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Effect of follicle-stimulating hormone on nuclear and cytoplasmic maturation of sow oocytes in vitro

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Abstract

A series of experiments were conducted to evaluate the effects of FSH supplementation during IVM on porcine oocyte nuclear maturation, and subsequent fertilization, cleavage and embryo development. Cumulus–oocyte complexes (COCs) were cultured 40 h without FSH (control), 40 h with FSH (FSH 0–40 h), or 20 h with FSH followed by a 20-h culture period without FSH (FSH 0–20 h). Nuclear stage of oocytes was assessed at intervals from 12 to 40 h of IVM. Furthermore, oocytes were in vitro fertilized, fixed and stained to determine normally fertilized and polyspermic oocytes. Additionally, COCs were matured with FSH, fertilized and zygotes cultured in NCSU-23. The percentage of cleaved embryos and blastocysts were determined and the number of nuclei was counted.

The presence of FSH during the first 20 h of IVM retarded germinal vesicle breakdown. After 40 h of culture 84, 67 and 58% MII oocytes were observed in the FSH 0–20 h, FSH 0–40 h and control groups, respectively. After IVF, penetration rates were similar at 27, 26 and 29%, while the proportion of polyspermic oocytes was 7, 19 and 11% of penetrated oocytes for control, FSH 0–40 and FSH 0–20 h groups, respectively. Cleavage and blastocyst rates differed among treatments (21, 29 and 38%, and 7, 15 and 20% for control, FSH 0–40 and FSH 0–20 h groups, respectively). No differences in blastocyst cell number were found among groups. Blastocyst rates, based on number of cleaved embryos, were 51 and 52% for the FSH 0–40 and FSH 0–20 h groups, which differed significantly from the control group (31%). The results indicate that FSH has a stimulatory effect on nuclear and cytoplasmic maturation of sow oocytes. Addition of FSH for the first 20 h of culture was most beneficial, based on cleavage and blastocyst development rates.

Keywords: IVM; Oocytes; Sow; FSH

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1. Introduction

Oocytes are arrested in the diplotene stage of the first meiotic division and resume meiosis immediately before ovulation. During this period of maturation, the oocyte changes from a developmentally incompetent cell to one with the capacity to direct and support the events of fertilization and early embryonic development. In the same period, cumulus cells lose contact with the oocyte and intercellular communication between these cells undergoes a progressive reduction. When oocytes from small and medium sized follicles are removed from their follicular environment and matured in vitro, spontaneous nuclear maturation does occur; only a low proportion of oocytes develop to blastocysts following IVF and early culture in vitro. These deficiencies may be attributed to abnormalities in cytoplasmic maturation, even though apparently normal nuclear maturation is observed. To increase the developmental ability of in vitro matured oocytes, gonadotropic hormones are added to the maturation medium. Although most in vitro maturation protocols currently utilize LH, FSH, or a combination of both, the effect of gonadotrophins on IVM and subsequent fertilization and early embryo development is still controversial [1].

Exposure of porcine cumulus–oocyte complexes (COCs) to a combination of eCG, hCG and porcine follicular fluid (pFF) [2] or to eCG and pFF [3] during the first half of the maturation period increases male pronuclear formation following IVF. Addition of FSH during in vitro maturation increases the proportion of metaphase II (MII) pig oocytes [4–7]. Rath et al. [8] observed an increase of MII-stage oocytes only when FSH together with pFF was added to the maturation medium, while Bing et al. [7] reported that FSH affects nuclear progression only when cysteamine is added to the maturation medium. Maturation in the presence of FSH has no effect on male pronuclei formation following fertilization of in vitro matured pig oocytes [4,8,9]. However, the positive effect of pFF in the maturation medium on male pronuclei formation [9] or cleavage rate [8] is further enhanced in the presence of FSH. Singh et al. [5] observed a significant increase in the proportion of polyspermic oocytes when maturation medium was supplemented with FSH. It remains to be investigated whether FSH is required for only the first half or the whole maturation period. Moreover, results from the studies mentioned above [4–8] were obtained by using an IVM system where fetal calf serum (FCS) was added to the maturation medium. Naito et al. [9] reported that FCS at concentrations higher than 1% interferes with the effect of FSH with the progression of meiosis. Hence, the present study was undertaken to investigate the effect of duration of a physiological concentration of recombinant FSH, supplemented to a chemically defined maturation medium, both on the progression of meiosis of porcine oocytes and on the developmental competence of the matured oocytes following IVF.

2. Material and methods

2.1. Culture media

All chemicals for the preparation of culture media were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise indicated. The basic medium for IVM (OMM) was