Animal Reproduction Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

In vitro culture of isolated preantral and antral follicles of goats using human recombinant FSH: Concentration-dependent and stage-specific effect

Anna Clara A. Ferreira[a], Jesús Cadenas[a], Naiza A.R. Sá[a], Hudson H.V. Correia[a], Denise D. Guerreiro[a], Carlos H. Lobo[b], Benner G. Alves[c], Carolina Maside[d], Eduardo L. Gastal[e], Ana Paula R. Rodrigues[a], José Ricardo Figueiredo[a,*]

[a] *Laboratory of Manipulation of Oocytes and Preantral Follicles, Faculty of Veterinary, State University of Ceará, Fortaleza, CE, Brazil*
[b] *Laboratory of Animal Physiology, Department of Animal Science, Federal University of Ceará, Fortaleza, CE, Brazil*
[c] *Institute of Biomedical Sciences, Federal University of Uberlândia, Uberlândia, MG, Brazil*
[d] *Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, University of Murcia, Murcia, Spain*
[e] *Department of Animal Science, Food and Nutrition, Southern Illinois University, Carbondale, IL, United States*

ARTICLE INFO

Keywords:
Goat
Oocyte maturation
Human recombinant FSH

ABSTRACT

The present study aimed to investigate a concentration-response curve of human recombinant FSH (hrFSH) for *in vitro* culture of isolated preantral and early antral follicles of goats. Isolated follicles were cultured for 18 days using the following treatments: basic culture medium (control); or control medium supplemented with 10, 50, and 100 mIU/mL of hrFSH. At the end of the culture, cumulus-oocyte complexes were recovered and subjected to *in vitro* maturation. The following endpoints were evaluated: follicle morphology, growth rate and antrum formation, oocyte viability and meiotic stage, and estradiol production, as well as relative expression of FSH receptor (*FSHR*), and steroidogenic enzyme (*3β-HSD*, *CYP17*, and *CYP19A1*) genes. In antral follicles, the FSH addition at 50 mIU/mL increased follicular diameter and growth rate, percentage of fully developed oocytes, and oocyte diameter ($P < 0.05$), and tended to increase the percentage of MII oocytes when compared to the control ($P = 0.07$). With preantral follicles, FSH addition at 100 mIU/mL increased relative abundance of mRNA for CYP19A1 when compared to the control ($P < 0.05$). At the same FSH concentrations of 100 and 50 mIU/mL, there was a greater relatively abundance of mRNA for 3β-HSD and CYP17 in preantral than in antral follicles ($P < 0.05$). For preantral and antral follicle comparisons when the same treatments were imposed, there were greater concentrations of estradiol for antral follicles ($P < 0.05$). In conclusion, hrFSH enhanced in a concentration-dependent manner the *in vitro* development of caprine antral follicles; however, there was no positive effect in the culture of preantral follicles.

1. Introduction

Follicle-stimulating hormone (FSH) is a gonadotropin produced by the pituitary gland that promotes ovarian follicular survival and development (Brown and McNeilly, 1999; Richards et al., 2002). The mRNA for FSH receptor (FSHR) has been detected in granulosa cells of preantral and antral follicles in several species (*e.g.*, mice: O'Shaughnessy et al., 1996; Sokka and Huhtaniemi,

* Corresponding author.
E-mail address: jrf.lamofopapapers@gmail.com (J.R. Figueiredo).

https://doi.org/10.1016/j.anireprosci.2018.07.004
Received 27 March 2018; Received in revised form 28 June 2018; Accepted 16 July 2018

1990; cattle: Xu et al., 1995; goats: Saraiva et al., 2011; pigs: Durlej et al., 2011). The FSH affects the *in vitro* development of follicles at early stages of folliculogenesis (Adriaens et al., 2004). The effects of FSH *in vitro,* however, may be determined by several factors such as species (Rocha et al., 2014; Xu et al., 2010), type of culture system (Ihm et al., 2015), concentration used (Javed et al., 2008), association with other hormones and/or growth factors (Saraiva et al., 2012; Ferreira et al., 2016), and sources of FSH (*i.e.*, recombinant, pituitary, urinary; Magalhães et al., 2009).

Human recombinant FSH (hrFSH) is used more often because of its relatively greater purity (Calder et al., 2003). The use of hrFSH in *in vitro* culture maintained survivability and promoted granulosa cell proliferation in rat (Adriaens et al., 2004) and human (Wright et al., 1999) preantral follicles. Furthermore, hrFSH has prevented apoptosis of human preantral follicles and antral follicles (Wright et al., 1999). In livestock such as goats, hrFSH at 10 mIU/mL is one of the components of a base medium used in *in vitro* culture of preantral follicles (Rocha et al., 2014). This concentration of FSH has been established in mice (Adriaens et al., 2004; Sánchez et al., 2011). To the best of our knowledge, however, there have been no studies investigating a concentration-response curve of hrFSH during *in vitro* culture of preantral and antral follicles of goats.

A concise body of knowledge has suggested that follicular requirements *in vivo* change as a follicle develops through the early stages of folliculogenesis until the preovulatory stage (Sánchez et al., 2012). In this regard, Magalhães-Padilha et al. (2013) studied gene expression in late developmental stages of secondary follicles and early stage development of antral follicles using microarray assay analyses in goats. It was reported that although the two follicular categories differed in diameter by only 150 µm, 2,466 genes were stage-specific, and up- and down-regulated. A recent *in vitro* culture study in goats has supported the aforementioned differences in gene expression between follicle categories (Cadenas et al., 2017); furthermore, from this study it was reported that the addition of growth hormone during culture of early antral follicles positively affected oocyte growth and maturation, but had no effect on the development of preantral follicles. Stage-specific concentrations of growth factors and hormones (*e.g.*, FSH) necessary during *in vitro* folliculogenesis, therefore, are relevant and should be defined and taken into consideration when proposing adequate culture medium for any species (Fortune, 2003; Peng et al., 2010). In addition, hrFSH has been used extensively in assisted reproductive technologies in humans (Agarwal et al., 2000). Because the goat has been considered as an adequate model to study early folliculogenesis *in vitro* in women (Faustino et al., 2011), studies aiming to evaluate the effect of hrFSH concentrations of follicles in different development stages are important.

The present study aimed to investigate for the first time a concentration-response curve of hrFSH for the *in vitro* culture of isolated preantral and early antral follicles of goats. The following endpoints were evaluated: follicle morphology and growth, oocyte growth and chromatin configuration, estradiol production, and relative abundance of mRNA for *3β-HSD*, *CYP17*, *CYP19A1*, and *FSHR*.

2. Materials and methods

2.1. Chemicals and media

The reagents and chemical used in this study were obtained from Sigma Chemical Co. (St. Louis, MO, USA), unless otherwise indicated.

2.2. Collection of ovaries, isolation, selection, and culture of preantral and early antral follicles

Ovaries (n = 120) were collected at a local slaughterhouse from adult crossbred goats (ages 1 to 3 years), and transported to the laboratory in MEM-HEPES within 1 h at 4 °C, as described previously (Chaves et al., 2008). In the laboratory, cortical slices (1 to 2 mm thick) were transferred into holding medium (MEM-HEPES). Two follicular categories (preantral and early antral follicles) were visualized under a stereomicroscope (SMZ 645 Nikon, Tokyo, Japan) and manually dissected from slices of ovarian cortex using needles (26 G). Preantral follicles (~250 µm in diameter, with visible oocyte surrounded by at least two granulosa cell layers, intact basement membrane, and no antral cavity) and early antral follicles (~350 µm in diameter and presence of translucent cavity filled with follicular fluid) were selected for culture (Cadenas et al., 2017). After selection, follicles were individually cultured in 100 µL drops of culture medium in Petri dishes (60 x 15 mm, Corning Incorporated, Corning, NY, USA). The base culture medium (control treatment) consisted of α-MEM (pH 7.2–7.4, Gibco; Invitrogen, Karlsruhe, Germany) supplemented with 3 mg/mL bovine serum albumin (BSA), 5.5 µg/mL transferrin, 5 ng/mL selenium, 2 mM glutamine, 2 mM hypoxanthine, and 50 µg/mL ascorbic acid, and 10 µg/mL human recombinant insulin, referred to as α-MEM$^+$ (Ferreira et al., 2016). The culture was conducted at 39 °C, in 5% CO_2 in air for 18 days. Fresh medium was prepared immediately before use and pre-equilibrated for at least 1 h prior to use, with 60 µL of medium being replaced in each drop every 2 days. The experiment was replicated five times, and approximately 50 follicles were used per treatment.

2.3. Experimental design

For the experimental conditions, preantral and early antral follicles were randomly distributed into the following treatments: α-MEM$^+$ alone (control treatment); or α-MEM$^+$ supplemented with 10 mIU/mL, 50 mIU/mL, and 100 mIU/mL of hrFSH, designated as FSH10, FSH50, and FSH100 treatments, respectively.