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Effect of sequential medium with fibroblast growth factor-10 and follicle stimulating hormone on *in vitro* development of goat preantral follicles

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ABSTRACT

A sequential medium with fibroblast growth factor-10 (FGF-10) and follicle stimulating hormone (FSH) was evaluated on the survival, ultrastructure, activation and growth rate of caprine preantral follicles submitted to long-term culture, aiming to establish an ideal *in vitro* culture system. Ovarian fragments were cultured for 16 days in α-MEM$^+$ alone or supplemented with FGF-10 and/or FSH added sequentially on different days of culture. Ovarian fragments were cultured during the first (days 0–8) and second (days 8–16) halves of the culture period, generating 10 treatments: α-MEM$^+$/α-MEM$^+$ (cultured control), FSH/FSH, FSH/FGF-10, FSH/FSH + FGF-10, FGF-10/FGF-10, FGF-10/FSH, FGF-10/FSH + FGF-10, FSH + FGF-10/FSH + FGF-10, FSH + FGF-10/FSH and FSH + FGF-10/FGF-10. Follicle morphology, viability and ultrastructure were analyzed. The FSH/FGF-10 treatment showed a higher ($P < 0.05$) percentage of normal follicles compared to all other treatments. In addition, follicles from the FSH/FGF-10 treatment maintained ultrastructural integrity after the culture period. After 16 days of culture, the FSH/FGF-10 and FSH/FSH treatments showed a higher percentage of activation compared to the cultured control (α-MEM$^+$/α-MEM$^+$). Moreover, the FSH/FGF-10 treatment promoted greater follicular and oocyte diameters compared to the fresh control. In conclusion, this study showed that a sequential medium with FSH followed by FGF-10 (FSH/FGF-10 and FSH/FSH) maintains follicular viability and ultrastructure and promotes transition from the primordial to primary stage (activation) and growth in goat preantral follicles cultured *in vitro*.

1. Introduction

The development of a culture system using primordial follicles is very important for developing an understanding of the early stage of folliculogenesis and for preserving

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female germ cells for a long period (Mao et al., 2002). In this context, several studies have been conducted to investigate the effect of many components, such as hormones and growth factors, during the *in vitro* culture of preantral follicles in multiple species to establish efficient culture systems that enable follicular growth and development (Smitz and Cortvrindt, 2002). Among the growth factors and hormones that act by modulating the development of ovarian follicles, fibroblast growth factor-10 (FGF-10) and follicle stimulating hormone (FSH) stand out.

FGF-10 is an approximately 26-kDa protein belonging to the fibroblast growth factor (FGF) family and is implicated in several biological processes such as growth, motility, cell differentiation and scarring processes (Yamasaki et al., 1996; Min et al., 1998). Studies investigating the gene expression of FGF-10 using PCR in bovine fetal ovaries showed that FGF-10 is associated with the activation of primordial follicles and the formation of primary follicles (Buratini et al., 2007; Castilho et al., 2007).

Ovarian function, besides being influenced by the presence of growth factors, undergoes strong regulation by gonadotropin hormones such as FSH (Fortune, 2003). Studies have shown that the addition of FSH to *in vitro* culture systems for goat preantral follicles maintained the ultrastructural integrity of follicles and promoted follicular growth (Matos et al., 2007a; Magalhães et al., 2009). Until recently, it was believed that primordial follicles lacked receptors for FSH but the hormone could act indirectly. This indirect effect was believed to occur via paracrine factors such as IGF-I and activin released by larger follicles or ovarian stroma cells (Joyce et al., 1999; Thomas et al., 2005). However, a recent study in goats showed the presence of FSH receptors in oocytes from primordial follicles, enabling the existence of a direct action of this hormone during this follicular phase (Patel et al., 2013).

Although some studies suggest an important role for FGF-10 and FSH in the survival of preantral follicles, the association of both of these substances in a sequential medium for long-term *in vitro* culture of caprine primordial follicles has not been studied. Thus, this study aimed to investigate the effects of FGF-10 and FSH, alone and in combination, at the beginning of follicular development during a long-term *in vitro* culture of goat preantral follicles.

2. Materials and methods

2.1. Source of ovaries

Ovaries ($n = 10$) were collected at a local slaughterhouse from five adult (1–3 years old) cross-breed goats (*Capra hircus*). Immediately postmortem the ovaries were washed in 70% alcohol for 10 s followed by two washes in Minimum Essential Medium (MEM) supplemented with 100 μg/ml penicillin and 100 μg/ml streptomycin. The pairs of ovaries were transported to the laboratory within 1 h while stored in MEM at 4 °C (Chaves et al., 2008). Unless mentioned otherwise, the culture media and other chemicals used in the present study were purchased from Sigma Chemical Company (St. Louis, MO, USA).

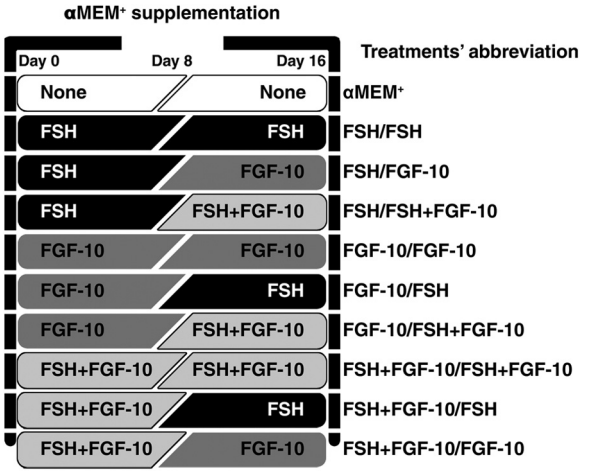

Fig. 1. α-MEM+ alone and α-MEM+ containing fibroblast growth factor 10 (FGF-10) and/or follicle-stimulating hormone (FSH) in different combinations during the *in vitro* culture of caprine preantral follicles.

2.2. Experimental protocol

The organ culture system utilized herein was previously described in detail (Matos et al., 2007a; Celestino et al., 2010). In the laboratory, the ovaries from each animal were stripped of the surrounding fat tissue and ligaments. Subsequently, ovarian cortex tissue samples from each ovarian pair were cut under sterile conditions into 19 slices (approximate size, $3 \times 3\,mm^2$ and 1 mm thick) using a needle and scalpel. One slice was immediately fixed for histological and ultrastructural analysis (fresh control) and the other 18 were placed in culture for 1, 8 or 16 days in individual wells. The cortex tissue samples were transferred to 24-well culture dishes containing 1 ml of culture medium. Culture was performed at 39 °C in 5% CO_2 in a humidified incubator and all media were incubated for 1 h prior to use. The basic culture medium (control medium) consisted of α-MEM (pH 7.2–7.4) supplemented with ITS (insulin 10 μg/ml, transferrin 5.5 μg/ml and selenium 5 ng/ml), 2 mM glutamine, 2 mM hypoxantine, 1.25 mg/ml bovine serum albumin (BSA) and 50 μg/ml ascorbic acid, and was called α-MEM+. For experimental conditions, the medium was supplemented with recombinant human FGF-10 (50 ng/ml; PeproTech Inc., Rocky Hill, NJ, USA), recombinant bovine FSH (50 ng/ml; rFSH®114, Nanocore, Brazil) or a combination of FGF-10 and FSH, as shown in Fig. 1. Each treatment was repeated five times and the culture media was replenished every other day. The concentrations of FGF-10 and FSH used in this work were chosen based on previous research conducted in our laboratory (Chaves et al., 2010; Magalhães et al., 2009).

2.3. Morphological analysis and assessment of in vitro follicular growth

Before culture (fresh control) and after 1, 8 or 16 days in culture, all ovarian fragments were fixed in Carnoy's solution for 12 h and then dehydrated in increasing concentrations of ethanol. After paraffin embedding (Synth, São