

## EMBRYO/PREGNANCY

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### Pain management in non-surgical embryo recovery in Santa Inês ewes: effects on animal welfare

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**Application:** Pain management during non-surgical embryo recovery (NSER) can promote better welfare conditions in sheep.

**Introduction:** Non-surgical embryo recovery, although less invasive than surgical techniques, still triggers responses that affect animal welfare (i.e., increased heart rate and cortisol concentrations), probably explained by cervical manipulation. Thus, this study evaluated the effectiveness of meloxicam with dipyrrone on the welfare of ewes subjected to non-surgical embryo recovery.

**Materials and Methods:** A total of 29 multiparous Santa Inês ewes received a standard oestrus synchronisation treatment and a superovulatory protocol. Non-surgical embryo recovery was performed after a standard hormonal protocol for cervical dilation (Leite et al., 2018). The animals were either administered Meloxicam and Dipyrrone ( $n = 15$ ), which received both meloxicam before (1 mg/kg, i.v.) and 24 h after cervical transposition (1 mg/kg, i.m.), and dipyrrone (50 mg/kg, i.m.) before, 12 h, and 24 h after cervical transposition, or not (control;  $n = 14$ ) which were treated with saline solution. Heart and respiratory rates, cortisol, glucose, total proteins, albumin, and globulin blood concentration were recorded before sedation, after sedation, after cervical transposition, immediately after collection, and 0.5, 1.5, 3, 6, 12, 24, and 48 h after embryo collection. Data were compared using a mixed model (SAS on Demand for Academics), including treatment, time, and their interaction as main effects. For all tests,  $P < 0.05$  was considered significant, and  $0.051 > P \leq 0.1$  were considered as tendencies.

**Results:** Glycaemia had a significant interaction between treatments and time ( $P < 0.0001$ ), tending to be greater in the control group after sedation ( $P = 0.052$ ), and being greater at 3 h ( $P < 0.0001$ ) and 6 h ( $P = 0.03$ ) after embryo collection. In ewes in the Meloxicam and Dipyrrone group, blood glucose increased from before sedation to after sedation ( $P = 0.02$ ), from after sedation to after cervical transposition ( $P < 0.0001$ ), returning to baseline values at 6 h after embryo collection ( $P < 0.05$ ); and in the control group increased from before sedation to after sedation ( $P < 0.0001$ ), peaking at 1.5 h after embryo collection and 3 h after embryo collection, returning to baseline at 12 h after embryo collection ( $P < 0.05$ ). Cortisol values tended ( $P = 0.1$ ) to be greater and serum total proteins and globulins values were greater ( $P < 0.0001$ ) in control ewes. The other variables were not affected ( $P > 0.1$ ) by treatments, varying only with time.

**Conclusions:** The combination of drugs used for supporting NSER recovery in sheep induced transient changes indicating stress and possibly pain. Although the treatment applied reduces pain, it apparently only had minimal effects on reducing the negative responses triggered by NSER.

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#### References

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### MicroRNA-665 derived from bovine conditioned media is a potential non-invasive biomarker for preimplantation embryos

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**Application:** MicroRNA-665 is a potential non-invasive biomarker for preimplantation embryo developmental competence.

**Introduction:** In recent years, there has been a surge of interest in using embryonic microRNAs (miRNAs) as non-invasive biomarkers. However, these signalling molecules have only been profiled in media conditioned with blastocyst-stage embryos. We previously identified 363 miRNAs derived from bovine embryo-conditioned media or extracellular vesicles isolated from embryo-conditioned media (Lin et al., 2019; Pavani et al., 2022). The current study aimed to test the functionality of miR-665.

**Materials and Methods:** To study the functionality effect of miR-665, *in vitro* produced bovine presumptive zygotes ( $n = 1925$ , 18 replicates, Holstein) were allocated in five treatments groups with supplementation of bta-miR-665 (mimics, inhibitor, mimics negative-control, inhibitor negative control) with concentration of 1  $\mu\text{M}/25 \mu\text{l}$  to the SOF medium (containing insulin, transferrin, and selenium supplemented with 0.4% BSA (Sigma A9647)) along with the control group. Embryo cleavage was assessed on Day 2. At Day 8, the blastocyst rate was determined, and the effect of the miRNA on the expression of selected target genes was determined using RT-qPCR ( $n8181$ , 3