Animal Reproduction Science 176 (2017) 64–69

Contents lists available at ScienceDirect

Animal Reproduction Science

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

Effect of GnRH and hCG on progesterone concentration and ovarian and luteal blood flow in diestrous mares

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ARTICLE INFO

Article history:
Received 7 September 2016
Received in revised form
20 November 2016
Accepted 21 November 2016
Available online 23 November 2016

Keywords:
Ovary
Corpus luteum
Blood flow
GnRH
LH
hCG

ABSTRACT

The objective of the present study was to investigate the effect of reproductive hormones (GnRH, hCG, LH and progesterone) on the regulation of corpus luteum (CL) and ovarian blood flow. Diestrous mares received a single treatment of saline, 100 μg gonadorelin (GnRH), or 1500 IU hCG 10 days after ovulation. Plasma LH and progesterone concentrations, resistance index (RI) for ovarian artery blood-flow, and percentage of corpus luteum (CL) with color-Doppler signals of blood flow were determined immediately before treatment (hour 0) and at hours 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, and 6. In the GnRH group, LH increased ($P < 0.0001$) between hours 0 and 0.25 and then progressively decreased; concentration of LH was not affected in the saline and hCG groups. Progesterone concentration was not different among groups. In the GnRH group, RI tended ($P < 0.07$) to decrease between hours 0 and 1.5 and increased ($P < 0.01$) between hours 1.5 and 4. In the hCG group, two transient RI decreases ($P < 0.05$) occurred before hour 2. The percentage change from hour 0 in the percentage of CL with blood-flow signals was greater at hour 0.5 in the GnRH group than in the saline group and was intermediate in the hCG group. The similarity among groups in progesterone concentration indicated that changes in progesterone were not involved in the GnRH and hCG stimulation of ovarian vascular perfusion. Effects of treatment might have been mediated through LH; however, since hCG biological activity is primarily LH-like, the differences in timing and degree of ovarian and luteal blood flow changes after GnRH or hCG administration in the present study suggest that GnRH might have a direct effect on ovarian blood vessels and vascular control.

1. Introduction

Remarkable changes in local vasculature are involved in the cyclic remodeling of the ovarian tissue that occurs during follicular growth, ovulation, corpus luteum (CL) formation, and luteolysis. Blood supply enables the follicles and CL to receive the required supply of nutrients, oxygen, and hormonal support as well as facilitating the release of steroid hormones. However, the dynamics of these vascular events are not fully understood. Understanding the mechanisms that regulate ovarian and luteal blood supply might provide new insight into ovarian physiology and physiopathology..

Studies investigating the regulation of ovarian blood supply have focused primarily on the development (angiogenesis) and regression of new blood vessels. Angiogenic factors such as members of the vascular endothelial growth factor family (VEGF), angiopoietin, fibroblast growth factor 2, and platelet-derived growth factor have been shown

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http://dx.doi.org/10.1016/j.anireprosci.2016.11.010
0378-4320/

to stimulate endothelial proliferation, migration, and tube formation and, to alter vascular permeability in the ovary. Local changes in blood flow within the ovary are also closely related to changes in the biosynthesis of prostaglandins and steroids and local factors such as insulin-like growth factors and oxygen tension likely modulate the angiogenic processes (Stouffer et al., 2001; Acosta and Miyamoto, 2004; Robinson et al., 2009). Studies on the role of reproductive hormones on short-term changes in ovarian blood flow have been done primarily in laboratory animals. Most of these studies have demonstrated a rapid increase in ovarian blood flow after LH or hCG administration (Wurtman, 1964; Janson, 1975; Lee and Novy, 1978; Varga et al., 1985; Wiltbank et al., 1989); however, the mechanisms behind the changes in hemodynamics produced by these hormones remain elusive.

Studies using the mare as a model have suggested that progesterone is not involved in regulating ovarian blood flow (Bollwein et al., 2004; Castro et al., 2016). Increased ovarian and luteal blood flow differed in magnitude when different doses of GnRH were administered to mares, but differences in progesterone were not significant (Castro et al., 2016). Human chorionic gonadotropin is chemically different from LH, but its biological activity is primarily LH-like; when 750–1500 IU hCG is administered to mares with follicles >35 mm during estrus, ovulation occurs within 48 h in more than 92% of the cases (Voss, 1993; Davies Morel and Newcombe, 2008). Therefore, GnRH and hCG were used in the present experiment to test the hypothesis that these reproductive hormones produce distinct, and progesterone-independent changes in ovarian blood flow.

2. Materials and methods

2.1. Animals and treatments

Animals were handled according to the United States Department of Agriculture Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching (http://www.fass.org/docs/agguide3rd/Ag_Guide_3rd_ed.pdf). Non-lactating, cycling mares of various breeds, aged 3 to 8 years and weighing 300 to 600 kg were used. Mares were housed in an open shelter with free access to grass hay, trace-mineralized salt, and water. The study was conducted during June and July in the northern temperate zone. Mares were examined daily by transrectal ultrasonography to determine the day of ovulation (Day 0); only mares with a single ovulation were used. On Day 10, mares were randomly assigned to receive 5 mL of saline, iv (control; n = 6), 100 μg gonadorelin, iv (Fertagyl®; Intervet, Roseland, NJ, US; n = 7), or 1500 IU hCG, iv (Chorulon®; Merck Animal Health, Summit, NJ, USA; n = 7). Plasma progesterone and LH concentrations, ovarian artery resistance index (RI), and percentage of CL with blood flow signals were determined immediately before treatment and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, and 6 h after treatment.

2.2. Hormone concentrations

Blood samples were collected into heparinized vacutainer tubes by venipuncture of the jugular vein. Samples were immediately placed in ice water for 10 min, followed by centrifugation (2000 × *g* for 10 min), plasma separation, and storage (−20 °C) until assayed. The assay procedure for each hormone has been validated and described for mare plasma in our laboratory as indicated. Concentrations of LH were determined by RIA (Ginther et al., 2005b). The intra-assay and interassay CVs and sensitivity were 5.4%, 21.9%, and 0.3 ng/mL, respectively. Progesterone concentrations were determined by a solid-phase RIA kit containing antibody-coated tubes and 125I-labeled progesterone (Ginther et al., 2005a) (ImmuChem Coated Tube Progesterone 125 RIA kit, MP Biomedicals, Costa Mesa, CA, USA). The intra-assay and interassay CVs and sensitivity were 3.5%, 5.8%, and 0.1 ng/mL, respectively.

2.3. Ovarian and luteal blood flow

Ovaries were examined by transrectal ultrasonography using a duplex B-mode (grayscale) and color-Doppler ultrasound scanner (Aloka SSD 3500; Aloka American, Wallingford, CT, USA) equipped with a linear-array 7.5 MHz transducer. All exams were performed by the same operator, who was blind to the treatments. The number and diameter of follicles and CL were noted prior to treatment. Color-Doppler scans were performed at a constant gain setting for color at a pulse-repetition frequency of 8 Hz. A color-Doppler setting of 9.96 cm/s for minimal flow velocity and a filter setting of 4 were used to minimize detection of venous flow and extraneous movement. The color-Doppler mode was used to display signals for blood flow in the ovarian artery close to the attachment of the ovarian plexus or mesovarium to the ovarian surface and in the vessels of the CL. The resistance index (RI) of the ovarian artery within 1 cm of the base of the ovary was determined by placing a cursor gate with a 1 mm opening over the most prominent color-Doppler signal (Ginther, 2007). A Doppler spectrum for each of three cardiac cycles was generated and the most appropriate spectrum (clearer systole/diastole signal) was used for determining ovarian RI; the process was repeated twice and the two RI measurements were averaged for analysis. An image of a section of the CL that appeared to represent the maximum area with color-Doppler signals uncomplicated by artifactual color displays was selected. The percentage of CL covered with color signals was subjectively estimated during real-time exam as previously described (Ginther et al., 2007a).

2.4. Statistical analysis

In addition to the use of actual data, end points that were different among groups at hour 0 were normalized to the time of treatment and transformed to percentage change from hour 0. Data for each analyses were normally distributed on the basis of a Shapiro-Wilk test. Data processing used the statistical analysis system (SAS) PROC MIXED with a REPEATED statement to minimize autocorrelation among sequential measurements (Version 9.4; SAS