Equine

THE PATHWAY OF EQUINE GROWTH HORMONE ON EQUINE OOCYTE MATURATION AND RECEPTOR LOCALIZATION BY IMMUNOCHEMISTRY AND REAL-TIME PCR

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In previous studies, equine growth hormone (eGH) had a positive effect on equine oocyte maturation. The objective of this study was to determine the location of eGH-receptor (eGH-R) on equine ovarian structures, including follicular wall, oocyte and cumulus oocyte complexes. Additionally, we observed if the eGH influences on oocyte maturation is removed when specific inhibitors against growth hormones are added to the culture in vitro. Real-time reverse transcription-PCR (RT-PCR) and immunocytochemistry methods were used to localize eGH-R in equine follicles and oocytes. Paraffin sections comprising the equine oocyte attached to the follicular wall were treated by streptavidin-biotin method for immunochemical detection. Briefly, primary antibodies (α-growth hormone; 1:20 dilution) were incubated overnight at 4 °C in a humidified chamber, later incubated with biotinylated secondary antibodies (α-mouse IgG; 1:200 dilution), streptavidin complex (1:400 dilution) and counterstained with haematoxilin. Quantification of the relative transcript levels of eGH-R was made by *Taq*-Man PCR Reagent kit and performed using an ABI Prism 7700 Sequence Detection System. Primers for eGH receptor precursor were based on cDNA sequence published at Genbank (AF097588). Pools of 15 cumulus oocyte complexes, denuded oocytes and cumulus cells alone were used for RT-PCR experiment. Addition of a specific adenylate cyclase inhibitor (DDA) and a specific inhibitor against cyclic AMP-dependent protein kinase (H-89) were used during culture in vitro to observe whether this inhibitory effect can block the eGH action on oocyte maturation. Results of different treatments groups were compared by χ^2-test for trend (significance was set at $P < 0.05$). Cumulus oocytes complexes incubated with eGH, 29/61 oocytes (47.5%), were classified as mature versus 18/64 oocytes (28.1%) in the control group ($P = 0.02$). The H-89 inhibitor used at 10×10^{-6} μM/mL decreased the number of oocytes reaching maturity when compared with control (5/13 versus 9/10; respectively; $P = 0.05$). The DDA inhibitor also reduced the number of oocytes reaching maturity (4/19 versus 9/12; $P = 0.05$). Results showed that: (1) significant positive immunostaining for eGH-R in cumulus cells, oocyte and granulosa cells of the follicle; (2) quantification by *Taq*-Man PCR showed that relative gene transcription for eGH-R was higher in cumulus cells and cumulus oocyte complexes at the start of in vitro maturation; (3) H-89 and DDA can be used in vitro to block the action of eGH on equine oocyte maturation. We inferred that the presence of eGH-R in equine ovarian follicular structures might mediate a positive effect in response to eGH in equine in vitro oocyte maturation.

Keywords: Equine growth hormone; Growth hormone receptor; Oocyte maturation

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EVALUATION OF KISSPEPTIN IN THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS OF THE MARE

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Kisspeptin and its receptor, GPR54, are crucial elements in the onset of pubertal development and may play a role in seasonal estrus and induction of ovulation via a mechanism of signaling GnRH release at the level of the hypothalamus. The purpose of this series of experiments was to evaluate the role of kisspeptide and GPR54 in the seasonally estrous mare. In a preliminary study, no difference was observed in LH response profiles following intravenous administration of 1.0 and 10.0 mg of rat kisspeptide (KiSS-10, AnaSpec, Inc., San Jose, CA, USA) in diestrous mares ($n = 3$ per treatment). To further elucidate a physiologic LH response to kisspeptide administration, three dose rates of kisspeptide (1.0 μg, 0.5 mg, 1.0 mg, iv) were compared to that of native GnRH (25 μg LHRH, iv;