Effect of hormonal cervical dilation protocol on the serum progesterone concentration, embryo development and quality in sheep

Efeito do protocolo hormonal de dilatação cervical sobre a concentração sérica de progesterona, desenvolvimento e qualidade de embriões ovinos

<u>Juliana Dantas Rodrigues Santos^{1,*}</u>, Mário Felipe Alvarez Balaro¹, Augusto Ryonosuke Taira¹, Caroline Gomes do Espírito Santo¹, Clara Vieira de Souza¹, Viviane Lopes Brair¹, Ana Luiza Cunha Bade¹, Joanna Maria Gonçalves Souza-Fabjan¹, Jeferson Ferreira da Fonseca², Felipe Zandonadi Brandão¹

¹Faculdade de Veterinária da Universidade Federal Fluminense, Niterói, RJ, Brasil; ²Embrapa Caprinos e Ovinos, Coronel Pacheco, MG, Brasil.

*E-mail: julianadantas_rodrigues@hotmail.com

The complex sheep cervical structure has been an obstacle to uterine access and to non-surgical embryo collection by transcervical route. Thus, hormonal protocols aiming at the relaxation of the cervix become important for the development of this technique. However, the hormonal environment of embryo donors needs to be compatible with embryo formation, and little is known about the effect of hormones used in the cervix dilatation protocols on progesterone (P4) concentration, as well as on embryo development and quality (EQ). Thus, this study aimed to evaluate the influence of a hormonal protocol of cervical dilatation, on the P4 serum concentration, stages of development and EQ. For this, 40 Santa Inês sheep were submitted to day zero protocol (Pinto P et al., 2018. Theriogenology, 113: 146-152) followed by natural mating. The animals were allocated into: treated group (Treat; n = 20), which consisted in administration of estradiol benzoate (100 µg, i.v.) and cloprostenol (0.12 mg, i.m.) 12 h prior to embryo collection associated with the application of oxytocin (100 IU, i.v.) 15 min prior to collection procedure (Leite L et al. Arg Bras Med Vet Zootec, 70:1671-1679); the other group was the control (Cont; n = 20), with saline administration. All embryos were collected by laparotomy and classified according to criteria established by the IETS. Blood samples were collected prior to protocol (dilation or control; 0 h), 3 h, 6 h, 9 h, and 12 h after hormone or saline administration. Serum was used for the P4 measurement by radioimmunoassay. Data were tested for normality and homoscedasticity with the Lilliefors and Bartlett's tests, respectively. P4 concentrations were compared by paired ANOVA followed by t test (normal data), and by the Mann Whitney or Friedman test for non-parametric data. Embryo data were evaluated by Fisher's test, all with a significance level of 5%. The control group had a tendency to a higher total number of embryos recovered per female (Treat: $3.8 \pm 3.6 \text{ vs.}$ Cont: 5.4 ± 2.7 ; P = 0.058). For the viable embryos by female, the control group (3.4 ± 2.4) was superior (P < 0.05) when compared to the treated group (2.0 ± 2.9) . Regarding the frequency distribution at each stage of development, there were no differences between groups (P > 0.05). In each grade of EQ there was an uniform distribution between the groups (Grade I Treat: 46.8 vs. Cont: 57.1; Grade II Treat: 38.3 vs. Cont: 33.3; Grade III Treat: 8.5 vs. Cont: 7.9; Grade IV Treat: 6.4 vs. Cont: 1.6, P > 0.05). However, analysis restricted in control group, obtained a higher frequency of grade I embryos (P < 0.05), while in the treated group prevailed grade I and II (P < 0.05). Serum concentrations of P4 at 0 h were Treat: 10.54 ± 10.03 vs. Cont: 8.54 ± 6.20 ; P > 0.05. The control group had higher concentrations compared to treated at 6 h (Treat: 3.70 ± 3.29 vs. Cont: 9.22 ± 5.73 ng/mL), 9 h (Treat 2.24 ± 2.01 vs. Cont: 9.11 ± 6.56 ng/mL) and 12 h (Treat 1.92 ± 1.69 vs. Cont: 11.57 \pm 7.22 ng/mL) after protocol or saline administration (P < 0.05). In the control group, P4 concentrations did not differ during the evaluations (P > 0.05), whereas in the treated group there was a decrease from 6 h to 12 h after the protocol (P < 0.05). Thus, we can conclude that cervical dilation hormonal protocol promotes a drop in P4 concentrations after 6 h of administration, although it does not affect the development of ovine embryos.

Support: EMBRAPA (Project 02.13.06.026.00.03) and CNPq (400785/2016-1).

Keywords: cervix, embryo collection, hormonal dosage, embryonic quality, sheep. *Palavras-chave:* cérvix, coleta de embrião, dosagem hormonal, qualidade embrionária, ovelhas.