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Correlations between ovarian follicular blood flow and superovulatory responses in ewes

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ABSTRACT

The primary goal of this study was to employ ultrasonography to examine the ovaries of ewes undergoing superovulatory treatment for correlations between antral follicular blood flow and ovarian responses/embryo yields. Five Santa Inês ewes were subjected to a short- (Days 0–6, Group 1) and five to a long-term progesterone-based protocol (Days 0–12, Group 2) to synchronize estrus and ovulations after the superovulatory treatment. Porcine FSH (pFSH, 200 mg) was administered in 8 decreasing doses over 4 days, starting on Days 4 and 10 in Groups 1 and 2, respectively. After CIDR removal, all ewes were bred by a ram and embryos were recovered surgically 7 days later. Transrectal ovarian ultrasonography was performed the day before and on all 4 days of the superovulatory treatment. Both an arbitrary-scale [(0) non-detectable; (1) small; (2) moderate; (3) intense blood flow] and quantitative analysis of the blood flow area were used to assess the follicular blood flow in color Doppler images. There were no significant correlations between the arbitrary blood flow scores and superovulatory responses in the ewes of the present study. However, there was a positive correlation between the quantitative estimates of follicular blood flow on the final day of the superovulatory treatment, and the number (DA: $r = 0.68$, $P < 0.05$; DA/TA × 100%: $r = 0.85$, $P < 0.05$) and percentage (DA: $r = 0.65$, $P < 0.05$; DA/TA × 100%: $r = 0.91$, $P < 0.001$) of unfertilized eggs (DA: Doppler area, TA: total area of the largest ovarian cross section). This experiment presents a commercially practical tool for predicting superovulatory outcomes in ewes and evidence for the existence of follicular blood flow threshold that may impinge negatively on oocyte quality when surpassed during hormonal ovarian superstimulation.

1. Introduction

The outcome of superovulatory treatments in sheep is dependent on a number of different intrinsic and extrinsic factors including, but not limited to, the season, breed, age, plane of nutrition, follicle-stimulating hormone (FSH) products and doses used, type of estrus

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synchronization and insemination, and interval between successive treatments (Gordon, 1997; Cognie et al., 2003; Gonzalez-Bulnes et al., 2004; Shipley et al., 2007). In recent years, the methods of superovulation in sheep have evolved from upgrading hormones to technological advancements (Alexander et al., 2010). Follicle-stimulating hormone (FSH) has been used since the 1980s when studies demonstrated that it resulted in a higher production of transferable quality embryos than pregnant mare serum gonadotropin that was the common choice at the time (PMSG; D'Alessandro et al., 1996). A few modified protocols have been devised to maximize superovulatory yields in small ruminants (Shipley et al., 2007; Menchaca et al., 2010). Despite these and several other improvements, the lack of predictability and tremendous variation in ovarian response and embryo yields/quality continues to be a critical issue in multiple ovulation and embryo transfer (MOET) programs. Predicting the outcome of hormonal ovarian superstimulation is financially vital to commercial MOET ventures, has important practical advantages, and is imperative in expanding the application of superovulation in reproductive research (Bartlewski et al., 2008).

Color Doppler sonography models vascularity and blood flow in body tissues and organs on ultrasound imaging (Fleischer and Andreotti, 2005). It is currently used in a wide range of clinical and diagnostic applications, particularly in obstetrics and gynecology when assessing the ovaries, uterus, fetus or placenta. It has also been used to monitor ovarian blood flow and ovarian responses to hormonal treatments during *in vitro* fertilization protocols in women (Zaidi et al., 1996).

During antral follicular growth, there is an increased blood supply to the theca layer comprising the outer wall of ovarian antral follicles (Lass and Brinsden, 1999; Matsui and Miyamoto, 2009). Increased blood flow and velocity are also associated with the ovulatory process, and there are no apparent differences in these two parameters between spontaneous and induced ovulations (Lass and Brinsden, 1999). The use of color Doppler sonography to predict ovarian responses after hormonal ovarian superstimulation has recently been studied in cows (Matsui and Miyamoto, 2009) and horses (Witt et al., 2012), but to the best of the authors' knowledge, a similar study has not been conducted in sheep or any other polyovulatory species.

The follicular blood flow velocity prior to ovulation and the duration of the preceding estrus/ovulation synchronization method may both affect ovarian responses and oocyte competence. Hence, the objective of the present study in Santa Inês ewes was to assess antral follicular blood flow during superovulatory porcine FSH (pFSH) treatment and to compare a semi-quantitative and quantitative approach to analyzing color Doppler sonography images for predicting the superovulatory response after a short- (6 days) or long-term (12 days) progesterone-based synchronization protocol.

2. Materials and methods

The present study was performed on ten anestrous Santa Inês ewes (aged 2–3 years, mean body weight: 42.0 ± 1.8 kg) housed in the research facility in Jaboticabal, São Paulo, Brazil (21°15′ S, 48°17′ W). All ewes were kept in an outdoor paddock with an easy access to a covered pen protecting them from extensive sunlight and heat. The ewes of the present study had lambed twice prior to the present experiment. Animals received daily balanced feed ratios containing corn silage (200 g/ewe/day) and had unlimited access to water and mineral salt licks.

Five ewes were subjected to a short- (Days 0–6, Group 1) and five to a long-term, progesterone-based protocol (Days 0–12, Group 2; CIDR®, Pfizer-New Zealand) to synchronize estrus and ovulations after follicle-stimulating hormone (pFSH) superovulatory regimen (Fig. 1). All ewes received two injections of 37.5 μg of D-cloprostenol (Prolise®, Arsa, Argentina) on Day 0 and at CIDR removal. The superovulatory regimen consisted of 8 i.m. injections of pFSH (Folltropin®-V; Bioniche Animal Health, Belleville, ON, Canada) administrated twice daily (40, 40, 30, 30, 20, 20, 10 and 10 mg). A single i.m. dose of 300 IU of equine chorionic gonadotropin (eCG; Novormon®, Syntex, Buenos Aires, Argentina) was given at the time of CIDR withdrawal. Subsequently, all ewes were bred by a fertile ram and embryos were recovered surgically 7 days later.

The ova/embryos were collected by laparotomy under general anesthesia. Each uterine horn was flushed with 40 ml of flushing media (DPBS®, Cultilab, Brazil) at 37 °C. Flushing media were injected *via* a 20 G catheter inserted at the proximal portion of the uterine horn and collected *via* a no. 10 Foley catheter inserted at the uterine bifurcation. All recovered eggs and embryos were enumerated and the embryos were maintained in holding media (Holding Plus®, Cultilab, Brazil). Morphological evaluation was performed under a stereomicroscope (40× magnification) using the International Embryo Transfer Society (IETS) criteria. Briefly, the embryos that developed to the morula or blastocyst stage at the time of collection were graded from one to four (1–4), with Grade 1 being excellent, Grade 2-good/fair, Grade 3-poor and Grade 4-degenerated (Lindner and Wright, 1993; Rubianes et al., 1995).

Transrectal ovarian ultrasonography was performed the day before and on all 4 days of the superovulatory treatment. Ultrasonographic examinations were carried out by one experienced operator using the color Doppler and B-mode ultrasound system (MyLab VET 30, ESAOTE, Italy) equipped with a stiffened, variable frequency (6–8 MHz), linear-array transducer. Ewes were restrained in a standing position and the abdominal wall was compressed to facilitate the visualization of the uterus and ovaries. The rectum was lubricated using hydrosoluble contact gel prior to insertion of the transducer; the probe was positioned perpendicularly to the abdominal wall and the bladder was identified to orientate the visualization of the uterine horns. The probe was then rotated laterally to both sides to obtain the images of the ovaries and ovarian antral follicles.

B-mode ultrasonography was used to visualize and measure all ovarian antral follicles ≥3 mm in diameter at 1.5–3× image magnification and variable depths. The mean diameter (average of two dimensions, vertical and horizontal) of each identified follicle was taken from still images. After identification of the follicles, the color Doppler was used to determine intraovarian blood flow. The settings of the scanner (Doppler sampling frequency (PRF) = 1.4 kHZ