360 EMBRYONIC DEVELOPMENT AND STEROIDOGENESIS OF BOVINE CUMULUS-OOCYTE COMPLEXES MATURED IN α-MEM MEDIUM SUPPLEMENTED WITH PVA OR PVP-40

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Abstract

The influence of the culture medium and its supplements on IVM and steroid secretion by bovine COCs was examined. Immature COCs were matured for 24 h in a-MEM supplemented with IGF-1 (10 ng mL^{-1}), insulin (100 ng mL^{-1}) and 0.1% polyvinyl alcohol (PVA; Sigma Chemical Co., St. Louis, MO, USA) or 0.1% polyvinylpyrrolidone-40 (PVP; molecular weight of 40 000; Sigma). Neither FSH nor LH was used in either treatment. The control group consisted of COCs matured in TCM supplemented with FSH (20 μg mL^{-1}) and 10% estrous cow serum. After fertilization, presumptive zygotes were co-cultured with cumulus cells until 224 h post-insemination. Steroid production was measured in culture medium after IVM and cumulus cells (CC) aromatase activity was estimated by estradiol (E2) production and by the determination of the ratio of E2 to testosterone (T). Cleavage, blastocyst, and hatching rates were evaluated 168-224 h post-insemination. Hormone determination data were analyzed using the GraphPad 5.0 for Windows software (GraphPad Software, San Diego, CA, USA). The means and standard deviations were first calculated for all variables, followed by the Kolmogorov-Smirnov normality test. Since the values were not normally distributed, the treatments were compared by the Kruskal-Wallis test. Blastocyst formation, cleavage, and hatching rates were analyzed by the chi-square test. The level of significance was set at $P < 0.05$ in all analyses. High aromatase activity (E2:T ratio > 1.0) was detected in the culture medium of both chemically defined IVM systems. E2 concentrations were 22.86 ng mL^{-1} and 22.46 ng mL^{-1} for PVA and PVP-40, respectively, and 0.27 ng mL^{-1} ($P < 0.001$) for the control group. Progesterone secretion was lower in a-MEM + PVP-40 medium. Testosterone was not secreted by COCs matured in control medium. There was a significantly higher cleavage rate in the control group, but no differences ($P > 0.05$) in blastocyst (48.92%, 49.56%, and 44.21%) or hatching (38.46%, 41.96%, and 40.78%) rates between the PVA, PVP, and control groups, respectively. Our results show that CC of COCs cultured in serum-supplemented medium show decreased aromatase activity. Also, the addition of IGF-I/insulin and PVA or PVP-40 to IVM medium had no significant effect on the rates of oocyte maturation and embryonic development when compared with results obtained in medium supplemented with estrous cow serum and FSH.

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