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Effect of follicular fluid supplementation on in vitro maturation of porcine oocytes

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The reproductive process that occurs in the female is controlled by a series of critical events. One such event that must occur is the "maturation" of the oocyte prior to its ovulation, so that it is capable of being fertilized. This maturation process occurs naturally in vivo and has been mimicked in vitro. Currently, there are two methods of determining oocyte maturation after being subjected to an oocyte in vitro maturation (IVM) protocol. The first method involves fixing and staining the oocytes to identify the developmental stage of each oocyte. The second method is based on visually determining whether each oocyte has extruded a polar body, which indicates that the oocyte has resumed meiosis and reached metaphase II. Follicular fluid (FF) is believed to play an important role in the in vivo maturation of oocytes. Our hypothesis was: 1) that the addition of FF to the IVM medium would increase the number of oocytes reaching metaphase II, and 2) that the success rate was dependent on the percentage of FF in the culture media. To test this hypothesis, we utilized porcine oocytes and porcine follicular fluid (pFF). Porcine ovaries were collected from a nearby slaughter facility and transported to the lab where the oocytes were aspirated from follicles on the ovaries of prepuberal gilts. Oocyte culture media consisted of: 1) TCM 199 as a control medium, 2) TCM 199 + 10% pFF, 3) TCM 199 + 25% pFF, or 4) TCM 199 + 50% pFF. Oocytes were randomly allocated to one of the four culture media and incubated at 37.5°C for approximately 46 hours. After incubation the cumulus complex was removed using hyaluronidase, and oocytes were examined for the presence of an extruded polar body. The control medium incubation resulted in 54.25% of oocytes reaching metaphase II (i.e. they had an extruded polar body). Incubation of oocytes in TCM 199 + 10% pFF resulted in a 57.96% maturation rate; incubation in TCM 199 + 25% pFF resulted in a 54.61% maturation rate and incubation in TCM 199 + 50% pFF resulted in a 48.10% maturation rate. Although there was no significant difference among the treatments, we suggest that these data provide evidence that the addition of pFF to the IVM culture of porcine oocytes may be beneficial, but not when added in excess of 25%.