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The use of different progestin devices in ovarian stimulation protocol affects gene expression in sheep cumulus-oocyte complexes (COC)

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Progesterone (P_4) and its analogues (progestins) are commonly used in estrus synchronization protocols and to hold the LH surge during ovarian stimulation to allow oocyte recovery in live donor ewes. However, recent evidence suggests that some progestins may have a deleterious effect on embryo quality after long-term use. Thus, the present study aimed to evaluate the effect of two progestin devices used during ovarian stimulation on the COC quality in donor ewes. A total of 30 pluriparous ewes had their estrus and follicular wave synchronized by a short-term protocol (Bragança et al., Reprod., Fertil. Dev., published online, 2018). At 80 h after sponge removal, all ewes received 80 mg of pFSH (Folltropin-V, Bioniche Animal Health, Ontario, Canada) in three applications (50%, 30% and 20%) every 12 h. For stimulation, the ewes were allocated into three groups (n = 10 each): MAP, in which ewes received intravaginal sponges containing 60 mg of medroxiprogesterone acetate (Progespon, Zoetis, São Paulo, Brazil); P_4, in which a silicone device impregnated with 0.33 mg of natural P_4 (CIDR, Eazi-Breed, Zoetis) was applied; and Control, in which the ewes did not receive any device (only luteal P_4). COCs were recovered by laparoscopy and morphologically graded as viable (GI/II, homogeneous ooplasm and at least a complete cumulus cells layer; and GIII, homogeneous ooplasm and/or partially denuded) or poor quality (GIV, heterogeneous ooplasm or degenerated). To infer development competence, viable COCs were stained with brilliant cresyl blue (BCB) and classified as BCB^+ (competent) and BCB^- (non-competent). Pools of 10 BCB^+ COCs/group were used for gene expression analysis by real-time PCR of oocyte competence markers (ZAR1, zygote arrest 1; MATER, maternal antigen that embryo requires; GDF9, growth differentiation factor 9; BMP15, bone morphogenetic protein 15; RELN, reelin; Bcl-2, B-cell lymphoma 2; and BAX, Bcl-2 associated X protein) and steroidogenic pathway-related genes (ERα, estrogen receptor α; LHr, LH receptor; FSHr, FSH receptor; and StAR, steroidogenic acute regulatory protein). An ANOVA was then conducted to compare the variables followed by a Tukey test. No significant difference ($P > 0.05$) was observed for the number of viable COCs per ewe (MAP: 5.7 ± 1.0, P_4: 7.7 ± 0.7 and Control: 5.7 ± 1.1) or the percentage of BCB^+ (MAP: 61%, P_4: 58% and Control: 65%). However, the gene expression profile was affected by the type of progestin used. FSHr, LHr and RELN genes were up-regulated ($P < 0.05$) in the P_4 as compared with the MAP group, while LHr and RELN genes were down-regulated in the MAP as compared with the Control ($P < 0.05$). Finally, FSHr, LHr, Erα, as well as the Bcl-2, ZAR1 and GDF9 were up-regulated in the P_4 as compared with the Control group ($P < 0.05$). In conclusion, the progestin device alters the expression of genes related to quality and the steroidogenesis pathway in fully-grown COCs, and the use of a natural P_4 device may improve the development competence of COCs. Further studies including IVP are necessary to confirm our findings.