

## Temporal associations of B-mode, power doppler, and ovarian steroid changes of the periovulatory follicle and corpus luteum during luteogenesis and luteolysis in jennies

Humberto Magalhaes,<sup>a,b</sup> Jose Dell-Aqua Jr,<sup>b</sup> Igor Canisso<sup>a</sup>

<sup>a</sup>Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL; <sup>b</sup>Department of Animal Reproduction, São Paulo State University (UNESP), Brazil

Assessing the relationships between B-mode and power doppler ultrasonography of the periovulatory donkey follicle and respective corpus luteum (CL) could prove useful in clinical practice to predict impending ovulation and determining CL viability. This study's objectives were to assess the associations between B-mode and power doppler ultrasonography and ovarian steroids of the periovulatory follicle and respective CL during luteogenesis and luteolysis in jennies. We hypothesized that information on changes in the granulosa are useful to detect impending ovulation and on blood flow are useful to differentiate an active versus inactive CL. One inter-ovulatory interval between 2 subsequent ovulations of 12 jennies (144 ± 22.5 kg; height 95.5 ± 113 cm) was used. Jennies were teased daily to a mature jack. B-mode ultrasonography was carried out until the detection of a periovulatory follicle (≥ 28 mm, endometrial edema, and estrous signs). Thereafter, jennies were monitored every 4 hours by B-mode and power doppler transrectal ultrasonography. Once presumed signs of impending ovulation (thickened and irregular follicular wall, hyper-echogenicity of granulosa layer in transrectal ultrasonography and softened follicle in transrectal palpation) were detected, jennies were reexamined at 1 hour intervals until ovulation. After ovulation, the CL was examined daily until the completion of luteolysis (progesterone < 1.5 ng/ml). Plasma estradiol and progesterone concentrations were assessed daily with chemiluminescence assays (Immulate 1000, Siemens, US). Data were analyzed using RM-ANOVA followed by Tukey's test (steroid concentrations, follicle and corpus luteum parameters), Friedman test adjusted by Dunn's test (edema score and behavior), and Pearson's coefficient correlations (thickness and echogenicity of the granulosa layer). Mouth-clapping, a species-specific estrous sign, was the first and the last sign to be detected (± 24 hours postovulation). The diameter of the ovulatory follicle was 34.6 ± 3.3 mm (31 - 38 mm). The echogenicity and the thickness of the granulosa layer increased ( $p < 0.05$ ) from 36 to 1 hour before ovulation in 70% of jennies; strong correlations between thickness ( $r = 0.70$ ), granulosa echogenicity ( $r = 0.80$ ), and impending ovulation were noted. Follicular wall blood flow increased ( $p < 0.05$ ) from 72 to 24 hours before ovulation and estradiol concentrations declined from 42 pg/ml at 72 hours to 31.6 pg/ml at 24 hours before ovulation. Vascularization of periovulatory follicle decreased ( $p < 0.05$ ) from 62% (36 hours before ovulation) to 37% (1 hour before ovulation); 75% of the jennies had a homogenous CL echogenicity with a white hyperechogenic central lacuna. The maximum CL size represented 76% of the periovulatory follicle diameter. Vascularization of the CL and progesterone concentrations had a gradual rise, reaching the

peak at 11 and 10 days after the ovulation, respectively ( $p < 0.05$ ). Luteal echo-genicity increased ( $p < 0.05$ ) 4 days after luteolysis as a consequence of corpus albicans formation). Vascularization of the CL started to decline ( $p < 0.05$ ) 3 days before luteolysis and progesterone concentrations had a sharp reduction ( $p < 0.05$ ) for 4 days before luteolysis. In conclusion, the structural changes of the periovulatory follicle detected on B-mode can be used to detect impending ovulation in donkeys; however, B-mode ultrasonography cannot be used to assess CL functionality. Conversely, power doppler can be used to differentiate a functional versus nonfunctional CL in jennies.

**Keywords:** Periovulatory period, luteogenesis, luteolysis, steroid

## Suitability of noncycling recipient mares for in vitro produced equine embryos

Charles Scoggin, Etta Bradecamp, Jamie Kaczor, Erin Lohbeck, Crystal Howard, Holly Hersey, Alaina Broach

Rood and Riddle Equine Hospital, Lexington, KY

Suitable cycling recipient mares are not always available at an equine embryo transfer station (ETS). Noncycling recipients primed with estrogen followed by progesterone before transfer of in vivo embryos can have similar pregnancy rates as those that are cycling. However, in vitro produced (IVP) embryos may be less hardy and require a more precise window of synchrony with recipient mares compared to in vivo embryos.<sup>1</sup> To our knowledge, no data exist on the suitability of noncycling recipient mares when transferring IVP embryos. A retrospective study was conducted whereby data from a single ETS during the 2020 breeding season were analyzed and pregnancy rates were compared between cycling and noncycling recipients receiving IVP embryos. All embryos were derived from commercial donors and transferred at the ETS. Cycling recipients (CRs) were examined via transrectal ultrasonography until emergence of a dominant follicle, then evaluated daily until ovulation and subsequently received an IVP embryo 4 days postovulation. Noncycling recipients (NCRs) were determined to be anestrus or transitional by a lack of luteal tissue and the presence of small follicles based on transrectal ultrasonography and serum progesterone concentrations. Initially, NCRs were treated intramuscularly with 10 mg estradiol-17 $\beta$  in oil ( $E_2$ ) on the first day, 6.6 mg on the second day, and 3.3 mg  $E_2$  on the third day. The day after the last  $E_2$  treatment, NCRs were treated intramuscularly with 200 mg progesterone in oil ( $P_4$ ); once a day for 3 consecutive days. On the fourth day (at transfer), NCRs received intramuscularly 500 mg of  $P_4$  and were supplemented with  $P_4$  at least until their first pregnancy examination. Data were analyzed using Chi-Square or Fisher's Exact tests, and values considered significant at  $p < 0.05$ . During the study, 78 IVP embryos were shipped to the ETS. Of these, 60.3% (41/78) were transferred into CRs and 39.7% (31/78) were transferred into NCRs. Overall transfer rate for IVP embryos was 52.6% (41/78). Day 42 pregnancy and embryo loss rates were 30.8% (24/78) and