

414 COLOR DOPPLER EVALUATION OF UMBILICAL BLOOD FLOW DURING PREGNANCY IN COWS

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There are few reports describing the use of Doppler for reproductive imaging in large animals; only one study (in mares) assessed umbilical blood flow (Bollwein *et al.* 2004 *Theriogenology* 61, 499–509). Conditions that constrict the placental vascular bed (i.e. hemorrhage, thrombosis, abnormal development, etc.) increase resistance to incoming blood. Thus, blood flow in umbilical arteries can be used to monitor placental development and function (Giles *et al.* 1985 *Brit. J. Obstet. Gynaecol.* 92, 31–38). The objective of the present study was to characterize the Doppler flow velocity waveform in umbilical arteries of cows with apparently normal pregnancies. Twenty-three multiparous, nonlactating Aberdeen Angus cows with pregnancies achieved after transfer of embryos derived by IVF ($n = 10$) or multiple ovulation and embryo transfer (MOET) ($n = 13$) were examined weekly from 5 to 38 weeks of gestation. Ultrasonography (Toshiba Nemio 20, Tokyo, Japan) was done using a 5–10 MHz intraoperative finger transducer (transrectal) from 5 to 17 weeks of pregnancy and thereafter using a 3–6 MHz linear-array transducer (transabdominal). The interrogation angle (between the ultrasound beam and the artery) ranged from 45° to 60°. Three resistance indices were calculated: A/B ratio, Resistance Index (RI) = $(A - B)/A$, and Pulsatility Index (PI) = $(A - B)/M$. [A = systole, B = diastole, and M = mean maximum Doppler-Shift frequency over the cardiac cycle.] The mean \pm SEM duration of pregnancy was 285 ± 1.6 days (range: 269 to 291 days). A repeated measure ANOVA was used to detect differences between groups for every week using the Wald-Wolfowitz test (InfoStat V1.5, FCA, Universidad de Córdoba, Córdoba, Argentina). All 3 resistance indices decreased (by $> 50\%$) until 26 weeks, with no substantial change thereafter (Table 1). From 5 to 18 weeks of pregnancy, blood flow was characterized by a systolic pattern (i.e. high resistance with absence of diastolic flow); at 20 weeks, all fetuses had a diastolic flow, consistent with low resistance. There were differences ($P < 0.05$) between IVF- and MOET-derived pregnancies for RI and PI at 38 and 26 weeks of gestation, respectively, indicating differences in the placental vascular development at these weeks of gestation. In conclusion, umbilical cord blood flow in cattle was characterized by high resistance (5 weeks of gestation), but resistance decreased until 26 weeks; consequently, blood flow was initially systolic but became diastolic. Doppler sonography was useful for assessment of umbilical blood flow from 5 to 38 weeks of pregnancy, and may be useful for assessing placental function in pregnancies under risk, e.g. clone-derived pregnancies (Bertolini *et al.* 2002 *Theriogenology* 57, 181–187).

Table 1. RI and PI at 19, 22, 26, 30, and 38 weeks of pregnancy in IVF- and MOET-derived pregnancies (mean \pm sd)

Week of pregnancy	RI IVF	RI MOET	PI IVF	PI MOET
19	0.88 \pm 0.06 ^a	0.94 \pm 0.02 ^a	1.71 \pm 0.28 ^a	2.17 \pm 0.36 ^a
22	0.78 \pm 0.06 ^a	0.85 \pm 0.06 ^a	1.34 \pm 0.19 ^a	1.51 \pm 0.16 ^a
26	0.69 \pm 0.04 ^a	0.69 \pm 0.08 ^a	1.08 \pm 0.16 ^a	1.11 \pm 0.21 ^b
30	0.61 \pm 0.05 ^a	0.65 \pm 0.06 ^a	0.88 \pm 0.11 ^a	1.00 \pm 0.15 ^a
38	0.56 \pm 0.09 ^a	0.71 \pm 0.07 ^b	0.81 \pm 0.19 ^a	1.25 \pm 0.25 ^a

^{a,b}Values with different superscripts are different ($P < 0.05$).

415 USE OF COMPUTER-ASSISTED ULTRASOUND IMAGE ANALYSIS IN EMBRYO RECIPIENT SELECTION

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Ultrasonography has been extensively used in the study of reproductive function in areas such as antral follicular dynamics, ovulation, luteal function, and early pregnancy. However, most studies with ultrasound evaluation are limited to measurement of diameter, length, or area of internal structures, and few groups use differences in image density (echotexture) as an evaluation parameter. For corpora lutea, echo-texture differences reflect variations in vascularization and luteal cell proportions. The aim of the present study was to evaluate image analysis as a tool for embryo recipient selection. Grades I or II embryos produced by conventional superovulation ($n = 55$) or IVF ($n = 87$) were nonsurgically transferred to primiparous cows or heifer recipients ($n = 142$). Estrous synchronization was performed using the heat-synch protocol (Day 0: CIDR[®]; Day 5: 400 IU of eCG; Day 7: 500 μ g of cloprostenol plus CIDR removal; Day 9: 2 mg of estradiol benzoate). Sonographic corpora lutea images were obtained on the day of embryo transfer (Day 7), using a portable ultrasound device (Aloka SSD 500; Aloka Co., Sao Paulo, Brazil) equipped with a linear rectal 5 MHz probe. Images were digitalized in .TIFF format at a resolution of 1500×1125 , using a video capture board (Pinnacle DC10; Pinnacle Systems, Mountain View, CA, USA). A representative elementary area (REA) of 2.704 pixels (25 mm²) was defined in the luteal tissue, using the criteria proposed by Van den Bygaart *et al.* (1999 *Can. J. Soil Sci.* 79, 149–160). Image analysis was performed using a custom software package (QuantPro[®]). Each

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image dot (pixel) received a numeric value ranging from 0 (black) to 255 (white). Data from cows and heifers later diagnosed as pregnant or not in the groups receiving SOV or IVF embryos were analyzed by ANOVA, and results are presented as means \pm SEM. As expected, the pregnancy rate was higher for conventional than for IVF embryos (58.2% vs. 31.0%; $P < 0.05$). There was a low correlation ($R = 0.33$) between luteal tissue area and pixel value for Day 7 corpora lutea. There was no difference ($P > 0.05$) in luteal tissue area or echotexture between pregnant and non-pregnant animals in the groups receiving superovulation embryos ($3.12 \pm 0.26 \text{ cm}^2$ vs. $2.63 \pm 0.25 \text{ cm}^2$, and 78.03 ± 2.25 vs. 79.73 ± 3.73 , respectively) and IVF embryos ($3.25 \pm 0.24 \text{ cm}^2$ vs. $3.03 \pm 0.14 \text{ cm}^2$, and 74.81 ± 1.93 vs. 70.82 ± 1.62 , respectively). However, in the IVF embryo group, no pregnancy was established in cows bearing a CL with mean pixel value lower than 60.25 or greater than 89.27 (total values ranged from 44.72 to 99.79). Corpora lutea image analysis, using the REA proposed in this approach, had limited value to predict pregnancy rate of embryo recipients. Further studies will investigate the accuracy of the REA established and other image characteristics, including the pixel distribution pattern.

416 EFFECTS OF THE CORPUS LUTEUM WITHIN THE OVARY ON THE FOLLICULAR DYNAMICS AFTER FOLLICULAR ASPIRATION AND ON THE DEVELOPMENTAL COMPETENCE OF ASPIRATED OOCYTES

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The use of ultrasound-guided transvaginal follicular aspiration combined with *in vitro* embryo production is increasing in the commercial cattle breeding industry. However, frequent puncture of follicles can induce the dysfunction of corpora lutea (CL) (Petyim *et al.* 2001 J. Vet. Med. A 48, 449–463), resulting in irregular estrous intervals or absence of estrus. This study was conducted to compare follicular and oocyte characteristics in ovaries ipsilateral vs. contralateral to the CL after follicular aspiration. The experiment was performed using a crossover design with each Japanese Black cow ($n = 7$) assigned to each of the 3 groups. The treatment period was 30 days for each group, and successive treatments were separated by a 2-month rest period. Hence the study spanned a period of 7 months (3 treatment periods and 2 rest periods). To verify ovulation prior to each treatment, the stage of the estrous cycle in each cow was synchronized with an ovulation synchronization (Ovsynch) program. Follicular aspirations were performed 6 times (every 3 or 4 days) between Day 3 and Day 20 after GnRH administration (Day = 0) of the Ovsynch program. All cows in each group ovulated the follicle before the initiation of follicular aspiration treatment. Follicles (≥ 3 mm in diameter) from the ovary ipsilateral (Group 1) or contralateral (Group 2) to the CL were aspirated using B-mode ultrasound scanner equipped with a 7.5 MHz probe. As a control (Group 3), the follicles from both ovaries were aspirated, irrespective of the presence of CL. In all groups, follicular development in each cow was monitored daily from Days 0 to 31. The cumulus-oocyte complexes (COCs) collected from each cow in Group 1 ($n = 158$), Group 2 ($n = 140$), and Group 3 ($n = 277$) were subjected to IVM/IVF/IVC. The numbers of embryos cleaved and developed to the blastocyst stage were recorded 3 days and 8 days after IVF, respectively. The mean profiles of total visible follicles (≥ 3 mm in diameter) in ovaries with follicular aspiration in Groups 1 and 2 were similar to those in Group 3, irrespective of the presence of CL. The average numbers of visible follicles in the ovaries with follicular aspiration 7 days after the end of follicular aspiration treatment were not different from those in the ovaries without follicular aspiration ($P > 0.05$; one-way ANOVA). Moreover, there were no significant differences among the groups with respect to the percentages of cleavage (57.5–65.7%) and development to the blastocyst stage (23.6–32.1%) of collected COCs ($P > 0.05$; chi-square analysis). These results indicate that the presence of CL within the ovaries does not affect the ovarian follicular dynamics after follicular aspiration and the developmental competence of collected COCs.

Reproduction, Fertility and Development

Proceedings of the Annual Conference of
the International Embryo Transfer Society,
Kyoro, Japan, 6–10 January 2007

Volume 19(1) 2007

www.publish.csiro.au/journals/rfd