Animal Reproduction Science xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Animal Reproduction Science

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

Biochemical composition and protein profile of alpaca (*Vicugna pacos*) oviductal fluid

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ARTICLE INFO

Article history:
Received 9 June 2014
Received in revised form
19 December 2014
Accepted 20 December 2014
Available online xxx

Keywords:
South American Camelids
Oviductal fluid
Oviduct
Protein

ABSTRACT

Knowledge and assessment of the constituents of the oviductal fluid (OF) in camelids is necessary for a correct formulation of specific culture media for the development of reproductive biotechnology. This study is the first describing the biochemical composition and SDS–PAGE protein profile of alpaca oviductal fluid in non-pregnant animals and animals that have completed the first month and second month of gestation. Samples were also classified into oviducts that were ipsilateral or contralateral to the ovary with corpus luteum. No differences were found between both oviducts, whereas pregnant and non-pregnant females displayed significant differences in the biochemical composition and protein profile of the oviductal fluid. Relative albumin content was higher in non-pregnant females. Relative creatinine content in OF from females that have completed the second month of gestation was lower than non-pregnant females and females that have completed the first month of gestation. Ion Na^+ concentration was higher in OF from non-pregnant females when compared with pregnant ones. The protein profile of non-pregnant females showed five protein bands of 70, 42, 25, 24 and 19 kDa that were significantly more intense compared with pregnant animals. Bands were identified as moesin, actin cytoplasmic 2, hydroxypyruvate isomerase, ferritin light chain and peroxiredoxin-6 with MALDI/MS. Our results encourage more thorough future studies, in order to unravel the complex reproductive processes of the South American camelid oviduct.

1. Introduction

The oviduct is considered a reproductive organ that carries out transport and secretion functions which are essential for early reproductive events. The organ provides a suitable environment for sperm transport, storage and capacitation, oocyte pick-up, transport and maturation,

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http://dx.doi.org/10.1016/j.anireprosci.2014.12.013
0378-4320/

fertilization and ultimately, early embryo cleavage (Hunter, 2005). Biochemical analysis shows that tubal secretion is a subtle mixture of a transudate, containing compounds originating from serum and specific compounds synthesized by tubal epithelium. The ionic composition and macromolecular content differ in many important aspects from that of plasma (Gandolfi et al., 1989). These differences demonstrate that oviductal fluid (OF) is not simply a filtrate of blood plasma (Leese, 1988).

Numerous compounds isolated from OF are now added to synthetic media for *in vitro* maturation/*in vitro* fertilization/embryo culture (IVM/IVF/EC) (Papanikolaou et al., 2008; Yoshioka, 2011). The rationale is now to resemble the biochemical composition of tubal fluid as much as possible even if interactions with gametes and embryo metabolism are still far from being understood.

Interest in the application of reproductive technologies in South American Camelids (SAC) has increased in the last decade; domestic and wild SAC species have become internationally known and there has been a greater diffusion of their productive characteristics. Physiologically, SAC exhibit several distinctive reproductive characteristics. They are induced ovulators (San-Martin et al., 1968), and in the absence of an ovulatory stimulus, ovarian activity has been proposed to occur in waves of follicular growth and regression. Follicular waves are usually associated with increased estrogens production during those waves (Vaughan and Tibary, 2006). In contrast, plasma progesterone levels remain below 1 ng/mL in the majority of unmated camelids (Aba et al., 1995), only increasing after ovulation and during pregnancy (Vaughan and Tibary, 2006).

Although knowledge and assessment of OF composition is necessary to develop specific sperm/oocyte/embryo culture media, currently the role of the SAC oviduct and its secretions are still poorly understood. A feasible reason may be the difficult access to the animals, and even more, the OF.

The current study describes the biochemical composition and protein profile of a domestic SAC, the alpaca, during different physiological states: non-pregnant uncopulated females, pregnant females that have completed the first month of gestation and pregnant females that have completed the second month of gestation. In addition, oviductal proteins that presented differential secretion patterns were identified. The underlying objective of this work was to gain a better knowledge of the microenvironment within the oviductal lumen in SAC, revealing differences between pregnant and non-pregnant animals.

2. Materials and methods

2.1. Animals and samples

The reproductive tissue of adult *Vicugna pacos* females (var. Huacaya) were collected from a slaughterhouse in Huancavelica (12°S, 74°W, and at 3676 m altitude), Peru. A total of 25 animals were used in this experiment: 5 were not pregnant (NP) with ovarian follicles smaller than 7 mm, 10 females were pregnant with 34–37 days of gestation (P1) and 10 with 60–64 days of gestation (P2). The gestational age of the animal was calculated measuring the crump-rump length or total fetal length of the fetuses according to Catone et al. (2006) and Olivera et al. (2003).

Oviducts, ovaries and mesenteries were collected at the time of slaughtering and immediately placed in PBS (136 mM NaCl, 2.68 mM KCl, 8.1 mM Na_2HPO_4, 1.47 mM KH_2PO_4; pH 7.4) at 4 °C. At the laboratory, oviducts were classified as follows: ipsilateral to the ovary with corpus luteum (I) or contralateral to the ovary with corpus luteum (C). Oviducts from NP females were considered as a single group with dominant follicles smaller than 7 mm. To obtain the OF, a sterile pipette was carefully introduced into the ampulla and air was insufflated several times. Then liquid was collected at the utero tubal junction level by slight pressure on the oviductal walls. Samples containing blood were rejected. Then, flushings from each experimental condition were grouped in three different pools ($n = 3$). Each pool was considered one sample and centrifuged at 5000 × *g* (10 min, 4 °C) to pellet any cellular debris. The OF thus obtained was stored at −20 °C until further analysis.

2.2. Biochemical analyses

Concentrations of the following parameters were assessed using an automated analyzer (BM Hitachi 911; Roche, Basel, Switzerland): glucose, creatinine, total protein, globulin (G), alkaline phosphatase (ALP), g-glutamine transferase (γ-GT), aspartate amino-transferase (AST), alanine amino-transferase (ALT), creatinine kinase (CK), lactate dehydrogenase (LDH), sodium (Na), potassium (K), magnesium (Mg), phosphorus (P) and calcium (Ca). Parameter values of proteins and enzymes were normalized to total protein concentration. Considering that about 40% of calcium is protein bound, usually to albumin (Azim et al., 2012), this parameter was also normalized to total protein concentration.

2.3. Protein profile using 1D electrophoresis

Total OF protein was determined using a Micro-BCA protein assay kit (Thermo Fisher Scientific, USA). Electrophoresis was carried out according to Gevaert and Vandekerckhove (2000) as follows: 20 μg of protein of each pool were diluted (v/v) with a sample buffer (0.1 M Tris–HCl, pH 6.8, 2% sodium dodecyl sulfate, 1% 2-β-mercaptoethanol, 30% glycerol, and 0.05% bromophenol blue), denatured at 95 °C for 10 min and loaded onto a 4% stacking polyacrylamide gel, which was overlaid on top of a 12% resolving gel. Molecular masses were determined by running standard protein markers (PageRuler Unstained Broad Range Protein Ladder, Thermo Fisher Scientific, USA) covering the range of 5–250 kDa. Gels were run in a PROTEAN II xi Cell (Biorad, CA, USA) at 2.5 mA per well at room temperature. After the run gels were fixed in a 30% methanol–10% acetic acid solution and stained with colloidal Coomassie Blue G-250 (Sigma, Chemical Co., St. Louis, MO, USA) according to Neuhoff et al. (1990). Gel images were obtained using a Pentax Optio M 90 camera (Pentax, Milan, Italy) and GelAnalyzer version 2010a software was used to determine the molecular weight and intensity of the detected bands on the digitized gel images. Three

Please cite this article in press as: Apichela, S.A., et al., Biochemical composition and protein profile of alpaca (*Vicugna pacos*) oviductal fluid. Anim. Reprod. Sci. (2015), http://dx.doi.org/10.1016/j.anireprosci.2014.12.013