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Original article

# Are the spectral Doppler indices of ovarian arteries indicative of antral follicular development and predictive of ovulatory responses and embryo yields in superovulated ewes?

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ABSTRACT

Nineteen ewes received 200 mg of pFSH administered in eight decreasing doses from Days 1 to 4, starting three days before CIDR® device removal. Ten ewes received an injection of 350 μg of estradiol benzoate at CIDR® device insertion (Group E) and nine animals served as controls (Group C). B-mode and spectral Doppler ultrasonographic examinations were performed daily throughout superovulatory treatment to enumerate ovarian antral follicles and to determine ovarian blood flow indices, respectively. There were no differences ($P > 0.05$) in superovulatory responses between left and right ovaries/uterine horns or the two groups of animals. End-diastolic velocity (EDV) and mean velocity (Vm) values were greater ($P < 0.05$) on Days 1 and 2, and peak systolic velocity (SVp) was greater ($P < 0.05$) on Day 3 in Group C than in Group E. In Group E 15 correlations was recorded among indices (SVp, Vm, EDV, flow velocity integral-FVI, and pulsatility index-PI) and follicles numbers in different size classes on Days 1, 2 and 4, and seven correlations among indices (SVp, EDV, Vm, and vascular resistance index-RI) and superovulatory/embryo results (numbers of regressing corpora lutea, numbers/percentages of degenerated embryos and viability rates) on Days 1, 2 and 3. In Group C, there were three correlations among EDV and RI and medium-sized/large follicle numbers on Days 1 and 3, and five correlations among indices (EDV, RI and PI) and superovulatory/embryo results (numbers of luteinized unovulated follicles, degenerated embryos and unfertilized eggs) on Days 2 or 4. There was a lack of consistency in the velocimetric correlates of antral follicle numbers and superovulatory responses between the left and right side. Therefore, the usefulness of ovarian arterial indices to predict ovine superovulatory outcomes remains equivocal and requires further confirmatory studies.

## 1. Introduction

The results of hormonal superstimulatory treatments in domestic ruminants are highly variable, which limits the more widespread application of superovulation in commercial MOET (multiple ovulation and embryo transfer) programs [1–8]. Identification of reliable predictors of superovulatory responses in ewes would help to determine the selection criteria for suitable donors and serve to prevent the needless waste on economic cost and animal stress endured by females who respond poorly to superovulatory treatments.

Spectral Doppler sonography is a valuable tool for measuring hemodynamic changes in internal organs and tissues [8]. This imaging technology is based on Doppler-shift frequencies wherein the ultrasound beams reflected by moving erythrocytes vary as the cells move with different velocities. Consequently, the blood flow velocities and other flow-related indices can then be computed during the Doppler scanning [9]. The growth and maturation of ovarian antral follicles is associated with a release of a vast array of angiogenic-like factors,

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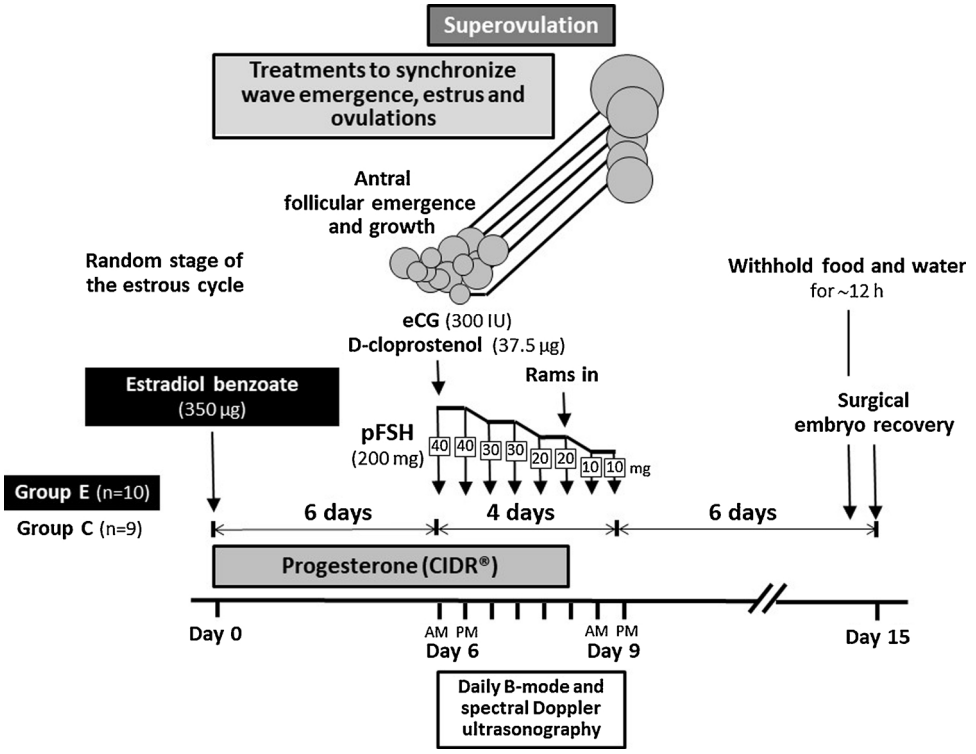


**Fig. 1.** Experimental design. eCG: equine chorionic gonadotropin; pFSH: porcine follicle-stimulating hormone; and CIDR®: controlled internal drug releasing device.

which leads to a rise in blood flow volume and velocity in follicular theca layer and perifollicular ovarian stroma [10–13]. In women, periodic changes in intraovarian vasculature that occur during normal (non-stimulated) ovarian cycles are accompanied by an increase in velocimetric indices measured in ovarian and uterine arteries [10,14,15]. Since superovulation propels the growth and metabolic activity of multiple antral follicles [11], it was suggested that ovaries of animals undergoing hormonal ovarian superstimulation would require a significantly greater blood supply [6].

Few attempts have been made to investigate the connection between the superovulatory response and ovarian blood flow. Honnens et al. [16] reported that blood flow volume (BFV) increased 3.8-fold while pulsatility index (PI) decreased 3.0-fold in the ovarian arteries 7 days after artificial insemination in superovulated cows, and both indices correlated significantly with the number of corpora lutea (CL) [17]. However, there were no correlations between BFV and PI of ovarian arteries before and during hormonal stimulation and the number of follicles and CL that developed after the treatment [17]. Moreover, even though the physical ablation of a dominant follicle before the superovulatory treatment that leads to an increase in embryo yields was associated with a significant change in PI of ovarian arteries, neither BFV nor PI recorded during the gonadotropin stimulation were predictive of superovulatory outcomes in cattle [17]. Alternatively, a study by Witt et al. [18] showed that both the uterine and ovarian arterial PI prior to ovulation were significantly correlated with the ovulation rate and the number of embryos retrieved after superovulation in horses. Similar studies do not exist for any polyovulatory species.

A single injection of estradiol-17β ($E_2$) during the period of pretreatment with progestin-soaked vaginal sponges significantly reduced the variability in ovarian responses and embryo yields without compromising embryo production in superovulated anestrous ewes [19,20]. These effects of the combined progestin-estrogen treatment were due to a transient suppression of FSH release followed by a synchronized re-emergence of multiple ovarian follicles [21,22]. However, the administration of a single dose of $E_2$ to medroxyprogesterone acetate- or progesterone-treated ewes in the breeding season failed to synchronize follicular wave emergence as it did in anestrus and there were no effects of $E_2$ pre-treatment on the superovulatory response [23]. There have been no previous studies of the combined progesterone-estrogen priming on the efficiency of superovulation in ewes raised under subtropical conditions.

Hence, the objectives of this study were to describe blood flow (velocimetric) indices of the ovarian arteries determined daily during the 4-day superovulatory treatment and to examine them for correlations with antral follicular numbers, ovulation rates and the numbers of recovered embryos in Santa Inês ewes with or without estrogen treatment during progesterone priming before the superovulatory regimen. We hypothesized that velocimetric indices measured in both ovarian arteries would be associated with a rise in antral follicle numbers and predictive of superovulatory outcomes in superovulated ewes.

## 2. Material and methods

### 2.1. Location and experimental outline

This study was conducted in the College of Agricultural and Veterinary Sciences (FCAV) situated in the municipality of Jaboticabal (latitude: 21°15'18"S, longitude 48°19'19"W), São Paulo State, Brazil, during the month of July (early period of increasing day lengths or the breeding season of ewes maintained in a subtropical climate). All experimental procedures on live animals had been approved by the Animal Care Committee of the School of Agricultural and Veterinary Sciences (FCAV), São Paulo State University, Jaboticabal, SP, Brazil (protocol no. 020995/12). The animals were kept in outdoor paddocks with easy access to the sheltered area to protect them from intense sunlight and heat. All ewes received daily corn silage supplemented with commercial feed pellets (200 g/ewe/day) and have unlimited access to drinking water and mineralized salt licks.

Nineteen clinically healthy, non-pregnant and non-lactating multiparous Santa Inês ewes (aged 2–3 years) were subjected to a short (8.5 days) progesterone (CIDR®; InterAg, Hamilton, New Zealand) priming

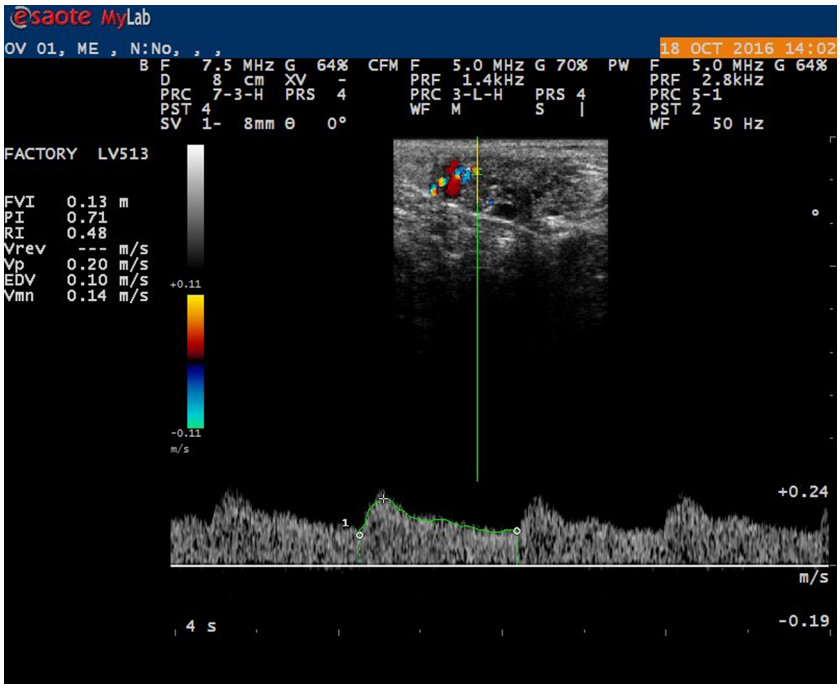


**Fig. 2.** A photographic reproduction of a spectral Doppler scan of the ovarian artery on the periphery of the subovarian vascular plexus and recorded in superovulated Santa Ines ewes. A green and yellow line denotes a position of a caliper placed in a central area of the blood vessel with the apertures to determine the spectral curve (bottom) and to compute velocimetric indices (left).

and a superovulatory treatment with a total dose of 200 mg of porcine follicle-stimulating hormone (pFSH) per ewe (Folltropin®-V; Bioniche Animal Health, Belleville, ON, Canada), given twice a day for four consecutive days in decreasing doses and initiated 6 days after CIDR® insertion (Fig. 1). Ten ewes received a single i.m. injection of estradiol benzoate (350 µg; Sincrodiol®, Ourofino, Brazil) at the time of CIDR® insertion (Group E); the remaining nine animals served as controls (Group C Fig. 2). Ewes were placed in a pen with fertile rams for three days after CIDR® withdrawal (rams to ewes ratio of 1:5).

### 2.2. Ultrasonographic examinations

Transrectal ultrasonographic examinations (B-mode and Doppler mode) using MyLab 20VET scanner equipped with a stiffened, variable frequency (6–8 − MHz) linear-array transducer (Esaote, Italy) were performed once a day throughout the superovulatory treatment (Days 1–4) to enumerate all ovarian antral follicles ≥ 2 mm in diameter and to determine the velocimetric indices of the left and right ovarian arteries (FVI: flow velocity integral; SVp: peak systolic velocity; Vm: mean velocity; EDV: end-diastolic velocity; RI: vascular resistance index (RI = [SVp − EDV]/SVp); and PI: pulsatility index (PI = [SVp − EDV]/ Vm). All spectral data were obtained from a longitudinal subovarian segment of the ovarian artery (Fig. 2); a Doppler gate ranging from 2 to 3 mm in length (~2/3 of the vessel's diameter) was positioned in a central area of the artery at the insonation angle ≤ 60°. Two spectral waves per each ovarian artery were analyzed and mean values recorded for subsequent statistical analyses.

### 2.3. Embryo recovery

Reproductive tracts were exposed, and embryos were recovered surgically (laparotomy) 6 days after the last pFSH dose. Each uterine horn was flushed separately with 40 ml of flushing medium (DPBS®; Cultilab, Campinas, SP, Brazil) at 37 °C injected with a 20 G catheter at the proximal end of the uterine horn and collected via a no. 10 Foley catheter inserted at the uterine bifurcation. All recovered structures were placed in holding media (Holding Plus®; Cultilab, Campinas, SP, Brazil) and counted; embryos that developed to the morula or blastocyst stage were classified based on the International Embryo Transfer Society (IETS) criteria described by Lindner and Wright [24] into four different classes (Classes I-III: transferable quality embryos and Class IV-degenerated embryos). The latter evaluation was done under a stereomicroscope at 40x magnification. In addition, the number of all luteal structures including luteinized unovulated follicles (LUFs) characterized by a lack of ovulatory stigmata and of prematurely regression corpora lutea (pale, ≤ 5 mm in diameter [25];) were recorded at the time of embryo recovery.

### 2.4. Statistical analyses

Initial inspection of the present data did not reveal any outliers (Grubb test). All statistical comparisons were carried out using the SigmaPlot® package (Systat Software Inc., Richmond, CA, USA). Velocimetric data that were not normally distributed were converted by $\log_n$ prior to analyses. Single time-point observations were compared between the left and right side and between the two groups of animals by two-way analysis of variance (ANOVA) whereas blood flow indices and daily numbers of ovarian antral follicles recorded during the study period were analyzed by three-way ANOVA (main effects of the group, side and day of the superovulatory treatment). As in a previous study of superovulatory yields in progestin/estradiol-treated ewes [5], the differences between sample standard deviations for superovulatory responses were analyzed by the F test for variance (http://www.statskingdom.com/220VarF2.html). Differences in proportions were analyzed by a chi-square test (the Brandt-Snedecor formula). Correlations among spectral Doppler indices of ovarian arteries, daily numbers of ovarian antral follicles in different size classes (small: 2–3.49 mm; medium-sized: 3.5–4.49 mm; and large: ≥ 4.5 mm in diameter) and superovulatory responses were examined with the Pearson Product Moment test and simple linear regression; correlation analyses were initially performed separately for the left and right side and then on a per animal basis. All results are given as mean ± standard error of mean (SEM). A P value ≤ 0.05 was considered statistically significant.

**Table 1**
Summary of superovulatory responses in Santa Inês ewes subjected to a short-term (8.5 days) estrous synchronization protocol with progesterone-releasing intravaginal devices, and a multiple-dose pFSH regimen with (Group E) or without (Group C) a single injection of estradiol benzoate on the day of CIDR® insertion (see Fig. 1 for details of experimental design). The same numbers of asterisks in the last row (*, **, ***) denote the mean values with significantly different sample standard deviations (F test for variances). LUFs: luteinized unovulated follicles; CL: corpora lutea; Classes I-IV: embryos classified based on the microscopic criteria as indicative of quality.

| Variable | Group E (n = 10) | | | Group C (n = 9) | | |
|---|---|---|---|---|---|---|
| | Right | Left | Overall | Right | Left | Overall |
| No. of CL | 7.8 ± 0.9 | 6.9 ± 1.4 | 14.6 ± 1.9 | 8.1 ± 1.7 | 6.5 ± 0.8 | 14.5 ± 2.3 |
| No. of LUF's | 0.7 ± 0.3 | 0.4 ± 0.3 | 1.1 ± 0.3 | 0.4 ± 0.2 | 0.6 ± 0.2 | 0.9 ± 0.3 |
| No. of regressing CL | 1.0 ± 1.0 | 0.7 ± 0.7 | 1.7 ± 1.7 | 1.0 ± 0.7 | 1.1 ± 0.7 | 2.1 ± 1.4 |
| No. of recovered structures | 6.5 ± 1.0 | 5.6 ± 1.6 | 12.1 ± 2.3 | 5.4 ± 1.2 | 5.8 ± 1.0 | 11.2 ± 2.1 |
| No. of viable embryos (Classes I-III) | 2.7 ± 0.7 | 3.3 ± 1.8 | 6.0 ± 2.1 | 3.1 ± 1.2 | 3.4 ± 1.1 | 6.5 ± 2.3 |
| No. of unfertilized eggs | 3.1 ± 1.1 | 1.9 ± 0.7 | 5.0 ± 1.7 | 2.2 ± 0.8 | 2.1 ± 0.8 | 4.4 ± 1.6 |
| No. of degenerated embryos (Class IV) | 0.7 ± 0.4 | 0.4 ± 0.2 | 1.0 ± 0.5 | 0.1 ± 0.1 | 0.2 ± 0.2 | 0.3 ± 0.2 |
| Recovery rate (%) | 85.9 ± 7.1 | 75.3 ± 7.1 | 80.0 ± 5.6 | 70.8 ± 10.3 | 85.6 ± 11.2 | 77.1 ± 10.2 |
| Viability rate (%) | 48.0 ± 14.6 | 46.6 ± 14.1 | 48.8 ± 13.8 | 47.4 ± 16.1 | 55.8 ± 14.9 | 51.5 ± 14.7 |
| % of unfertilized oocytes | 40.2 ± 11.4 | 37.4 ± 13.9 | 38.5 ± 11.6 | 51.3 ± 16.0 | 42.0 ± 15.6 | 47.6 ± 15.8 |
| % of degenerated embryos | 11.8 ± 6.6* | 16.0 ± 10.2** | 11.7 ± 6.6*** | 1.2 ± 1.2* | 2.3 ± 2.3** | 2.0 ± 1.4*** |

## 3. Results

### 3.1. Superovulatory outcomes

Superovulatory responses recorded in the ewes of the present study are summarized in Table 1. All ewes had ≥3 normal corpora lutea (CL; positive response to superovulatory protocol) at embryo flushing performed 6 days after the superovulatory pFSH treatment. Luteinized unovulated follicles were detected in 3/9 control ewes and 2/10 Group E animals ($P > 0.05$), and prematurely regression CL were seen in 2/10 Group C ewes and 1 control animal ($P > 0.05$). Only 4/19 collections contained no viable embryos (2 in Group C and 2 in Group E; $P > 0.05$) and 10/19 yielded less than the average number of class I-III embryos per ewe (5 in Group C and 5 in Group E animals; $P > 0.05$). There were no differences ($P > 0.05$) in ovarian responses and embryo yields/quality between left and right ovaries/uterine horns flushed or between estradiol benzoate-treated and control Santa Inês ewes. The variability in the percentage of degenerated embryos was consistently lower ($P < 0.05$) in control compared with that in EB-treated ewes.

### 3.2. Velocimetric indices of ovarian arteries

Mean EDV and Vm were greater ($P < 0.05$) in Group C compared with Group E on Days 1 and 2, and mean SVp was greater ($P < 0.05$) in Group C ewes on Day 3 of the superovulatory treatment compared with Group E (Table 2). In Group E ewes, mean SVp values increased ($P < 0.05$) from Day 1 to Day 4 by 38 %, and mean Vm and EDV rose ($P < 0.05$) from Day 2 to Day 4 of the superovulatory regimen (by 57 % and 75 %, respectively; Table 3).

### 3.3. Antral follicle numbers

Changes in daily numbers of ovarian antral follicles throughout the entire period of pFSH stimulation are shown in Fig. 3. In both groups of superovulated ewes, the number of small (2–3.49 mm in diameter; Fig. 3A) antral follicles was greater ($P < 0.05$) on Days 1 and 2 compared with Days 3 and 4. Overall, the mean number of medium follicles (3.5–4.49 mm in diameter; Fig. 3B) was greater ($P < 0.05$) on Days 3 and 4 compared with Days 1 and 2 of the superovulatory treatment but there were no significant differences in the follicle numbers within each group of ewes. Control ewes exceeded ($P < 0.05$) Group E animals in mean daily numbers of medium-sized follicles on Days 3 and 4 of the treatment; on those days, Group C animals had more ($P < 0.05$) medium-sized follicles than Group E ewes on the right ovary. In general, there were more ($P < 0.05$) medium-sized ovarian follicles on the right compared with the left ovary throughout the entire period of ovarian superstimulation of Santa Inês ewes. In Group E, the mean number of large (≥4.5 mm in diameter; Fig. 3C) antral follicles was greater ($P < 0.05$) on Days 3 and 4 compared with Day 2 and was greater ($P < 0.05$) on Day 3 compared with Day 1 of the superovulatory treatment. Control ewes exceeded ($P < 0.05$) Group E animals in daily numbers of large follicles on Days 3 and 4 and on both these days the overall difference between the left and the right ovary was also significant. Overall, the total number of detected antral follicles was significantly greater on Days 3 and 4 than on Days 1 and 2 of the superovulatory regimen and it was less ($P < 0.05$) on the left compared with right ovary of ewes (Fig. 3D).

### 3.4. Correlations among blood flow indices and antral follicle numbers

A total of 15 correlations for data analyzed on a per animal basis (i.e., overall correlations) was recorded among velocimetric indices of ovarian arteries and antral follicle numbers in Group E ewes (Table 3). Seven correlations were recorded on Day 1, six correlations on Day 2, and two correlations on Day 4 of the superovulatory treatment. Significant correlations were found for five blood flow indices (FVI, PI, EDV, Vm and SVp) and all three follicle size categories. The strongest correlation was recorded between Vm and medium follicle numbers on Day 4 of the superovulatory regimen ($r = 0.90$, $P = 0.0004$). There were 12 correlations recorded for the right ovary and ovarian artery (four on Day 1 and eight on Day 4) and six correlations for the contralateral ovarian artery and ovary (one on Day 2 and five on Day 4). Three significant correlations recorded on Day 1 for the right ovary/ovarian artery were also seen in the data analyzed on a per animal basis (between FVI and medium follicle numbers, between FVI and large follicle numbers, and between Vm and medium follicle numbers), and three correlations for the left ovary/ovarian artery on Days 2 and 4 were also recorded as overall correlations (between Vm and small follicle numbers on Day 1, between PI and large follicle numbers on Day 4, and between PI and medium follicle numbers on Day 4). Only one significant correlation was recorded on the same day for both ovaries/ovarian arteries (between PI and medium follicle numbers on Day 4; Table 3).

Three overall correlations were recorded among velocimetric indices of ovarian arteries and antral follicle numbers in Group C ewes (Table 4); two correlations were recorded on Day 1 and one on Day 3. Significant correlations were only found for two blood flow indices (RI and EDV) and medium/large follicle numbers. The strongest correlation was recorded between EDV and medium follicle numbers on Day 3 of the superovulatory regimen ($r = -0.79$, $P = 0.01$). There was one

**Table 2**
Ovarian artery velocimetric indices in superovulated Santa Inês ewes with (Group E) or without (Group C) an estradiol benzoate injection during the estrous synchronization protocol preceding the pFSH superovulatory treatment. FVI: flow velocity integral; SVp: peak systolic velocity; Vm: mean velocity; EDV: end-diastolic velocity; RI: vascular resistance index (RI = [SVp − EDV]/SVp); and PI: pulsatility index (PI = [SVp − EDV]/ Vm). P < 0.05 (between days (ab) and between treatment groups (*) for each velocimetric variable).

| Variable | Day | Group E (n = 10) | | | Group C (n = 9) | | |
|---|---|---|---|---|---|---|---|
| | | Right | Left | Overall | Right | Left | Overall |
| FVI | 1 | 0.05 ± 0.01 | 0.04 ± 0.004 | 0.05 ± 0.004 | 0.07 ± 0.01 | 0.06 ± 0.01 | 0.06 ± 0.01 |
| | 2 | 0.05 ± 0.01 | 0.05 ± 0.005 | 0.05 ± 0.005 | 0.09 ± 0.01 | 0.08 ± 0.01 | 0.08 ± 0.01 |
| | 3 | 0.13 ± 0.07 | 0.06 ± 0.006 | 0.1 ± 0.04 | 0.07 ± 0.01 | 0.08 ± 0.01 | 0.08 ± 0.01 |
| | 4 | 0.08 ± 0.01 | 0.06 ± 0.008 | 0.07 ± 0.01 | 0.06 ± 0.01 | 0.06 ± 0.01 | 0.06 ± 0.01 |
| SVp | 1 | 0.14 ± 0.01 | 0.12 ± 0.01 | 0.13 ± 0.01[a] | 0.15 ± 0.01 | 0.16 ± 0.01 | 0.15 ± 0.01 |
| | 2 | 0.16 ± 0.01 | 0.16 ± 0.01 | 0.16 ± 0.01[ab] | 0.19 ± 0.03 | 0.20 ± 0.02 | 0.20 ± 0.02 |
| | 3 | 0.16 ± 0.02 | 0.16 ± 0.02 | 0.16 ± 0.01[ab]* | 0.19 ± 0.03 | 0.20 ± 0.03 | 0.20 ± 0.02* |
| | 4 | 0.18 ± 0.03 | 0.19 ± 0.02 | 0.18 ± 0.01[b] | 0.16 ± 0.01 | 0.16 ± 0.02 | 0.16 ± 0.02 |
| Vm | 1 | 0.07 ± 0.01 | 0.06 ± 0.006 | 0.06 ± 0.004[a]* | 0.11 ± 0.03 | 0.09 ± 0.01 | 0.10 ± 0.02* |
| | 2 | 0.07 ± 0.01 | 0.07 ± 0.008 | 0.07 ± 0.006[a]* | 0.11 ± 0.02 | 0.11 ± 0.03 | 0.11 ± 0.01* |
| | 3 | 0.09 ± 0.01 | 0.08 ± 0.007 | 0.09 ± 0.006[ab] | 0.10 ± 0.02 | 0.11 ± 0.04 | 0.11 ± 0.01 |
| | 4 | 0.12 ± 0.02 | 0.1 ± 0.01 | 0.11 ± 0.01[b] | 0.09 ± 0.01 | 0.09 ± 0.01 | 0.09 ± 0.01 |
| EDV | 1 | 0.04 ± 0.01 | 0.03 ± 0.005 | 0.04 ± 0.005[a]* | 0.06 ± 0.01 | 0.05 ± 0.01 | 0.05 ± 0.01* |
| | 2 | 0.04 ± 0.01 | 0.04 ± 0.008 | 0.04 ± 0.007[a]* | 0.07 ± 0.01 | 0.07 ± 0.01 | 0.07 ± 0.01* |
| | 3 | 0.06 ± 0.01 | 0.05 ± 0.007 | 0.05 ± 0.005[ab] | 0.06 ± 0.01 | 0.07 ± 0.01 | 0.07 ± 0.01 |
| | 4 | 0.08 ± 0.02 | 0.06 ± 0.01 | 0.07 ± 0.01[b] | 0.05 ± 0.01 | 0.06 ± 0.01 | 0.06 ± 0.01 |
| RI | 1 | 0.71 ± 0.05 | 0.71 ± 0.05 | 0.71 ± 0.03 | 0.60 ± 0.07 | 0.71 ± 0.07 | 0.67 ± 0.05 |
| | 2 | 0.77 ± 0.05 | 0.75 ± 0.05 | 0.77 ± 0.04 | 0.58 ± 0.06 | 0.61 ± 0.04 | 0.60 ± 0.03 |
| | 3 | 0.62 ± 0.05 | 0.69 ± 0.04 | 0.65 ± 0.03 | 0.69 ± 0.05 | 0.60 ± 0.06 | 0.65 ± 0.04 |
| | 4 | 0.58 ± 0.04 | 0.69 ± 0.05 | 0.63 ± 0.04 | 0.65 ± 0.09 | 0.65 ± 0.04 | 0.65 ± 0.06 |
| PI | 1 | 1.7 ± 0.3 | 1.6 ± 0.2 | 1.6 ± 0.1 | 1.3 ± 0.3 | 1.6 ± 0.3 | 1.5 ± 0.2 |
| | 2 | 1.9 ± 0.3 | 1.7 ± 0.2 | 1.9 ± 0.2 | 1.2 ± 0.08 | 1.2 ± 0.2 | 1.2 ± 0.1 |
| | 3 | 1.2 ± 0.2 | 1.4 ± 0.2 | 1.3 ± 0.1 | 1.4 ± 0.3 | 1.2 ± 0.2 | 1.3 ± 0.2 |
| | 4 | 1.0 ± 0.3 | 1.6 ± 0.4 | 1.3 ± 0.2 | 1.5 ± 0.3 | 1.2 ± 0.1 | 1.3 ± 0.2 |

significant correlation for the right ovary/ovarian artery (between PI and large follicle numbers on Day 2) and six correlations for the contralateral ovarian artery and ovary (four on Day 1 and two on Day 4). Two significant correlations recorded on Day 1 for the left ovary/ovarian artery were also seen when the data were analyzed on a per animal basis (between RI and large follicle numbers, and between EDV and large follicle numbers; Table 4).

### 3.5. Correlations among blood flow indices and superovulatory outcomes

In Group E, there was a total of seven significant overall correlations among spectral Doppler indices of the ovarian arteries and superovulatory outcomes (Table 5); one correlation was recorded on Day 1, four on Day 2 and two on Day 3) of the 4-day superovulatory regimen. Significant correlations were found among four blood flow indices (SVp, EDV, Vm and RI) and four specific superovulatory responses, namely the percentage and number of degenerated embryos, the number of prematurely regressing CL and embryo viability rate. The strongest overall correlation was recorded between Vm on Day 2 and numbers of degenerated embryos ($r = 0.76$, $P = 0.01$) and between RI on Day 4 and numbers of inadequate CL ($r = -0.76$, $P = 0.01$). There were nine correlations recorded for the right uterine horn and ovarian artery (one for a velocimetric index recorded on Day 1, six for the indices recorded on Day 2, and two for the indices recorded on Day 3), and 10 correlations for the left uterine horn and ovarian artery (two for blood flow parameters recorded on Day 1, three for the parameters recorded on Day 3, and five for the parameters recorded on Day 4). Four significant correlations for velocimetric indices recorded on Days 2 and 3 in the right ovarian artery were also seen in the data analyzed on a per animal basis (Day 2: between EDV and the number/percentage of degenerated embryos and between Vm and numbers of degenerated embryos; and Day 3: between RI and numbers of regressing CL; Table 5).

Five overall correlations were recorded among velocimetric indices of ovarian arteries and superovulatory outcomes in Group C ewes (Table 6); two correlations were recorded for velocimetric indices determined on Day 2 and three for the indices determined on Day 4. Significant correlations were only found among three blood flow indices (RI, PI and EDV) and three types of superovulatory outcomes (number of unfertilized eggs, percentage of degenerated embryos, and number of LUFs). The strongest correlation was recorded between RI on Day 4 and the number of LUFs ($r = -0.79$, $P = 0.01$). Three significant correlations for velocimetric parameters recorded on Day 4 in the right ovarian artery were also seen when the data were analyzed on a per animal basis (between PI, RI and EDV and the number of LUFs; Table 6). All significant correlations and times at which they were recorded differed between the left and right side in both groups of superovulated ewes.

## 4. Discussion

A single injection of estradiol-17β ($E_2$) half-way through the 12-day treatment with medroxyprogesterone acetate (MAP)-releasing vaginal sponges in anestrous ewes in the Northern Hemisphere resulted in a transient suppression of FSH release, and accelerated regression of large antral follicles and synchronous follicle wave emergence 5–6 days after the treatment [5]. Ensuing superovulatory regimen, begun at the time of wave emergence, was associated with significantly reduced variability (2- to 3-fold) in the ovulatory response, numbers of LUFs and embryo yields. However, there was no similar effect of the combined $E_2$/MAP or $E_2$/progesterone pre-treatment in cyclic ewes raised in temperate climates [6]. The present experiment was conducted in April or the period of decreasing day lengths in a subtropical climate of Southern Brazil, which corresponds to the mid-breeding season of ewes in the temperate climate [26,27]. Unexpectedly, superovulated ewes that received estradiol benzoate injections at the time of CIDR® insertion showed significantly greater variability in the proportion of degenerated embryos compared with their untreated counterparts. The reasons for these differences in the variability of superovulatory responses between the ewes maintained in temperate and subtropical climatic zones remain to be elucidated.

Doppler ultrasonography has paved the way to exciting new

**Table 3**
Summary of correlations among velocimetric indices of ovarian arteries determined during the 4-day superovulatory treatment of Santa Inês ewes and daily numbers of ultrasonographically detected ovarian antral follicles (small: 2–3.49 mm; medium: 3.5–4.49 mm; and large: ≥4.5 mm in diameter) in estradiol-benzoate primed animals. FVI: flow velocity integral; SVp: peak systolic velocity; Vm: mean velocity; EDV: end-diastolic velocity; RI: vascular resistance index (RI = [SVp − EDV]/SVp); PI: pulsatility index (PI = [SVp − EDV]/ Vm); and r: coefficient of correlation.

| Day | Input (x) variable | Output (y) variable | r | P value | Regression equation |
|---|---|---|---|---|---|
| **Group E** (n = 9) Overall | | | | | |
| 1 | FVI | Small | −0.79 | 0.006 | y = 16.4−127.6x |
| 1 | FVI | Medium | 0.89 | 0.0005 | y = −2.5 + 62.9x |
| 1 | FVI | Large | 0.66 | 0.04 | y = −0.9 + 22.9x |
| 1 | PI | Small | 0.65 | 0.04 | y = 5.5 + 3.0x |
| 1 | EDV | Small | −0.71 | 0.02 | y = 13.7−96.8x |
| 1 | Vm | Small | −0.84 | 0.002 | y = 17.9−119.5x |
| 1 | Vm | Medium | 0.76 | 0.01 | y = −2.5 + 47.3x |
| 2 | FVI | Medium | 0.74 | 0.01 | y = −4.3 + 164.3x |
| 2 | PI | Medium | −0.69 | 0.03 | y = 9.5–3.0x |
| 2 | SVp | Small | −0.67 | 0.03 | y = 28.0−117.8x |
| 2 | Vm | Small | −0.83 | 0.003 | y = 23.4−198.4x |
| 2 | EDV | Medium | 0.69 | 0.03 | y = −0.1 + 103.4x |
| 2 | Vm | Medium | 0.90 | 0.0004 | y = −7.6 + 158.6x |
| 4 | PI | Large | −0.84 | 0.002 | y = 19.9−6.5x |
| 4 | PI | Medium | 0.73 | 0.02 | y = −0.9 + 2.9x |
| **Group E** Right | | | | | |
| 1 | FVI | Large | 0.85 | 0.003 | y = −0.6 + 13.2x |
| 1 | FVI | Medium | 0.88 | 0.002 | y = −1.1 + 28.9x |
| 1 | Vm | Large | 0.67 | 0.05 | y = −0.4 + 7.89x |
| 1 | Vm | Medium | 0.70 | 0.03 | y = −0.9 + 17.5x |
| 4 | FVI | Medium | 0.63 | 0.05 | y = −1.0 + 61.2x |
| 4 | PI | Medium | −0.69 | 0.03 | y = 4.8−1.4x |
| 4 | RI | Medium | −0.65 | 0.04 | y = 7.5−7.0x |
| 4 | SVp | Small | −0.66 | 0.04 | y = 11.2−37.3x |
| 4 | EDV | Small | −0.67 | 0.04 | y = 7.3−54.0x |
| 4 | EDV | Medium | 0.65 | 0.04 | y = 0.6 + 37.8x |
| 4 | Vm | Small | −0.86 | 0.002 | y = 10.5−73.1x |
| 4 | Vm | Medium | 0.63 | 0.05 | y = −0.7 + 39.0x |
| **Group E** Left | | | | | |
| 2 | Vm | Small | −0.69 | 0.04 | y = 9.9−81.9x |
| 4 | FVI | Large | 0.69 | 0.03 | y = 0.8 + 63.7x |
| 4 | PI | Large | −0.82 | 0.003 | y = 7.4−1.6x |
| 4 | PI | Medium | 0.83 | 0.003 | y = -0.7 + 1.2x |
| 4 | RI | Large | −0.86 | 0.001 | y = 13.5−12.5x |
| 4 | EDV | Large | 0.77 | 0.009 | y = 1.6 + 58.2x |

possibilities for the study of ovarian physiology as it has the potential to determine the patterns of ovarian blood flow and hence identify functional changes in ovarian antral follicles and luteal structures [28]. During the normal menstrual cycle, ovarian follicular and stromal peak systolic blood flow velocity (BFV) rose significantly in the ovary bearing a dominant follicle but with no concurrent changes in blood flow resistance or pulsatility, and there were no significant changes in BFV in the contralateral ovary [10]. In another study, it was shown that blood flow in the ovarian artery could be influenced by follicular estrogens as vascular impedance in the ovarian artery of polycystic ovarian syndrome (PCOS) patients remained high and did not change throughout the cycle [29]. Clearly, the increased serum concentrations of ovarian estrogens can cause vasodilatation in the ovarian artery and consequently a decrease in vascular impedance [30]. In the ewes of the present study, mean blood flow velocities increased by the last day of the superovulatory treatment only in estradiol benzoate-treated animals (Table 2). However, even though the numbers of growing, potentially estrogenic follicles increased in response to pFSH injections (Fig. 3B and C), there were no consistent correlations among blood flow velocity (SVp, EDV and Vm) and antral follicle numbers (Table 3). There was also a lack of consistency in the correlations found in the left and right ovary/ovarian artery and on various days of the superovulatory treatment in both groups of superovulated ewes in this study. One of the potential causes of such inconsistencies is the fact that the two ovaries function independently and ovarian antral follicles are histomorphologically different even if they attain similar diameters. A study by Dominguez et al. [31] examined the effects of unilateral ovariectomy in rats and determined that the right ovary is better than the right ovary at maintaining normal ovulation rates when the contralateral ovary is removed; they suggested that this asymmetry might be due to differential catecholaminergic and vagal innervation of the ovaries. An earlier study by McDonald [32] found that 55–65% of ovulations and pregnancies in sheep occurred on the right ovary/uterine horn, which is the side opposite to the rumen; the authors suggested that the presence of rumen decreased ovarian blood flow and the supply of gonadotropins to the ipsilateral ovary. In our experiment, the right and left ovaries had similar blood supply on the consecutive days of the superovulatory treatment yet towards the end of the superovulatory regimen, the total number of ovarian antral follicles was greater on the right compared with the left ovary of ewes; this difference appeared to be caused mainly by the lower number of medium-sized antral follicles on the left ovary in Group E animals.

The extent of vascular plexus development and permeability of capillary blood vessels (and hence the supply of hormonal growth promoters and cholesterol) can be regulated independently within each ovarian follicle [33–35]. In cattle, angiogenesis was observed mainly in the apical part of the inner capillary layer of medium-sized antral follicles and the middle or basal part of the capillary layer of healthy dominant follicles (i.e., ostensibly ovulatory sized follicles) whereas in atretic follicles, large avascular areas were observed in the inner thecal layer. Therefore, color Doppler sonography could be used to estimate the thickness of the follicle wall proper and antral follicular health status in cows [34]. Previous studies in superovulated ewes [12,13] have shown that blood flow velocity in the theca layer of ovarian antral follicles was highly heterogeneous. Histological studies in ewes revealed that the mean thickness of theca externa of Graafian follicle in left ovary of sheep was greater than that in the right ovary [36]. Collectively, the differences in antral follicular histomorphology between the left and right ovaries and highly variable health status/vascularity of individual antral follicles may, at least partly, explain a lack of consistent correlations among ovarian blood supply and antral follicle numbers in both groups of superovulated ewes studied.

Another possible reason for a lack of consistent correlations between Doppler indices in ovarian arteries and antral follicle numbers in this study is a variable collaborative influence of both central and local factors on ovarian blood flow. Central factors include nervous system stimulation and circulating hormone concentrations. Differences in animals' temperament may be responsible for rapid changes in blood pressure [37], especially during the short periods of handling and restraint when ultrasonographic examinations were performed. The movement of animals makes it difficult to record spectral blood flow parameters in the same region of the ovarian artery, which may also alter the velocimetric values [38]. Significant positive correlations have been found between ovarian blood flow and systemic concentrations of progesterone, luteinizing hormone and FSH [39]. Systemic estrogen has also been implicated in the regulation of blood flow, potentially by activating endothelial nitric oxide synthase, which may cause vasodilation [40]. Doppler ovarian blood flow indices correlate positively with serum vascular endothelial growth factor (VEGF) concentrations, which rise after ovarian stimulation [41]. It is feasible that in superovulated ewes ovarian blood supply is initially controlled by the central factors and once the ovaries have surpassed a certain threshold of locally produced substances, ovarian factors may exert effects on ovarian blood flow as well. The ovaries secrete these substances at different rates, which may not always be sufficient to sustain altered blood flow in the ovarian artery. The latter seems to be confirmed, at least partly, by the occurrence of "dominant ovaries" during the superovulatory treatment of ewes in this study. For example, the strength of correlations between FVI and Vm and medium/large follicle numbers on the

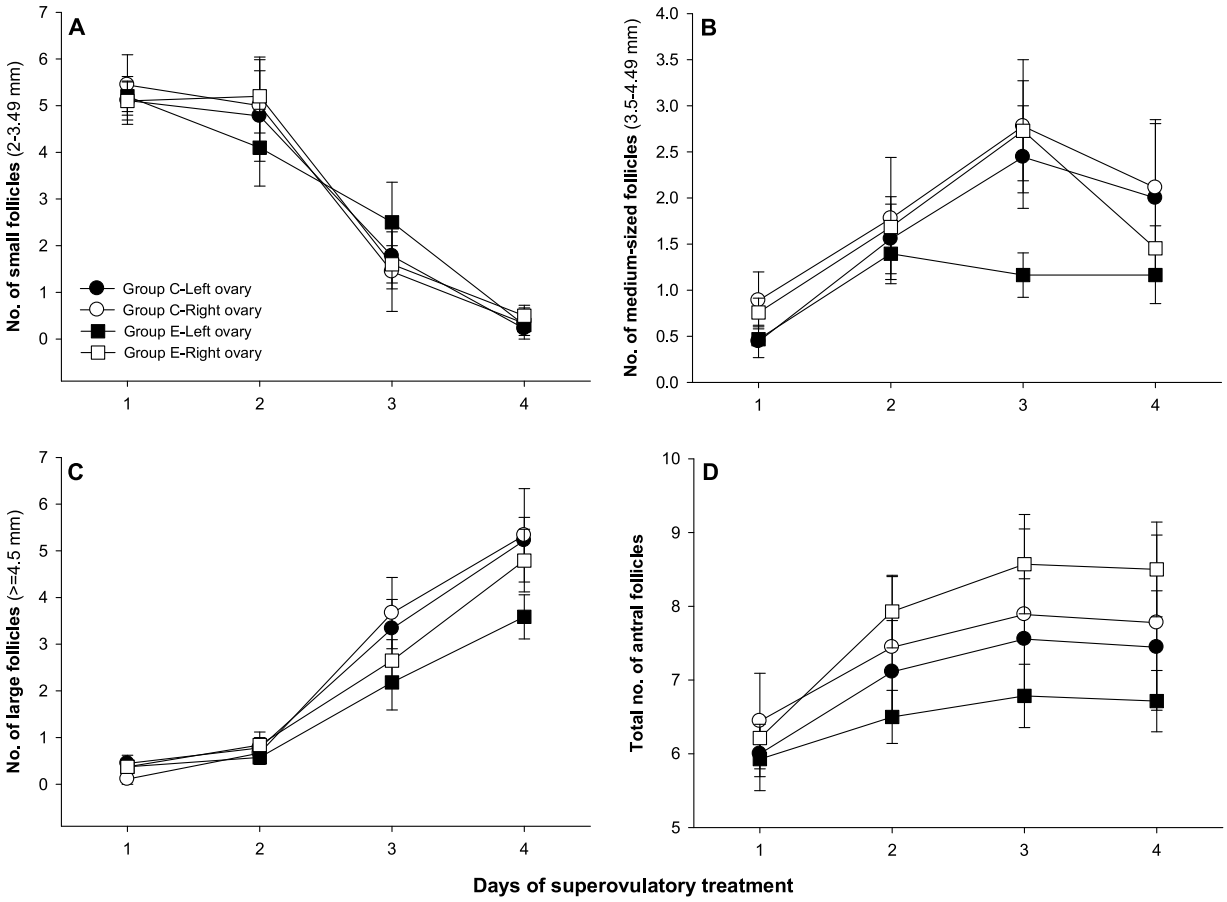


**Fig. 3.** Mean (SEM) daily numbers of ovarian antral follicles in three different size categories (small: 2–3.49 mm; medium-sized: 3.5–4.49 mm; and large: ≥4.5 mm in diameter) and total follicle counts determined ultrasonographically (B-mode) in Santa Inês ewes that underwent hormonal ovarian superstimulation (Days 1–4) with (Group E) or without (Group C) prior injection of estradiol benzoate given at the outset of progesterone treatment.

**Table 4**
Summary of correlations among velocimetric indices of ovarian arteries determined during the 4-day superovulatory treatment of Santa Inês ewes and daily numbers of ultrasonographically detected ovarian antral follicles (small: 2–3.49 mm; medium: 3.5–4.49 mm; and large: ≥4.5 mm in diameter) in control animals. SVp: peak systolic velocity; EDV: end-diastolic velocity; Vm: mean velocity; RI: vascular resistance index (RI = [SVp − EDV]/SVp); PI: pulsatility index (PI = [SVp − EDV]/ Vm); and r: coefficient of correlation.

| Day | Input (x) variable | Output (y) variable | r | P value | Regression equation |
|---|---|---|---|---|---|
| **Group C** (n = 9) Overall | | | | | |
| 1 | RI | Large | −0.76 | 0.02 | y = 2.2 − 2.5x |
| 1 | EDV | Large | 0.70 | 0.04 | y = −0.06 + 11.6x |
| 3 | EDV | Medium | −0.79 | 0.01 | y = 10.5 − 78.2x |
| **Group C** Right | | | | | |
| 2 | PI | Large | −0.80 | 0.01 | y = 3.4 − 2.3x |
| **Group C** Left | | | | | |
| 1 | PI | Large | −0.70 | 0.05 | y = 1.1 − 0.4x |
| 1 | RI | Large | −0.77 | 0.03 | y = 1.9 − 1.9x |
| 1 | SVp | Medium | 0.74 | 0.04 | y = −2.1 + 16.7x |
| 1 | EDV | Large | 0.74 | 0.04 | y = 0.03 + 9.9x |
| 4 | RI | Large | 0.72 | 0.03 | y = −0.4 + 8.7x |
| 4 | EDV | Large | −0.75 | 0.02 | y = 7.1 − 33.3x |

right ovary (Day 1) was associated with significant overall correlations in Group E animals (i.e., with data combined for both ovaries and ovarian arteries). Interestingly, the ovarian dominance was seen mainly for the right ovary, both regarding correlations with antral follicle numbers and superovulatory responses. Moreover, such instances of strongly influential correlations were primarily with medium/large follicle numbers or undesirable superovulatory outcomes (e.g., numbers or percentages of degenerated embryos, numbers of luteinized unovulated follicles or prematurely regressing CL). This might suggest that the events associated with abnormal follicular maturation and oogenesis are most strongly related to ovarian blood flow dynamics in superovulated ewes.

Inherent inequity of function and morphological features of ovarian antral follicles between the left and right ovary, and all the aforementioned influences on arterial blood flow may also explain a lack of consistent correlations between the changes in ovarian blood supply and superovulatory responses. In addition, even though several studies suggested that ovarian responses are related to the numbers of small antral follicles present at the beginning of the superovulatory treatment, other studies concluded that ovulatory rates after superovulation were only correlated with the numbers of healthy, gonadotropin-responsive antral follicles [6]. The latter is probably a major reason for the existence of significant correlations among high-velocity blood flow (determined in antral follicles with the use of color Doppler sonography) and superovulatory responses in ewes, since increased capillary perfusion is a marker of follicular health [13]. In addition, the preattachment embryos and possibly unfertilized eggs can migrate between the two uterine horns in ewes, potentially resulting in the difference between numbers of ovulating follicles and retrieved structures on each side of the reproductive tract. However, this should not preclude the detection of positive correlations between the two variables with the data analyzed on a per animal basis. Moreover, no embryos that apparently migrated to the uterine horn on the opposite side of the body to the ovary from which the egg originated were seen in cyclic ewes with CL in each ovary [42].

**Table 5**
Summary of correlations among velocimetric indices of ovarian arteries determined during the 4-day superovulatory treatment of Santa Inês ewes and superovulatory responses recorded in estradiol-benzoate primed animals 6 days after the end of the pFSH regimen. SVp: peak systolic velocity; EDV: end-diastolic velocity; Vm: mean velocity; RI: vascular resistance index (RI = [SVp − EDV]/SVp); PI: pulsatility index (PI = [SVp − EDV]/ Vm); and r: coefficient of correlation.

| Day | Input (x) variable | Output (y) variable | r | P value | Regression equation |
|---|---|---|---|---|---|
| **Group E** (n = 10) Overall | | | | | |
| 1 | SVp | % degenerated embryos | 0.65 | 0.04 | y = −80.3 + 707.3x |
| 2 | EDV | No. of degenerated embryos | 0.66 | 0.04 | y = −0.6 + 44.7x |
| 2 | EDV | % degenerated embryos | 0.63 | 0.04 | y = −10.7 + 604.6x |
| 2 | Vm | No. of degenerated embryos | 0.76 | 0.01 | y = −3.3 + 60.5x |
| 2 | Vm | % degenerated embryos | 0.72 | 0.02 | y = −45.7 + 808.3x |
| 3 | RI | No. of regressed CL's | −0.76 | 0.01 | y = 27.0−38.7x |
| 3 | Vm | Viability rate | 0.64 | 0.04 | y = −75.9 + 1449.7x |
| **Group E** Right | | | | | |
| 1 | Vm | No. of LUF's | −0.70 | 0.04 | y = 2.1−20.4x |
| 2 | FVI | Recovery rate | 0.70 | 0.02 | y = 42.3 + 853.2x |
| 2 | SVp | No. of CL | −0.63 | 0.05 | y = 14.7−42.8x |
| 2 | RI | No. of degenerated embryos | −0.64 | 0.04 | y = 4.4−4.8x |
| 2 | EDV | No. of degenerated embryos | 0.71 | 0.02 | y = −0.4 + 29.1x |
| 2 | EDV | % of degenerated embryos | 0.67 | 0.03 | y = −5.9 + 455.6x |
| 2 | Vm | No. of degenerated embryos | 0.73 | 0.02 | y = −1.6 + 31.4x |
| 3 | RI | No. of regressed CL | −0.71 | 0.02 | y = 7.3−10.5x |
| 3 | EDV | No. of viable embryos | 0.63 | 0.05 | y = −0.08 + 48.8x |
| **Group E** Left | | | | | |
| 1 | FVI | No. of regressed CL | −0.65 | 0.04 | y = 5.6−114.1x |
| 1 | SVp | No. of LUF's | 0.73 | 0.02 | y = −2.1 + 20.5x |
| 3 | PI | Recovery rate | −0.62 | 0.05 | y = 112.3−26.3x |
| 3 | RI | No. of unfertilized eggs | −0.63 | 0.05 | y = 10.3−12.2x |
| 3 | RI | Recovery rate | −0.63 | 0.05 | y = 154.3−115.3x |
| 4 | FVI | No. of CL | 0.79 | 0.007 | y = −2.7 + 149.6x |
| 4 | FVI | No. of recovered structures | 0.80 | 0.005 | y = −5.1 + 167.2x |
| 4 | FVI | No. of viable embryos | 0.71 | 0.02 | y = −7.4 + 167.6x |
| 4 | Vm | No. of regressed CL | 0.64 | 0.04 | y = −3.3 + 41.6x |
| 4 | Vm | Recovery rate | 0.69 | 0.03 | y = 31.3 + 453.6x |

**Table 6**
Summary of correlations among velocimetric indices of ovarian arteries determined during the 4-day superovulatory treatment of Santa Inês ewes (Days 1–4) and superovulatory responses recorded in control animals 6 days after the end of the pFSH regimen. SVp: peak systolic velocity; EDV: end-diastolic velocity; Vm: mean velocity; RI: vascular resistance index (RI = [SVp − EDV]/SVp); PI: pulsatility index (PI = [SVp − EDV]/ Vm); and r: coefficient of correlation.

| Day | Input (x) variable | Output (y) variable | r | P value | Regression equation |
|---|---|---|---|---|---|
| **Group C** (n = 9) Overall | | | | | |
| 2 | RI | No. of unfertilized eggs | 0.68 | 0.04 | y = −16.0 + 34.1x |
| 2 | EDV | % of degenerated embryos | 0.73 | 0.04 | y = −4.5 + 96.7x |
| 4 | PI | No. of LUF's | −0.69 | 0.04 | y = 3.1−1.6x |
| 4 | RI | No. of LUF's | −0.79 | 0.01 | y = 4.2−5.1x |
| 4 | EDV | No. of LUF's | 0.74 | 0.02 | y = −0.4 + 23.1x |
| **Group C** Right | | | | | |
| 2 | FVI | No. of LUF's | −0.67 | 0.05 | y = 1.5−12.2x |
| 2 | FVI | No. of degenerated embryos | 0.69 | 0.04 | y = −0.4 + 5.7x |
| 2 | SVp | No. of degenerated embryos | 0.82 | 0.007 | y = −0.5 + 3.4x |
| 2 | SVp | % of degenerated embryos | 0.84 | 0.008 | y = −5.4 + 34.8x |
| 3 | FVI | Recovery rate | −0.81 | 0.008 | y = 128.3−784.7x |
| 3 | SVp | No. of LUF's | 0.70 | 0.03 | y = 0.1 + 0.08x |
| 3 | SVp | No. of recovered structures | −0.72 | 0.03 | y = 11.0−29.3x |
| 3 | SVp | Recovery rate | −0.66 | 0.05 | y = 115.0−232.9x |
| 3 | EDV | No. of LUF's | 0.68 | 0.04 | y = −0.3 + 11.9x |
| 3 | EDV | No. of recovered structures | −0.76 | 0.02 | y = 9.4−65.1x |
| 3 | Vm | No. of LUF's | 0.66 | 0.05 | y = −0.6 + 10.5x |
| 3 | Vm | No. of recovered structures | −0.72 | 0.03 | y = 11.2−56.6x |
| 3 | Vm | Recovery rate | −0.66 | 0.05 | y = 116.2−448.9x |
| 4 | PI | No. of LUF's | −0.71 | 0.03 | y = 1.5−0.7x |
| 4 | RI | No. of LUF's | −0.75 | 0.02 | y = 1.8−2.0x |
| 4 | EDV | No. of LUF's | 0.79 | 0.01 | y = −0.3 + 14.1x |
| **Group C** Left | | | | | |
| 1 | RI | Recovery rate | 0.79 | 0.02 | y = −10.3 + 132.8x |
| 1 | EDV | Recovery rate | −0.82 | 0.01 | y = 118.4−728.7x |
| 3 | EDV | No. of unfertilized eggs | 0.76 | 0.02 | y = -2.0 + 55.8x |
| 3 | EDV | Viability rate | −0.70 | 0.05 | y = 124.0−866.4x |
| 3 | EDV | % of unfertilized eggs | 0.70 | 0.05 | y = −29.6 + 908.6x |
| 4 | PI | Recovery rate | −0.66 | 0.05 | y = 156.6−60.2x |

In summary, the administration of a single dose of estradiol benzoate to CIDR®-treated Santa Inês ewes during the breeding season in the subtropics failed to suppress the growth of large antral follicles. It remains to be determined if a lack of inhibitory effects of the combined estrogen/progesterone treatment on antral follicle numbers in Santa Inês ewes is due to: i. a difference in the time of estrogen administration (at the time of CIDR® insertion vs. half-way through the 12-day period of treatment with progestin-releasing intravaginal sponges); ii. progestin or estrogen used (natural progesterone vs. medroxyprogesterone acetate and estradiol-17β vs. estradiol ester); or iii. climate-dependent neuroendocrine differences in the functioning and sensitivity of the ewes' hypothalamo-pituitary unit. Ovarian responses and embryo yields following the superovulatory treatment started 5 days after estrogen injection did not vary between EB-treated and control ewes in this study but individual variability in the proportion of degenerated embryos was greater in estradiol benzoate-primed animals. Results of the present study also show that the 4-day superovulatory treatment with pFSH in ewes resulted in an overall increase in blood flow velocity indices. Small follicle numbers declined whereas daily numbers of medium-sized and large antral follicles detected with transrectal ultrasonography increased in response to a gonadotropic stimulation. We also observed differences (i.e., reduced populations of small antral follicles) between the left and right ovary of superovulated ewes. Multiple significant correlations were found between the velocimetric indices and superovulatory responses, but they varied among days, the two ovaries, and the two groups of animals studied. Several histophysiological factors and certain limitations of the spectral Doppler measurements in restrained animals may be a reason for its limited diagnostic and prognostic value in ewes undergoing hormonal ovarian superstimulation. More studies are needed to corroborate the ovarian and cardiovascular basis for potential correlations among arterial blood supply, antral follicular health status and superovulatory results in ruminant species.

## Declaration of Competing Interest

The authors have nothing to disclose.

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