Animal Reproduction

Abstracts - 37th Annual Meeting of the Association of Embryo Technology in Europe (AETE)

Embryology, developmental biology, and physiology of reproduction

Monounsaturated oleic acid addition during early embryonic development increases bovine blastocyst rates.

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Metabolic stress, characterized by elevated levels of free fatty acid (FFA), have been linked to reduced female fertility. Saturated FFA (stearic acid (SA)) appears to have a dose-dependent negative effect on oocyte developmental competence while monounsaturated oleic acid (OA) is shown to be harmless. Oocytes are protected against FFA by cumulus cells via stearoyl-CoA desaturase 1 (SCD1) activity, which converts saturated SA into mono-unsaturated OA. To study FFA effects on early embryonic development, embryos were cultured in the presence of SA and OA and SCD activity was analyzed.

Cumulus-oocyte-complexes (COCs), collected from 2-8 mm sized follicles of bovine slaughterhouse ovaries, were *in vitro* matured (n=400/run) and fertilized (Aardema et al., Biol Reprod; 85: 62-69, 2011). During the first five days of embryo culture (day 1-5; i.e. oviductal period), embryos were cultured in SOF without (control) or with FFA (FFA conc.= 25 and 50 µM OA; 25 and 50 µM SA; 25 or 50 µM OA + 25 or 50 µM SA). Fatty acids were complexed to fatty acid free BSA (10 mM) (FFA:BSA ratio of 5:1). FFA was conjugated to albumin, likewise the transport of FFA *in vivo*, to solubilize FFA in an aqueous solution. At day 8 the number of blastocysts was counted. With RT-qPCR the mRNA expression of *SCD1* was measured in all the conditions. The general linear model was used for statistical analysis with SPSS 27.0. The day 8 embryos, COCs (positive control) and oocytes (negative control) were fixated in 4% PFA and incubated with a primary antibody, SCD1, diluted 1:100 in PBST overnight at 4°C for immunostaining. Thereafter, embryos were washed 3 times in PBST for 15 min and incubated with the second antibody, goat anti-rabbit Alexa™ fluor 647, diluted 1:100 in PBST for 1h in the dark at RT. Confocal microscopy was performed using an inverted Nikon A1R confocal microscope to determine the presence of SCD1 protein.

Exposure to 25 and 50 µM SA from day 1-5 resulted in a significantly lower blastocyst rate of respectively 18.9 ± 1.6% and 2.6 ± 4.6%, compared to the control condition 25.9 ± 3.1% (n=3; $p<0.05$). Interestingly, exposure to 25 and 50 µM OA resulted in a significantly higher blastocyst rate, 36.4 ± 6.3% and 34.6 ± 7.3% respectively (n=3; $p<0.05$). Exposure to 25 µM OA + 25 µM SA resulted in a blastocyst rate of 26.0 ± 3.7% comparable to the control condition and was not significantly different from exposure to 50 µM OA + 50 µM SA (25.6 ± 5.5%) (n=3; $p>0.05$). Interestingly, exposure to 50 µM OA + 25 µM SA resulted in a blastocyst rate of 33.7 ± 9.3% comparable to the 50 µM OA condition (n=3; $p<0.05$). SCD1 was faintly expressed at RNA and protein levels in day 8 embryos. Nevertheless, SCD1 was stronger detected in embryos compared to oocytes.

Previously, our group demonstrated no detectable SCD1 protein levels in oocytes, but solely in cumulus cells. We here showed that day 8 embryos express SCD1, which may protect embryos against saturated FFA. The current culture data show that OA counteracted the negative effect of SA on embryos. Future studies should investigate the role of OA and SCD1 in embryos.

Keywords: embryo; free fatty acid; stearoyl-CoA desaturase.