network in mammalian species [14—16].

The effects of diet, arginine (Arg) and/or follicle stimulating hormone (FSH) on selected uterine functions have been demonstrated for several species, but rather limited information is available for non-pregnant sheep. Nevertheless, a few studies have demonstrated the effects of diet or dietary supplements on select uterine characteristics and fetal development in non-pregnant and early pregnant sheep and cows [17—25]. Arginine, a conditionally essential amino acid and possible dietary supplement, improved reproductive performance and the uterine environment for maintenance of pregnancy in sheep [26,27], and affected expression of lipid droplets (LD) in uterus of non-pregnant sheep [25].

Follicle stimulating hormone has been widely used in animal production and human medicine to induce multiple follicle development and/or superovulation [28—31]. However, FSH-treatment not only stimulates follicle development and enhanced steroid secretion but also affects uterine functions including endometrial receptivity; gene expression for steroids, other hormone and growth factors' receptors in endometrium; and endometrial secretory functions, morphology and vascular density in several species [32—38].

However, little is known about the effects of nutritional plane, Arg, and FSH on PGR expression and function in the uterus of non-pregnant animals. We hypothesized that expression of PGR protein in uterine tissues is affected by nutritional plane, Arg and/or FSH during the estrous cycle in sheep. Therefore, the objectives of this experiment were to 1) immunolocalize PGR protein in uterine tissues, and 2) determine the expression of PGR protein (using immunohistochemistry followed by image analysis) in endometrial compartments including luminal epithelium (LE), luminal (superficial) and deep endometrial glands (EG), luminal (superficial) and deep endometrial stroma (ES) and myometrium (Myo) obtained at early, mid and/or late luteal phases of the estrous cycle in ewes fed control (maintenance; C), excess (O; overfed) or restricted diets (U; underfed), and treated with vehicle (saline, Sal; control), Arg or FSH.

2. Materials and methods

2.1. Animal and experimental design

All animal procedures performed were approved by the North Dakota State University (NDSU) Institutional Animal Care and Use Committee (#A12013). The study was initiated during the normal breeding season in August and finished in December.

The experimental design was previously described [25,39—42]. Briefly, in Experiments 1 and 2, non-pregnant, non-lactating Rambouillet ewes were individually penned at the Animal Nutrition and Physiology Center on the NDSU campus. Ewes were stratified by body weight (BW) and randomly assigned into one of three dietary groups: maintenance-control (C; 100% National Research Council [NRC] requirements; 2.4 Mcal of metabolizable

energy [ME]/kg BW), overfed (O; 200% NRC requirements), or underfed (U; 60% NRC requirements). Dietary treatments were initiated 60 days prior to the onset of estrus (d 0). Ewes were fed their individual diets twice daily at 0800 and 1500 h for the duration of the experiment, and ewes were weighed once weekly. Estrus was synchronized by insertion of a controlled internal drug release (CIDR) device for 14 days. Ewes were in estrus approximately 36 h after removal of the CIDR, which was treated as d 0 of the estrous cycle [25].

2.2. Experiment 1

At d 0, ewes (n = 101) were randomly assigned into Sal (~10 ml) or Arg (L-Arg-HCl; Sigma, St. Louis, MO, USA; 155 μmol Arg/kg BW) treatment groups. Treatments were administered three times daily (0700, 1400, 2100 h) via jugular catheter beginning on day 0 of the first estrous cycle until completion of experiment. Uterine tissue were collected at the early (d 5) and mid (d 10) luteal phases of the second estrous cycle, and at the late (d 15) luteal phase of the first estrous cycle that corresponded to day 21, 26 and 15 of Arg treatment. Throughout the study, BW was maintained, increased ($P < 0.001$) and decreased ($P < 0.001$) for C, O and U, respectively. Initial and final BW for ewes in this experiment are reported by Kaminski et al. [39] and Bass et al. [42].

2.3. Experiment 2

Ewes (n = 34) were injected twice daily (morning and evening) with follicle stimulating hormone (FSH-P; Sioux Biochemical, Sioux Center, IA, USA) on d 13 to 15 of the first estrous cycle (5 mg/injection, 4 mg/injection, or 3 mg/injection, respectively [25]). Uterine tissues were collected at the early (d 5) and mid (d 10) luteal phases of the second estrous cycle. Throughout the study, BW was maintained, increased ($P < 0.001$) and decreased ($P < 0.001$) for C, O and U, respectively. Initial and final BW for ewes in this experiment are reported by Kraisoon et al. [41].

2.4. Tissue collection, immunohistochemistry and image analysis

Tissues were collected from 4 to 7 ewes/group. Cross sections of uterine horn were dissected from middle part of uterine horn ipsilateral to ovary with the CL, immediately fixed in 10% neutral buffered formalin, and then embedded in paraffin. Uterine tissues were cut into 4 μm thick slices, rinsed several times in PBS containing Triton-X100 (0.3%, v/v), and treated for 20 min with blocking buffer [PBS containing normal goat serum (2%, vol/vol)]. The tissue sections were then incubated with specific primary antibody against PGRAB (1: 100; mouse monoclonal, PGR Ab-8, Clone h PRa 2 + h PRa 3, Cat # MS-298-P0; Thermo Scientific, Fremont, CA). For control sections, primary antibody was replaced with mouse IgG at the same dilution and protein concentration as the primary antibody. Images were generated using an Axio Imager microscope (Zeiss Inc., Thornwood, NY) and subsequently analyzed (Image-ProPlus Premier software; Media Cybernetics, Inc., Rockville, MD) to determine the proportion (%) of PGRAB-positive cells and intensity of staining in several uterine compartments including LE, EG, ES and Myo. For each ewe, 6—9 images containing uterine compartments were generated and analyzed. Due to heterogeneous distribution and different sizes, image analysis for blood vessels was not performed.

2.5. Statistical analysis

Data were analyzed statistically using the GLM program of SAS. The model included plane of nutrition, Arg-vs. Sal-treatment, Sal vs.