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Short communication

# Inflammatory markers in ewes submitted to surgical or transcervical embryo collection

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ABSTRACT

The techniques currently available for embryo collection in sheep include surgical (laparotomy; LP) and transcervical (TC) methods Although both methods are well established, there is no data about the levels of inflammatory markers in ewes after using such procedures. The objective of this study was to compare the systemic reaction to inflammation in ewes submitted to superovulation and embryo collection through LP or TC methods Blood samples were collected before each procedure (at D0) and at 3, 6 and 9 days after procedures to determine the levels of total protein, haptoglobin, fibrinogen and paraoxonase 1. The levels of inflammatory markers did not differ ($P > 0.05$) between methods Serum levels of haptoglobin and total protein and paraoxonase 1 activity increased after D0 ($P < 0.05$) for ewes submitted to both methods In conclusion, although TC is a less invasive embryo collection method compared to LP, both methods induced similar inflammatory response over time.

## 1. Introduction

Due to the difficulties on cervical transposition, surgical collection through laparotomy (LP) is the most commonly used method for embryo recovery in ewes (Tervit and Havik, 1976). Although LP is efficient, allowing high embryo recovery (ER) rates, it invariably induces adherences in reproductive organs, compromising subsequent fertility and limiting the number of future procedures to be conducted in embryo donor ewes.

Alternatively, nonsurgical transcervical (TC) procedures were established based on induction of cervical dilatation (Gusmão et al., 2009; Masoudi et al., 2012) allowing cervical transposition in a greater number of ewes, reducing the occurrence of undesirable sequels. However, there is no information regarding the systemic reactions caused by any of such procedures. This study aimed to determine the impact of using LP or TC methods for embryo recovery in ewes on the levels of inflammatory markers such as haptoglobin, fibrinogen, paraoxonase 1 and total protein.

## 2. Material and methods

All procedures were approved by the UFPel's Ethics in Animal Experimentation Committee. Multiparous Crioula Lanada ewes were pre-synchronized, so they could be classified according to the ability of transposing their cervix during the estrus and diestrus periods of the breeding season (31°48′19.21″S − 52°24′44.53″W). In that trial, three cervical dilatation protocols were tested with administration of: a) subaracnoid ketamine (DeRossi et al., 2009), b) intravaginal misoprostol (Prostokos, Hebron) as prostaglandin E analog (Gusmão et al., 2009) and c) estradiol + oxytocin (Masoudi et al., 2012), modified by using estradiol benzoate via IM. It was used the methodology based on traction and fixation of the cervix with Pozzi tweezers and the cervical transposition with Hegar n°2 uterine dilator (Gusmão et al., 2009). The estradiol/oxytocin protocol provided the most satisfactory results (data not shown). Twenty days later, two groups of ewes (n = 5 each) selected as candidates for either the TC or LP procedures were submitted to a protocol of multiple ovulation and embryo transfer (MOET), as described by Menchaca et al. (2009), with modifications: 200 mg of pFSH (Folltropin V, Bioniche) were used for superovulation (SOV); 75 μg of D-cloprostenol (PGF analogue − Veteglan®, Calier) and 25 μg lecirelin (GnRH analogue, Gestran, Tecnopec) were administered to induce luteolysis and ovulation, respectively. Estrous behavior was assessed twice a day after PGF, using a teaser. After finishing the pFSH treatment at the time of estrus detection (at D-6), a dose of GnRH

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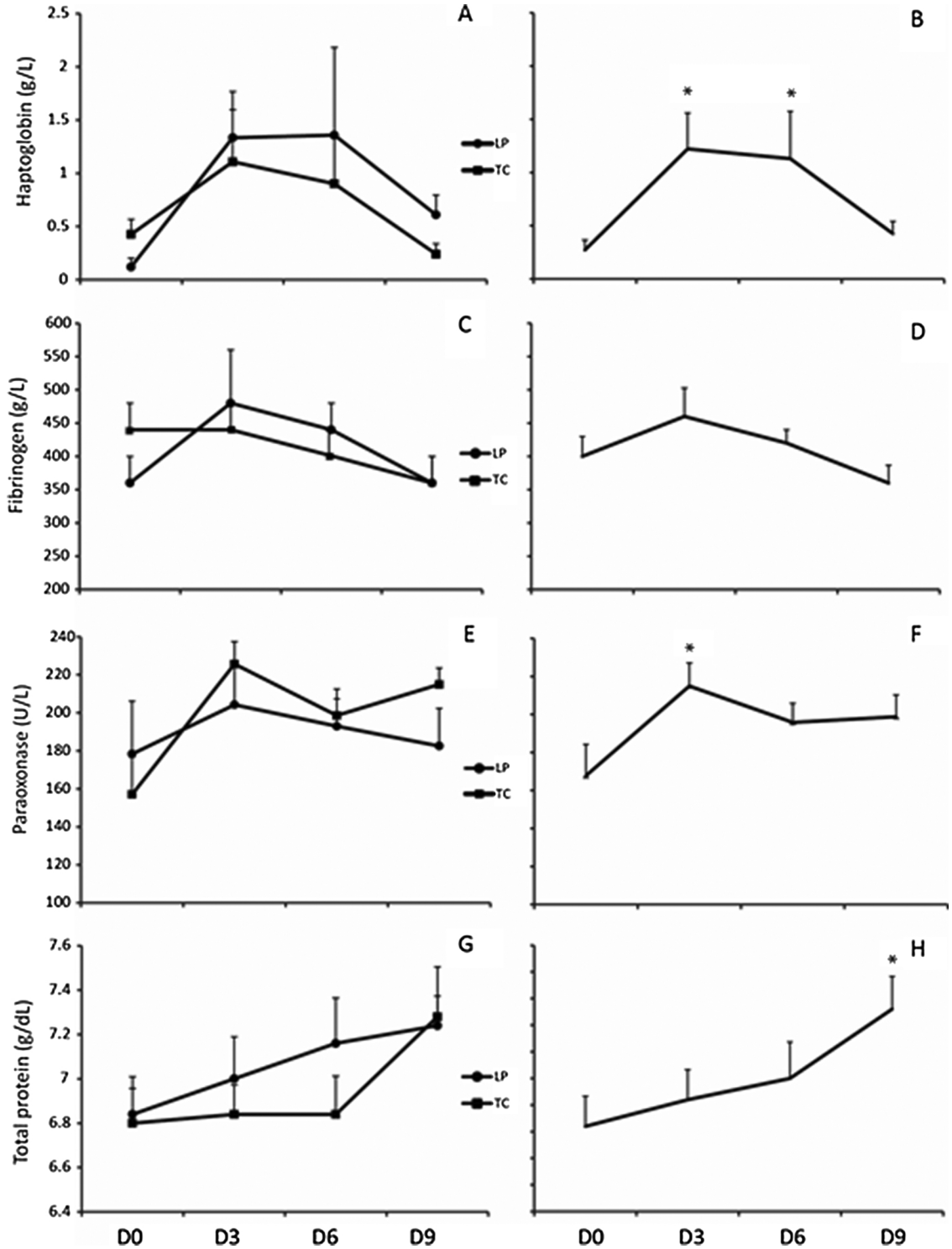


**Fig. 1.** Serum levels of haptoglobin, fibrinogen, paraoxonase 1 activity and total protein in ewes submitted to embryo collection. A, C, E and G: Comparisons between embryo collection methods; non-surgical transcervical (TC) and laparotomy (LP) (n = 5 ewes per group). B, D, F and H: Comparisons across periods regardless of the embryo collection method (n = 10 ewes). Blood samples were collected immediately before the procedures (D0) and 3, 6 and 9 days after. Error bars represent the standard error of the mean. *Differences from D0 ($P < 0.05$).

analog was administered, followed by cervical artificial insemination 12 and 24 h later, using fresh semen.

Ewes to be submitted to LP were fasted from food and water at D-1. At D0, prior the LP procedure (Candappa and Bartlewski, 2014), ewes were anesthetized with 0.2 mg/kg b.w. of xylazine i.m. (Anasedan, Ceva), followed by 5 mg/kg b.w. of ketamine i.v. (Dopalen, Vetbrands).

Ewes to be submitted to TC received 100 μg of estradiol benzoate i.m. (Gonadiol, Zoetis) at D-1 and 100 IU oxytocin i.m. (Placentex, Agener União) at D0. Ten min later, ewes were submitted to embryo collection by TC technique (Gusmão et al., 2009), as described above.

The time spent to perform each procedure was registered. At D0, a few minutes before the execution of both embryo recovery procedures, blood samples were collected from all ewes, from the jugular vein, in tubes with and without anticoagulant to determine basal levels of