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EFFECTS OF SUPPLEMENTATION OF HORMONES IN  
MATURATION MEDIUM ON MATURATION AND FERTILIZATION  
OF BOVINE OOCYTES IN-VITRO

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To examine the hypotheses that supplementation of hormones (FSH preparation and estradiol-17 $\beta$ ) in maturation medium stimulates cumulus cells to produce a sperm capacitation factor(s) which subsequently improves the fertilization rate in vitro, the following experiments were conducted using a serum-free maturation medium (TCM-199 + polyvinyl alcohol).

In the first series of experiments, the effects of hormones in the maturation medium on maturation and fertilization of cumulus oocyte complexes (COC) were investigated. The meiotic maturation rate as well as the penetration rate did not differ with either oocytes cultured with or without hormones. However, the normal fertilization rate was higher with hormones than without hormones (59.0 vs 43.4%,  $P < 0.05$ ).

In the next experiment, it was examined whether the presence of cumulus cells enhanced the in-vitro fertilization rate. COC cultured in the maturation medium with hormones were classified into the following: oocytes completely devoid of cumulus cells (denuded oocytes), denuded oocytes with cumulus cells isolated from COC in the fertilization medium (denuded oocytes with cumulus cells) and oocytes with intact cumulus cells (intact oocytes). The penetration and normal fertilization rates for the denuded oocytes (29.1 and 24.1%, respectively) were significantly lower ( $P < 0.05$ ) than those of the denuded oocytes with cumulus cells (65.0 and 47.0%) and the intact oocytes (86.6 and 57.1%).

Finally, after being cultured in the maturation medium with or without hormones, oocytes were isolated from COC. Denuded oocytes and isolated cumulus cells were divided into the following groups: both denuded oocytes and cumulus cells cultured with hormones, either denuded oocytes or cumulus cells cultured with hormones, neither denuded oocytes nor cumulus cells cultured with hormones. They were inseminated in vitro and their fertilization rates were analyzed by 2-way ANOVA. The results demonstrated that significantly improved penetration and normal fertilization rates ( $P < 0.05$ ) are due to the presence of cumulus cells cultured with hormones in the maturation medium, rather than the oocytes. The polyspermic rate was not influenced by the presence of cumulus cells cultured with hormones but was affected by the oocytes themselves if they were cultured with or without hormones.

The present study shows that the fertilization rate in vitro is improved by the presence of cumulus cells cultured in the maturation medium with hormones. Moreover, it is expected that supplementation of hormones in the maturation medium beneficially affects the oocyte's ability to prevent polyspermy.