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SOCIETA´ SCIENTIFICA DI RIFERIMENTO **SOFIVET**

TITOLO **Exogenous melatonin strengthens fetal-maternal cross-talk by ameliorating uterine microenvironment during the early stage of pregnancy in sheep**

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Testo e Riferimenti bibliografici

Early pregnancy loss is still the current cause of 25% of pregnancy failure in small ruminants due to asynchrony between conceptus and uterine signals [1]. In this context, melatonin plays a crucial role in sheep reproductive dynamic exerting its action by binding MT1 and MT2 receptors. Melatonin implants lessen oxidative stress and increase corpus luteum competence, which prolongs the breeding season [2]. However, little is known about its effect during the peri-implantation period. Thus, we hypothesized that melatonin supports embryo implantation by affecting the uterine microenvironment. This study aims to elucidate the exogenous melatonin effects on the endometrium and early placenta rearrangement, allowing pregnancy proceeding in sheep.
The experiment followed a protocol (PI47/21) approved by the Ethics Committee of the University of Zaragoza, Spain. Ten multiparous Rasa Aragonesa ewes were selected, 5 of which were treated with subcutaneous melatonin implants (18 mg, Melovine, CEVA) 50 days before synchronized and controlled natural mating (CTR: control; MEL: melatonin implanted). On Day 21 of pregnancy, pregnant status was confirmed by transrectal ecoscan, and sheep were euthanized. Blood, endometrium, and conceptus were collected. Plasma progesterone concentration (P4) was evaluated. Embryo viability was immediately checked, and crown-rump length was measured and placenta was fixed for histology and histochemistry. Moreover, placenta and endometrium were stored at -80°C for further gene (angiogenic factors) and protein (MT2) expression analyses.
MEL ewes showed a higher prolificity rate (1.8 vs 2.8 embryos/ewe) and an increase of P4 (2,9 vs 3,6 ng/mL; $p<0.05$) compared to the CTR group. No difference was observed in crown-rump length, notwithstanding the distribution of MEL embryos measurement appeared more restricted ($\sigma 2$ 1.32 vs 0.36). Immunocytochemistry revealed the expression of epithelial-cadherin at the chorionic level in both MEL and CTR placentas. Alpha-smooth muscle detection evidenced placental blood vessels only in the allantois part and no difference in the stage of vessel formation was observed between the two groups [3]. However, MEL placenta consisted of more binucleated trophoblast cells in the chorion region than CTR ($p<0.0001$), and ovine placental lactogen was upregulated in MEL placenta ($p<0.05$). Exogenous melatonin led to higher levels of angiogenetic factors expression (VEGFA, VEGFR1, IGF1R, $p<0.05$) in the caruncular endometrium, whereas no difference was observed either in the intercaruncular region and the placenta. Moreover, IFNAR2 was upregulated in MEL endometrium ($p<0.05$). Lastly, an increase of MT2 receptor mRNA was observed in the endometrium of MEL sheep, as well as in western blot analysis in placenta tissue ($p<0.05$). These findings suggest that melatonin implants differentially act on the uterus and placenta rearrangement. We propose that melatonin drives placenta toward differentiation while promoting vessel maturation at the endometrium level. In conclusion, exogenous melatonin seems to enhance the uterine microenvironment, improving the success of embryo implantation during the early stage of pregnancy in sheep.

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