Influence of Serum and Hormones on *In vitro* maturation of Buffalo Oocytes

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Abstract

The influence of different combinations of protein supplements in the presence of different concentrations of commercially available FSH (FSH Folltropin-V) and steroids (17-ß oestradiol) were studied in order to establish optimal condition for *in vitro* maturation of buffalo oocytes. The isolated 10 immature oocytes cultured in Ham's F-10 + sera (buffalo oestrus serum or fetal bovine serum) + different concentrations of FSH i.e. 1, 10 and 20 µg/ml and oestradiol at 39°C in 5% CO_2 in air and 95% relative humidity and incubated for 24 hrs. The oocytes matured in presence of 10% BES and 10% FBS showed higher maturation rate than the medium alone. The addition of different concentrations of FSH and oestradiol (E2) in the presence of serum to medium showed improved maturation rate over that serum alone. However higher maturation rate (71.05%) was observed in Ham's F-10 + FBS + E2 + 10 µg/ml FSH than Ham's F-10 + BES + E2 + 10 µg/ml FSH i.e. 51.16%, but no significant differences was observed. In the present study, FBS and 10 µg/ml concentration of commercially available crude FSH in the presence of oestradiol and suitable protein supplement found to be most suitable medium for maturation of buffalo oocytes

Keywords : Nuclear maturation, fetal bovine serum, germinal vesicle, metaphase.

Introduction

Mammalian oocyte are arrested at the dictyate stage of nuclear maturation in the ovary. Immature oocytes which have been removed from the follicle and placed in culture medium undergo germinal vesicle breakdown and the continuation of meiosis to metaphase II.

The selection of protein supplements and hormones for *in vitro* maturation (IVM) play an important role in subsequent *in vitro* fertilization and development. The medium supplemented with blood serum resulted in high frequency of nuclear maturation and cumulus expansion, when FSH was added (Leibfried et al. 1987). Now a days commercially produced crude FSH and LH are available in market. The addition of FSH, LH and oestradiol cause synergistic enhancement of nuclear maturation of buffalo oocytes (Totey et al. 1993).

The purpose of research was to investigate the effect of serum and hormones on *in vitro* maturation of buffalo oocytes.

Material and Methods

Buffalo ovaries were collected from local slaughter house and brought to the laboratory in normal saline at 30-35°C within 1-2 hrs of slaughter. The cumulus oocyte complexes (COCs) were isolated from follicles by slicing method and only good quality oocytes were used for maturation. The isolated oocytes were washed thrice with TL-Hepes medium and final two washings were given in maturation medium. The maturation was studied in two different groups (i) Ham's F10+10% BES (ii) Ham's F-10+10% FBS in the presence of three different concentrations of commercially available FSH (Folltropin -V, Vetrepharm Inc, Ontario, London Canada) and oestradiol (E2). The *in vitro* maturation was carried out in six different treatment groups (i) Medium only (control) (ii) Medium+10% serum (BES or FBS) (iii) medium+10% serum +1 µg/ml oestradiol (E2) (iv) medium+10% serum+1 µg/ml oestradiol (E2) +1 µg/ml FSH (v) medium +10% serum +1 µg/ml oestradiol (E2)+10 µg/ml FSH (vi) medium +10% serum +10 µg/ml oestradiol (E2)+20 µg/ml FSH.

10 oocytes were placed in 50 µl maturation drops, covered under sterile mineral oil at 390C in 5% CO2 in air and 95% relative humidity and incubated for 24 hrs. After 24 hrs of culture, the oocytes fixed overnight in ethanol : acetic acid (3:1) and stained with 1% aceto orecin stain. They were evaluated for germinal vesicle (GV), metaphase I (MI) and metaphase II (MII) and degenerated oocyte.

Results and Discussion

In the present study the effect of serum, different concentrations of commercially available FSH (Folltropin-V) and oestradiol on *in vitro* maturation was investigated.

The maturation rate of buffalo oocytes in Ham's F-10 alone was 33.33%. The addition of protein supplement in the form of BES and FBS improved the metaphase II percentage over control but no significant difference was observed. The addition of oestradiol to medium in the presence of BES was 40.00% and 41.66% respectively. The maturation was further increased after addition of different concentrations of FSH. The maximum maturation rate was found in 10 µg/ml FSH in the presence of FBS and oestradiol (1µg/ml) i.e. 71.05%. The maturation rate was higher in FBS supplemented group than BES, but no significant difference was observed.

In the present study we found that the oocyte matured in medium alone get matured only partially. The addition of sera to the culture medium during the maturation of oocytes promotes the rupture of the germinal vesicle and facilitates the oocyte maturation (Sanbuissho et al. 1990). The addition of 10% FBS in the presence of 1µg/ml E2 and 10 µg/ml FSH showed improved maturation rate as compared with 10% BES supplemented group, but no significant difference was observed. Fetal calf serum improves the maturation and fertilization over that of BES because it contains some unidentified growth promoting components that were absent in the serum of adult animals or fetal calf serum lacks components (hormones and immunoglobulins) present in the adult serum that retard the *in vitro* development of cells (Mochizuki et al. 1991). Our results were in agreement with the observations of Fukui and Ono (1989) who did not observe any significant difference between FCS and oestrus cow serum on bovine oocyte maturation.

In the present study, three different concentrations i.e. 1, 10 and 20 µg/ml commercially available FSH were used. The higher maturation rate was observed in 10 µg/ml FSH (71.05%). The overall maturation rate in hormone treated groups was found lower in our study as compared to maturation observed by Pawshe et al. (1993). They observed maturation rate (91.86%) in presence of 10 µg/ml FSH (Folltropin - V) in goat.

Our results were in agreement with results observed by (Chauhan and Anand, 1991) who reported (69.1%) maturation rate in Ham's F-12 supplemented with BSA and FCS in the presence of FSH.

In conclusion, medium containing FBS and 10 µg/ml commercially available FSH and oestradiol can be successfully used for *in vitro* maturation of buffalo oocyte.

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