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Technical note

Effect of various growth factors on the in vitro development of goat preantral follicles

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Abstract

The effect of transforming growth factor-α (TGF-α; 10 ng ml^{-1}), insulin-like growth factor-II (IGF-II; 20 ng ml^{-1}), epidermal growth factor (EGF; 50 ng ml^{-1}) and FSH (1 μg ml^{-1}) was investigated in the in vitro development of goat preantral follicles (PFs). The control medium was bicarbonate buffered Tissue Culture Medium 199 (TCM 199B), without supplementation. Small (40–60 μm) and large (61–100 μm) PFs were cultured for 6 days. In vitro growth of the PFs was assessed by the increase in follicle diameter, oocyte diameter, DNA synthesis by follicular cells and follicular cell number. All growth factors studied induced DNA synthesis in the small and large goat PFs. A significant increase in diameter of the follicles (EGF: 51.18 ± 0.71 versus 58.19 ± 1.41 and FSH: 53.14 ± 0.71 versus 58.30 ± 1.30) and follicular cell number (EGF: 39.21 ± 1.20 versus 55.96 ± 1.32 and FSH: 40.34 ± 1.25 versus 51.75 ± 1.81) was observed in small PFs cultured in EGF and FSH supplemented media. In the large PFs, only EGF induced a significant increase in follicle diameter and follicular cell number. It was concluded that EGF is mitogenic to goat PFs, FSH is capable of inducing growth in small but not large PFs and TGF-α and IGF-II are stimulatory to goat PFs in culture.

Keywords: Goat; Preantral follicles; In vitro culture; Growth factors

1. Introduction

A major constraint for the optimum utilization of the genetic potential of female farm animals is the limited number of ova produced at each oestrous cycle. Although superovulation and in vitro embryo

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production (IVEP) have improved access to female germplasm, these techniques are beset with problems such as refractoriness to hormones, unpredictable response and poor fertilization rate. It is known that the ovaries of mammals contain hundreds of thousands of oocytes in primordial and preantral follicles (PFs). If a technological method to access this large pool of gametes could be developed it would radically improve the utilization of female germplasm. Several important benefits of in vitro maturation of PFs in farm animals have been previously elaborated (Mao et al., 2002; Hemamalini et al., 2003; Tamilmani et al., 2005).

While in vitro culture systems for PFs have been developed in different mammalian species (Hemamalini et al., 2003), only mouse offspring have been produced from oocytes in cultured primordial follicles (Eppig and Schroeder, 1989; Carroll et al., 1990; Eppig and O'Brien, 1996; Liu et al., 2001). Isolation of PFs from sheep and goat ovaries has been standardized (Chelikani et al., 1998). Recently, success in DNA synthesis and in vitro growth (Hemamalini et al., 2003) and maturation of sheep oocytes from in vitro cultured PFs to the metaphase II stage were achieved (Tamilmani et al., 2005). The present study was conducted as an initial step towards achieving similar success in goat PFs. TGF-α, IGF-II, EGF and FSH are known to influence in vitro PF development in other species (Roy and Greenwald, 1988; Nayudu and Osborn, 1992; Jewgenow and Pitra, 1993; Roy, 1993a, 1993b; Smyth et al., 1994; Hulshof et al., 1995; Wandji et al., 1996; Nuttinck et al., 1996; Cecconi et al., 1999; Hemamalini et al., 2003). To elucidate the restricted effects of TGF-α, IGF-II, EGF and FSH on in vitro development of goat PFs, these growth factors were individually included in a medium free of serum, insulin, transferrin, selenium and BSA.

2. Materials and methods

Unless otherwise mentioned, all the media, hormones, growth factors, fetal calf serum (FCS) and other chemicals were obtained from Sigma Chemical Co. (St.Louis, USA) and plastics from Nunclon, (Roskilde, Denmark). Epidermal growth factor used in these studies was a tissue culture product obtained from mouse sub-maxillary glands (cat no. E4127, Sigma, USA; cell proliferation BALB/MK cells EC_{50} = 0.02 ng/ml). Insulin-like growth factor II (cat no. I2139, Sigma, USA; cell proliferation MCF-7 cells EC_{50} = 6.5 ng/ml)) and Transforming growth factor-α (cat no. T7924, Sigma, USA; cell proliferation BALB/3T3 cells EC_{50} = 0.61 ng/ml) were recombinant human analogues expressed in *E. coli*. FSH was from the porcine pituitary glands (cat no. F2293, Sigma USA; 50 U/vial).

Procedures for collection of the ovaries, isolation of PFs (Chelikani et al., 1998), classification of PFs (small: 40–60 μm in diameter; Plate 1: A and large: 61–100 μm in diameter; Plate 1: B) selection of PFs for culture, preparation of culture media and in vitro culture protocols were previously described by Hemamalini et al. (2003). Briefly each ovary was dissected into halves along its longitudinal axis, the ovarian medulla having been scooped out and the remaining ovarian cortex was sliced into small pieces of ∼1 mm^3 using a sterile scalpel blade. The cortical fragments were placed in 20 ml of 50 U ml^{-1} Hyaluronidase solution at 37 °C. Digestion was facilitated by intermittent vigorous in and out movements of the solution through a narrow glass pipette followed by gentle mixing on a hot magnetic stirrer for 15 min at 400 rpm. At the end of mixing, 2 ml PBS containing 10% goat serum was added to stop the enzyme action. The suspension was then filtered through a 500 μm nylon mesh. Subsequently, the solution was transferred to a 100 ml square polystyrene dish (Intergrid 1012, Falcon plastics, USA) and follicles were isolated under an inverted phase contrast microscope (Labovert FS, Leitz, Germany). Goat PFs having a clear oocyte surrounded by one to three layers of granulosa cells and with an intact basement membrane were considered acceptable and selected for culture (Plate 1: A and B). All the small and large PFs isolated from several caprine ovaries on each day of experimentation and selected for culture were randomly allotted to different treatment groups (Table 1). The PFs were washed three times in appropriate culture medium and placed in 20–30 μl droplets of the culture medium in 35 mm plastic tissue culture dishes (5–7 follicles per dish) and overlaid by a lightweight mineral oil that was preequilibrated overnight with the medium. The day on which the PFs were placed in the culture was designated as day 0 and subsequent days as day 1, day 2, etc. The culture medium was replenished with fresh medium on day 3.

The morphology of each follicle during culture was evaluated using an inverted microscope and number