

POSTER PRESENTATION

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THE EFFECT OF 17A-ETHINYLESTRADIOL EXPOSURE OF *IN VITRO* CULTURED BOVINE MORULAE ON SUBSEQUENT EMBRYONIC DEVELOPMENTAL COMPETENCE AND QUALITY.

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17- α -Ethinyl estradiol (EE2), a derivate of 17 β -estradiol (E2), is an important component of most oral birth-control pills, making it one of the most used medicines worldwide. The major concern is that EE2 can act as a xeno-estrogen, after being released into the environment through urine and feces. While this emphasizes the need for the evaluation of its toxicity, several oocyte and embryo-toxic effects have been reported (Beker-Van Woudenberg *et al.*, 2012). Therefore, the aim of the present study was to evaluate the effect of EE2 exposure during early embryonic development, more specific at morula stage (18h), on subsequent embryonic development and quality.

Cumulus oocyte complexes (COCs) from 2-6mm diameter follicles were matured in groups of 50 in 500 μ l TCM with 20ng/ml EGF for 24h and subsequently fertilized in groups of 100 in 500 μ l fertilization medium for 22h (5% CO₂, 38.5°C). Presumptive zygotes were denuded and cultured in groups of \pm 25 in 50 μ l SOF with ITS (5 μ g/ml insulin, 5 μ g/ml transferrin, 5ng/ml selenium) and 2% BSA, covered with mineral oil (5% O₂, 5% CO₂, 38.5°C). 135h pi, embryos at morula stage were selected and randomly allocated to treatment groups (n) divided over 5 replicates: 1) Control (67), 2) Solvent control: 0.1% ethanol (49), 3) 10ng/ml EE2 (49) or 4) 10 μ g/ml EE2 (63). The morulas were cultured individually in 30 μ l standard SOF medium supplemented with the desired concentrations ethanol or EE2 (5% O₂, 5% CO₂, 38.5°C) in 96-well half area culture plates, without oil coverage during 18h. Following exposure, embryos were cultured singly in standard culture medium during 2 more days. Subsequently, developmental competence was evaluated and blastocyst rates calculated (blastocyst rate = total blastocyst / number of grade 1 selected morula). Expanded (EB) and hatched blastocysts (HB) were fixed with 4% paraformaldehyde and total cell number and apoptotic cell ratio were determined by DAPI and TUNEL staining (13 EB and 7 HB per treatment).

Comparable blastocyst rates were obtained in all treatment groups: Solvent control (77.8%), 10ng/ml EE2 (69.0%) and 10 μ g/ml EE2 (84.0%), as compared to the Controls (88.2%) ($P > 0.05$; Bin. log. reg.). In addition, no significant effect of treatment could be found on total cell number or apoptotic cell ratio: solvent control (149.70 \pm 23.47 and 3.46 \pm 1.73), 10ng/ml EE2 (154.75 \pm 23.26 and 3.22 \pm 1.35) and 10 μ g/ml EE2 (150.50 \pm 26.69 and 4.23 \pm 1.85), as compared to the Controls (145.02 \pm 24.71 and 3.07 \pm 2.03) ($P > 0.05$; two-way ANOVA).

Although, our results show no immediate statistical significant effect of short-term EE2 exposure during the morula stage in *in vitro* culture on subsequent embryonic development and quality, additional research is necessary to find out if EE2 may affect gene-expression patterns, eventually resulting in unknown embryo-toxic effects that might turn up during later embryonic developmental stages.