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Proteomic analysis of follicular fluid from tropically-adapted goats

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ABSTRACT

The present study was conducted to characterize the major proteome of ovarian follicular fluid from locally-adapted, "Canindé" goats in the northeast of Brazil. Eight estrous cycling goats received a hormonal treatment consisting of medroxyprogesterone acetate, D-cloprostenol and FSH. Fluid was collected by laparoscopy from small (< 3 mm), medium (3–4 mm) and large (> 4 mm) follicles and then, proteins were analyzed by 2-D SDS-PAGE and tandem mass spectrometry. Thirty-six proteins were identified in the goat follicular fluid, including albumin, immunoglobulins, ceruloplasmin, complement factor B, alpha-1B-glycoprotein precursor, serotransferrin, complement C3 and serpins, among others. Albumin and immunoglobulins were the most abundant proteins. Protein concentrations were similar in the fluid from small (45.3 ± 3.1 mg/mL), medium (44.2 ± 3.3 mg/mL) and large follicles (45.1 ± 2.3 mg/mL). The intensities of spots identified in 2-D gels as serotransferrin, zinc-alpha-2-glycoprotein-like, complement factor B and complement protein C3 differed ($P < 0.05$) among follicle categories. The amount of serotransferrin was greater in the medium than small follicles ($P < 0.05$). Content of zinc-alpha-2-glycoprotein-like, complement factor B and complement C3 was greater ($P < 0.05$) in the fluid of large follicles than in medium follicles. Based on gene ontology, the major molecular functions associated with goat follicular fluid proteins were binding and catalytic activity, while the main biological processes were related to regulation, cellular processing, location and the immune system. In conclusion, the major proteome of the follicular fluid from goats subjected to hormonal stimulation was elucidated in the present study. Also, molecules associated with follicle development are potential biomarkers of oocyte competence were prevalent.

1. Introduction

In mammals, the follicular fluid provides a biological microenvironment for the development of oocytes. Follicular fluid accumulates during the formation of antral cavity and it is mainly formed by the secretory activity of theca and granulosa cells and by

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diffusion of components from capillaries to the antrum (Gosden et al., 1988). Follicular secretion has an important role during nuclear and cytoplasmic maturation of the oocyte as well. For *in vitro* fertilization (IVF) of several species, ovaries are hormonally stimulated to enable the collection of as many oocytes as possible. Currently, morphology is the only criteria used for oocyte selection as there is limited information about the molecular components of female gametes (Virant-Klun and Krijgsveld, 2014). Studies about the proteome of ovarian follicular fluid become important as these will provide information about the follicular microenvironment and knowledge about factors that potentially influence oocyte growth and maturation. Furthermore, follicular fluid is a useful biological entity for the assessment of potential markers of fertility in all animals including humans (Powell et al., 2010).

When Europeans discovered Brazil more than 500 years ago, the settlers brought to the new land the first ruminant species of farm animals. As natural selection occurred over the years, these animals developed morphological and physiological characteristics adapted to environmental conditions of the different regions of Brazil, forming the "criollo", "local" or "naturalized" breeds (Mariante and Cavalcante, 2006). In the semi-arid region of Brazil, the majority of naturalized goats are endangered, including breeds known as "Canindé", "Moxotó", "Marota" and "Repartida". Such breeds are well adapted to semi-arid climate and need to be preserved because of their unique attributes and to favor biodiversity. The animals of the Canindé breed have a remarkable adaptation to heat stress and dairy performance in low-input production systems in comparison to highly demanding European goats (Mariante et al., 2009). A study reported that the number of fertilized oocytes was greater ($P < 0.0001$) in Canindé than in Saanen females (98.9 compared with 36.2 oocytes, respectively) after hormonal treatment and natural service (Moura et al., 2010). Such results indicate that local breeds have some unique reproductive capacity as compared with non-adapted breeds in a tropical environment. Efforts are currently being made to prevent the extinction of locally-adapted breeds, using reproductive biotechnologies such as IVF. Efficiency of *in vitro* production of goat embryos is, however, still low due to, among other factors, limited knowledge of the specific processes and molecular pathways that regulate oocyte development (Souza-Fabjan et al., 2014). Studies with different approaches in proteomics have been conducted to identify proteins in the ovarian follicular fluid of several species, such as cattle (Maniwa et al., 2005), swine (Bijttebier et al., 2009), dogs (Fahiminiya et al., 2010), horses (Fahiminiya et al., 2011) and humans (Ambekar et al., 2013). The present study was, therefore, conducted to characterize the major proteome of follicular fluid as related to follicle development in locally-adapted Canindé goats subjected to hormonal stimulation.

2. Material and methods

2.1. Locality of study and experimental animals

The experiment was conducted at the Ceará State University, located in Fortaleza, Brazil (3°47′38″S, 38°33′29″W) and used eight estrous cycling Canindé goats that were 1.9 ± 0.3 years of age and weighing 23.1 ± 1.5 kg. Animals were kept in shaded pens with free access to unshaded areas and received diets containing Tifton (*Cynodon dactylon*) hay and commercial concentrate (0.2 kg/day; 20% crude protein), with free access to water and mineralized salt. Experimental protocols and animal handling were approved by the Ethics Committee of the Ceará State University (3246402/2014). In addition, this study was conducted in accordance with the guidelines for animal care (Association for the Study of Animal Behaviour, 2006).

2.2. Hormonal treatment and collection of follicular fluid

As part of the hormonal treatment, goats initially received vaginal sponges with 60 mg medroxyprogesterone acetate (Progespon, Syntex, Buenos Aires, Argentina) for 10 days. On Day 7, 75 μg D-cloprostenol (Prolise, ARSA S.R.L., Buenos Aires, Argentina) were given to all animals and ovarian stimulation was achieved with five pFSH injections (30, 30, 20, 20 and 20 mg/goat; Folltropin-V, Bioniche, Belleville, Canada). The pFSH injections were given at 12-h intervals, starting in the morning of the day D-cloprostenol was injected (Sanchez et al., 2014). Laparoscopy was performed 24 h after goats received the last pFSH injection.

For collection of follicular fluid, goats were subjected to food (36 h) and water (24 h) deprivation, followed by anesthesia with 20 mg/kg thiopental (Tiopentax 2.5%, Cristália, São Paulo, Brazil) and 3% isoflurane (Isoforine, Cristália, São Paulo, Brazil). Follicles were punctured and fluid was collected using a 5-mm Hopkins laparoscope (Karl Storz, Tuttlingen, Germany), and a 22-G needle and a vacuum pump (WTA, Cravinhos, Brazil) adjusted to 35 mmHg. Follicles selected for fluid collection were determined as small (< 3 mm), medium (3–4 mm) and large (> 4 mm). Definition of these groups was based on a study previously conducted for evaluation of follicle categories in locally-adapted goats (Sousa et al., 2011). Based on such study, follicles larger than 4 mm in Canindé goats are considered mature, preovulatory follicles (Sousa et al., 2011). Each follicle was punctured separately to prevent mixing of follicular fluid from follicles with different sizes. Considering all females used in the present study, 222 follicles were punctured and follicles were defined as large ($n = 95$), medium ($n = 85$) and small ($n = 42$). The average number of follicles punctured per goat was 6.3 ± 4.5, 5.7 ± 2.4 and 6.0 ± 1.4 for large, medium and small follicles, respectively. Fluid from the same follicle sizes and same animal were pooled and centrifuged at 3000*g* for 20 min at 4 °C. The supernatant was subsequently pipetted into 1.5-mL tubes and stored at −80 °C until further analysis.

2.3. Two-dimensional gel electrophoresis

Reagents for electrophoresis were purchased from GE Life Sciences (Piscataway, NJ, USA) and Sigma-Aldrich (St. Louis, MO, USA). Soluble protein content in follicular fluid samples was determined according to Bradford method (Bradford, 1976). Aliquots containing 500 μg protein were mixed with re-hydration buffer (7 M urea, 2 M thiourea, 2% CHAPS, 0.5% immobilized pH gradient