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Immunohistochemical distribution of estrogen receptor alpha (ERα) in the uterus of sows under different hormonal protocols

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The use of fixed-time artificial insemination (FTAI) in sows aims to minimize errors, labor related to estrus detection,estrus cycle variation, interval of ovulation (KNOX, Theriogenology, v.75, p.308-19, 2011), and semen doses per sow in each estrous,consequently, decreasing production cost. Studies on effects of the exogenous ovarian stimulation in sows are scarce and many questions of the possible effects of its use remain unanswered. The uterus is one of the most affected organs under the influence of steroidal hormones. The hormonal changes are regulated by estrogen, mainly ERα and progesterone receptors (Sukjumlong et al., 2009). The distribution of these receptors in the uterus could be related to the ideal environment for the embryo development. The aim of this study was to evaluate the effects of different FTAI protocols on the distribution and quantification of ERα in the uterus of sows using immunohistochemistry (IHC). Thirty-eight sows were randomly assigned into groups: control, eCG (eCG IM 600UI at weaning), GnRH56h (600UI eCG IM at weaning, 50 mcg GnRH IM 56h after eCG) and GnRH80h (600UI eCG IM at weaning, 50 mcg GnRH IM 80h after the eCG). At day 6.5 after AI, animals were euthanised and samples of the uterus were fixed in 10% neutral buffered formalin for 48 hours and routinely processed for histology/IHC. Tissue sections were incubated with a primary antibody (ERα, #SC-7207, rabbit polyclonal, 1:200, Santa Cruz Biotechnology, Dallas, TX, USA) for 1 hour, followed by secondary antibody incubation with UV LP HRP polymer (Thermo Fisher Scientific, Fremont, California, USA) for 15 minutes, and visualized using a chromogen complex 3, 3'-diaminobenzidine. For each section, 10 randomly selected high power fields (400x) of the following areas of the uterus were examined for nuclear immunolabeling: superficial epithelium, endometrial stroma, endometrial glands and myometrium. The IHC reactivity was scored as follows: (-) absent, (+) ≤ 30% of nuclear immunolabeling in each area; (++) 31-60% of nuclear immunolabeling in each area, (+++) > 60% of nuclear immunolabeling in each area. Data were analyzed by One-way ANOVA and Tukey test ($P \leq 0.5$). The results are written as mean ± SD as follows for control, eCG, GnRH56h and GnRH80h groups, respectively: superficial epithelium (0.33 ± 0.16, 0.22 ± 0.14, 0.20 ± 0.13 and 0.50 ± 0.26 (P=0.66); endometrial stroma (1.1 ± 0.35, 1.55 ± 0.57, 1.6 ± 0.4, 1.5 ± 0.34) (P=0.78); endometrial glands (1.77 ± 0.49, 2.44 ± 0.37, 2.00 ± 0.33, 2.3 ± 0.30) (P=0.61), and myometrium (0.33 ± 0.16, 0.66 ± 0.16, 0.60 ± 0.16; 0.7 ± 0.15 (P= 0.39). No significant differences were observed among experimental groups for any of the evaluated uterine areas. The estrus cycle synchronization using the proposed AI protocols does not interfere with distribution of ERα in the uterus 6.5 days after AI.

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