Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

OPU-IVF and ET

Neuregulin 1 modulates nuclear maturation during amphiregulin-induced IVM of bovine cumulus-oocyte complexes and improves post-IVF embryo production

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Resumo

Gradual activation of the ovulatory cascade during in vitro oocyte maturation (IVM) has been proposed to enhance nuclear-cytoplasmic synchrony and cumulus-oocyte communication, thus enhancing oocyte developmental competence and post-IVF in vitro embryo production (IVP). In the present study, we assessed the effects of neuregulin 1 (NRG1), an EGF-like factor that modulates EGFR signaling and thus the activation of the ovulatory cascade, on oocyte nuclear maturation dynamics, cumulus expansion, expression of mRNAs regulating these processes and post-IVF embryo development. Bovine cumulus-oocyte complexes (COCs) were aspirated from 2-8 mm follicles of slaughterhouse ovaries, pooled in groups of 20-25, and subjected to IVM in serum-free TCM containing physiological concentrations of FSH, IGF1, steroids, and 100 ng/mL AREG ("IVM Follicular System"); supplemented with 1 ng/mL NRG1 (NRG1 group) or not (Control group) for 6, 9, 12, 20, and 24 h. Oocyte chromatin/meiotic status was assessed by fluorescence microscopy following Hoechst staining at each time-point. Cumulus expansion degree (1 to 3) was assessed after 24 h of IVM and cumulus mRNA expression (real-time RT-PCR) after 9 and 20 h of IVM. Embryo production rates and embryo cell number (fluorescence microscopy/Hoechst) were assessed after standard IVF using frozen semen of a single bull/batch and standard embryo culture for 7 days. All experiments were performed with 5 replicates. Data in percentages were arcsine transformed and all the data were first tested for normality (Shapiro-Wilk test) before assessing treatment effect with the Student's t-test. Data are presented by mean ± SEM and differences were considered significant when $P < 0.05$. NRG1 decreased the percentage of oocytes undergoing germinal vesicle breakdown at 6h of IVM (GVBD; 52.24 ± 4.70 vs. 70.37 ± 5.10; $P = 0.02$), without altering later meiotic dynamics, nor the percentage of oocytes achieving meiosis II at the end of culture. NRG1 did not affect cumulus expansion, but increased the percentage of expanded and hatched blastocysts (39.31 ± 2.56 vs. 32.60 ± 1.03; $P = 0.03$), as well as blastocyst total cell number (202.30 ± 10.52 vs. 169.18 ± 10.55; $P = 0.03$). NRG1 decreased EGFR mRNA abundance (0.82 ± 0.05 vs. 1.00 ± 0.03; $P = 0.02$), while increasing mRNA levels of NPR2 (1.64 ± 0.22 vs. 1.03 ± 0.13; $P = 0.04$) and PTX3 (5.73 ± 2.74 vs. 1.09 ± 0.24; $P = 0.04$) at 9h, as well as those of TNFAIP6 (1.77 ± 0.23 vs. 0.82 ± 0.04; $P = 0.02$) at 20h of IVM. This is the first study to report the regulatory role of NGR1 during oocyte maturation in a mono-ovulatory species, and to demonstrate that this action may be applied during IVM to improve post-IVF embryo development.

Acknowledgements

Supported by FAPESP (2019/14588-6) and CAPES.