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

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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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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

different images, belonging to the electrophoresis of three different pools of each experimental group were analyzed and compared.

One-way ANOVA (Infostat Statistical Software, Córdoba, Argentina) was used to determine statistical differences between bands intensities among the samples assayed and to create a band picking list. Protein bands that exhibited clear differences between pregnant and non-pregnant females were excised for characterization using matrix-assisted laser desorption–ionization mass spectrometry (MALDI-MS), performed on an Ultraflex II TOF/TOF (BrukerDaltonics, Bremen, Germany) mass spectrometer, at the CEQUIBIEM mass spectrometry facility (CEQUIBIEM service, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina). Proteins were identified by peptide mass fingerprinting with MASCOT v. 2.2.03. Fragmentation was carried out with the most intense MS peaks (MS/MS). When possible, MS and MS/MS information was combined for one or more peptide searches. *De novo* sequencing was inferred from BLAST results when peak fragmentation was allowed. The percentage of protein coverage was determined for each spot using the MASCOT search.

3. Results

Protein parameters are shown in Table 1. Relative albumin content was highest in NP females. The lowest relative creatinine values were found in P2 samples. Glucose levels were very variable (Table 2). Ion content is shown in Table 3. Sodium concentration in OF was higher in NP than in pregnant females.

Three different images from three different electrophoresis assays were analyzed and compared for protein profile analysis (Fig. 1). In total 35 protein bands were identified in NP animals, 33 in CP1 animals, 32 in IP1 and CP2 animals, and 33 protein bands were observed in IP2 animals (Table 4). Statistical analysis revealed that 12 bands that, although present in all samples, showed significant differences in intensity between groups. One of these, a protein band of 50 kDa, was more intense in pregnant females, but no hit was found when peak fragmentation was analyzed using BLAST. Five protein bands, 70, 42, 25, 24 and 19 kDa, showed significant more intensity in non-pregnant females. The search of these five selected protein bands against the MASCOT database yielded matches with moesin actin, cytoplasmic 2, hydroxypyruvate isomerase isoform 1, peroxiredoxin-6 and ferritin light chain. The identity of the matches is summarized in Table 5.

4. Discussion

Knowledge of the composition of OF in camelids is of fundamental importance to simulate *in vitro* physiological conditions for gamete conditioning, fertilization and early development. The *in vitro* accomplishment of these processes probably improves under physiological conditions that closely resemble the natural environment. The present study reports for the first time the biochemical composition and protein profile of OF in camelids species.

Because of its importance to gamete and embryo metabolism, the most commonly examined substrate in OF is glucose (Brinster, 1973; Leese, 1988). The glucose concentration found for alpaca OF presented great variation between groups. Nevertheless, the range agrees with previous reports in cows (Carlson et al., 1970), and resulted lower when compared with glucose content in mare (Campbell et al., 1979), ewe (Hamner, 1973) and mouse (Gardner and Leese, 1990).

Albumin is probably the protein of major importance considering its ability to bind lipids (Ehrenwald et al., 1990), peptides (Ménézo and Khatchadourian, 1986) or catecholamines (Khatchadourian et al., 1987). The concentration of this protein was higher in NP females. Taking

Table 1
Media values and standard error ($n = 3$) of protein content of alpaca oviductal fluid. NP: non-pregnant females. P1: pregnant females with 34–37 days of gestation. P2: pregnant females with and 60–64 days of gestation. I: fluid from oviduct ipsilateral to the ovary with corpus luteum. C: fluid from oviduct contralateral to the ovary with corpus luteum. Data statistically different ($P < 0.05$) are in bold and marked with capital letters. ALP, alkaline phosphatase; γ-GT, gamma-glutamyl transpeptidase; AST, aspartate amino-transaminase; ALT, alanine amino-transaminase; CK, creatinine kinase; LDH, lactate dehydrogenase.

	NP	C-P1	I-P1	C-P2	I-P2
Total protein (g/dL)	4.67 ± 0.33	5.40 ± 0.83	4.34 ± 0.72	4.67 ± 0.33	5.00 ± 0.58
Albumin (g/g total prot)	**2.23 ± 0.18^{A}**	0.42 ± 0.05	0.50 ± 0	0.40 ± 0.003	0.45 ± 0.03
Creatine (mg/g total prot)	**0.53 ± 0.01^{B}**	**0.58 ± 0.04^{B}**	**0.52 ± 0.02^{B}**	**0.35 ± 0.01^{A}**	**0.34 ± 0.01^{A}**
Globuline (g/g total prot)	0.54 ± 0.05	1.04 ± 0.22	0.63 ± 0.13	0.60 ± 0	0.62 ± 0.04
ALP (U/g total prot)	4463 ± 450	3513 ± 823	3428 ± 937	2726 ± 245	3133 ± 67
γ-GT (U/g prot)	**4505 ± 386^{AB}**	**6492 ± 44^{C}**	**6015 ± 599^{BC}**	**3642 ± 63^{A}**	**3023 ± 738^{A}**
AST (U/g prot)	19 890 ± 3719	22 622 ± 3894	18 400 ± 786	15 829 ± 437	15 660 ± 595
ALT (U/g prot)	339 ± 44	536 ± 82	312 ± 16	**301 ± 44^{A}**	327 ± 8.53
CK (U/g prot)	36 741 ± 67 608	277 059 ± 5304	437 804.14 ± 3762.54	293325.83 ± 43094.61	308 873 ± 31 851
LDH (U/g prot)	188 917 ± 11 708	203 366 ± 4712	221 813 ± 6979	235 647 ± 49 591	177 412 ± 5873

Table 2
Glucose content of alpaca oviductal fluid. NP, non-pregnant females. P1: pregnant females with 34–37 days of gestation, P2: pregnant females with and 60–64 days of gestation. I: fluid from oviduct ipsilateral to the ovary with corpus luteum, C: fluid from oviduct contralateral to the ovary with corpus luteum. Statistical differences ($P < 0.05$) are marked with capital letters.

	NP	C-P1	I-P1	C-P2	I-P2
Glucose (mg/dL)	65 ± 4.7^{AB}	119 ± 28.4^{C}	25 ± 1.4^{A}	94 ± 6^{BC}	68 ± 13.5^{AB}

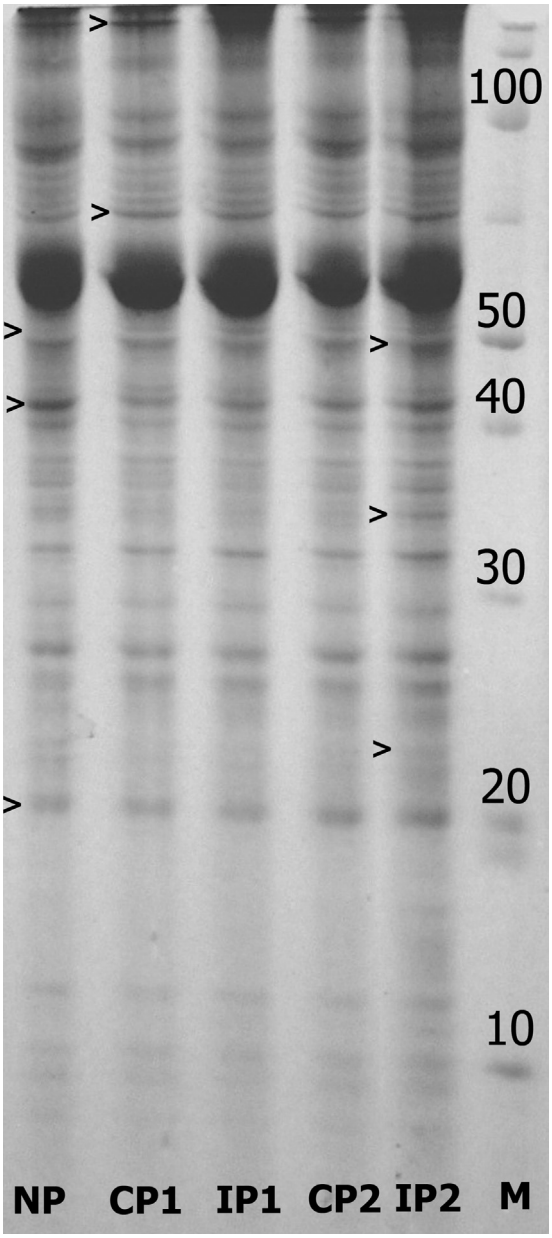

Fig. 1. 1D SDS–PAGE of alpaca oviductal fluid. NP: non-pregnant females. P1: pregnant females with 34–37 days of gestation, P2: pregnant females with and 60–64 days of gestation. I: fluid from oviduct ipsilateral to the ovary with corpus luteum, C: fluid from oviduct contralateral to the ovary with corpus luteum. M: molecular weight marker. The arrows indicate protein bands statistically different ($P < 0.001$).

into account the key role of serum albumin in mammalian sperm capacitation (Yanagimachi, 1994), it is probable that the concentration observed ensures the environment for an adequate sperm function.

Lactate dehydrogenase (LDH) is essential for continuous glycolysis. The levels of LDH described in this work agree with those in pig, rat, rabbit and mouse OF; with values similar to some of the largest mammalian tissues, such as the heart (Gibson and Masters, 1970). The present study also describes enzymes involved in several important biochemical pathways, providing insights into the normal OF protein composition.

Ion concentrations are essential for the regulation of enzyme activity and the oviduct pH. The composition of inorganic elements in the mammalian oviduct differs among species (Leese et al., 2011). Potassium and sodium were the major ions found in the present study, whereas Hugentobler et al. (2004) and Grippo et al. (1992) observed chloride and sodium as the main ions in bovine OF during the estrous cycle. The sodium concentration range recorded was 5 times higher than that reported for bovine and potassium 10 times higher. The addition of potassium to semen extenders has been shown to improve motility of stallion (Padilla and Foote, 1991) and human sperm (Karow et al., 1992). On the other hand, in rams, increasing potassium levels has been reported to be negatively correlated with progressive motility, while the reverse is true for sodium (Abdel-Rahman et al., 2000). Calcium concentrations observed in the current study ($\leq$0.8 mM) were lower to those that facilitate sperm capacitation, acrosome reaction, and hyperactivation in several species (0.9–2.0 mM) (Grippo et al., 1992).

The OF protein profile showed a complex mixture of components. It is well known that tubal secretion is a subtle mixture of a transudate of compounds originating from serum and specific compounds synthesized by tubal epithelium, and consequently, a great number of protein bands can be expected. From the 35 protein bands identified, only 5 showed clear differences between pregnant and non-pregnant females, whereas no differences were found between oviducts with or without corpus luteum. Even though proteins were isolated with 1D electrophoresis, bands showed unique matches and four proteins could be identified. In all cases they were more abundant in non-pregnant females. Moesin, an actin-binding protein, and actin cytoplasmic 2 have been found in other reproductive fluids (Di Quinzio et al., 2007; Ametzazurra et al., 2009; Bhutada et al., 2013). The origin of this protein is probably related to different processes such as epithelial cell renewal or the secretory activity by apocrine and holocrine processes (Steffl et al., 2008). Moreover, evidence of apocrine secretion has been described in llama oviductal epithelial cells (Apichela et al., 2009). Actin has been detected in culture supernatants of endometrial epithelial cell lines, thereby providing evidence of its secretion from endometrial epithelial cells (Bhutada et al., 2013). Abundance of actin in OF from non-pregnant females could be attributed to a higher secretory activity of epithelial cells, but presence due to contamination of blood or oviductal cells cannot be ruled out.

Hydroxypyruvate isomerase is an enzyme involved in carbohydrate transport and metabolism. It catalyzes the conversion of hydroxypyruvate to 2-hydroxy-3-oxopropanoate. Its presence has been reported in secretions from *in vitro* cultured human amniotic epithelial cells (Liu et al., 2009). Nevertheless, its role in the reproductive function is has not been elucidated yet.

Ferritin light chain is a protein with ferroxidase activity, important for iron homeostasis. It has been proposed that ferritin could serve as cytotoxic protector against iron oxidative damage (Geiser et al., 2003; Tsuji et al.,

Table 3
Ion content of alpaca oviductal fluid by biochemical assessment. NP, non-pregnant females. P1: pregnant females with 34–37 days of gestation, P2: pregnant females with and 60–64 days of gestation. I: fluid from oviduct ipsilateral to the ovary with corpus luteum, C: fluid from oviduct contralateral to the ovary with corpus luteum. Data statistically different ($P < 0.05$) are in bold and marked with capital letters.

	NP	C-P1	I-P1	C-P1	I-P2
Na^{+} (mmol)	**860 ± 23^{B}**	**599 ± 226^{A}**	**521 ± 52^{A}**	**466 ± 18^{A}**	**479 ± 12^{A}**
K^{+} (mmol)	37.6 ± 1.45	52 ± 10.8	44.6 ± 3	39 ± 0.3	39.5 ± 0.3
Ca^{++} (mg/dL)	**3.2 ± 0.4^{B}**	**2.27 ± 0.8^{AB}**	**1.5 ± 0.05^{A}**	**1.3 ± 0.36^{A}**	**2.9 ± 0.23^{B}**
Mg^{++} (mg/dL)	4.6 ± 0.23	7.4 ± 1.45	5.36 ± 0.7	5.3 ± 0.36	6 ± 0.58
Ca^{++} (mg/g prot)	1.70 ± 1.15	0.4 ± 0.1	0.36 ± 0.07	0.27 ± 0.06	0.62 ± 0.12
P (mg/dL)	28 ± 2	35.4 ± 2	33.45 ± 7.89	26 ± 1.05	26.3 ± 1.2

2000). This is important for oviductal environment and, considering that iron has been reported to be capable to induce oxidative damage in mouse sperm (Mojica-Villegas et al., 2014), it may play a protective role in gametes.

Probably the most interesting protein identified in the present study is peroxiredoxin-6 (PRDX6), a unique antioxidant that operates independently of other peroxiredoxins and antioxidant proteins (Manevich and Fisher, 2005). In the uterus, PRDX6 plays an antioxidant role during early pregnancy (Hirota et al., 2010). Bovine oocytes also produce PRDX6, and the expression of transcripts is upregulated during *in vitro* maturation (Leyens et al., 2004). The authors stated that PRDX6 might also be accumulated in oocytes to be used during fertilization or early embryo development. It is known that gametes and embryos are sensitive to oxidative stress (OS) (Aitken et al., 2014; Dennery, 2007). Presence of PRDX6 in alpaca OF could play a role as critical antioxidant against exogenous OS during sperm transport, fertilization or embryo development, but this hypothesis has to be examined more thoroughly.

In the mouse uterus, neither P4 nor estradiol-17β (E2) significantly altered PRDX6 expression levels, but P4 changed the expression domain from the epithelium to the stroma (Hirota et al., 2010). This change probably explains the differential abundance of PRDX6 in OF from non-pregnant alpaca females, when it is well known that progesterone levels are basal.

Table 4
Intensity of band proteins obtained by 1D SDS–PAGE of alpaca oviductal fluid. MW: deduced molecular weight. NP: non-pregnant females. P1: pregnant females with 34–37 days of gestation, P2: pregnant females with and 60–64 days of gestation. I: fluid from oviduct ipsilateral to the ovary with corpus luteum, C: fluid from oviduct contralateral to the ovary with corpus luteum. Protein bands statistically different ($P < 0.001$) are in bold.

	MW	NP	CP1	IP1	CP2	IP2
1	**187**	**0.423 ± 0.204^{ab}**	**0.841 ± 0.210^{c}**	**0.2103 ± 0.044^{a}**	**0.519 ± 0.141^{b}**	**0.503 ± 0.181^{ab}**
2	**153**	**0.292 ± 0.012^{abc}**	**0.448 ± 0.032^{c}**	**0.1529 ± 0.105^{a}**	**0.206 ± 0.068^{ab}**	**0.334 ± 0.141^{bc}**
3	110	0.386 ± 0.153	0.496 ± 0.088	0.453 ± 0.103	0.368 ± 0.107	0.342 ± 0.071
4	95	1.112 ± 0.345	1.066 ± 0.178	0.721 ± 0.156	1.104 ± 0.223	1.293 ± 0.406
5	84	0.074 ± 0.040	0.070 ± 0.024	0.072 ± 0.007	0.061 ± 0.015	0.068 ± 0.018
6	79	0.082 ± 0.040	0.114 ± 0.047	0.121 ± 0.021	0.138 ± 0.047	0.095 ± 0.041
7	74	0.025 ± 0.010	0.043 ± 0.023	0.068 ± 0.024	0.037 ± 0.005	0.027 ± 0.018
8	**70**	**0.181 ± 0.037^{a}**	**0.588 ± 0.062^{c}**	**0.365 ± 0.077^{b}**	**0.379 ± 0.062^{b}**	**0.406 ± 0.139^{b}**
9	56	14.5 ± 5.4	17.2 ± 5.1	18.7 ± 5.4	12.2 ± 3.3	14.5 ± 4.7
10	**50**	**0.006 ± 0.006^{a}**	**0.031 ± 0.007^{b}**	**0.036 ± 0.013^{b}**	**0.041 ± 0.006^{b}**	**0.040 ± 0.005^{b}**
11	**48**	**0.312 ± 0.077^{a}**	**0.522 ± 0.055^{a}**	**0.382 ± 0.086^{a}**	**0.429 ± 0.068^{a}**	**0.870 ± 0.360^{b}**
12	47	0.004 ± 0.006	0.037 ± 0.062	0.016 ± 0.025	0.013 ± 0.014	0.013 ± 0.017
13	44	0.013 ± 0.017	0.158 ± 0.190	0.137 ± 0.219	0.226 ± 0.364	0.282 ± 0.446
14	43	0.029 ± 0.011	0.230 ± 0.202	0.342 ± 0.156	0.461 ± 0.246	0.608 ± 0.345
15	**42**	**0.684 ± 0.113^{b}**	**0.211 ± 0.069^{a}**	**0.098 ± 0.068^{a}**	**0.126 ± 0.083^{a}**	**0.201 ± 0.159^{a}**
16	41	0.194 ± 0.073	0.144 ± 0.051	0.075 ± 0.071	0.115 ± 0.107	0.172 ± 0.150
17	38	0.040 ± 0.042	0.027 ± 0.015	0.040 ± 0.053	0.126 ± 0.097	0.140 ± 0.121
18	37	0.084 ± 0.073	0.044 ± 0.048	0.038 ± 0.055	0.062 ± 0.035	0.163 ± 0.143
19	36	0.006 ± 0.008	0.029 ± 0.025	0.043 ± 0.037	0.141 ± 0.154	0.013 ± 0.013
20	35	0.137 ± 0.018	0.090 ± 0.071	0.054 ± 0.086	0.088 ± 0.139	0.199 ± 0.046
21	34	0.009 ± 0.008	0.157 ± 0.255	0.033 ± 0.029	0	0.019 ± 0.015
22	**33**	**0.166 ± 0.184^{a}**	**0.203 ± 0.166^{a}**	**0.016 ± 0.0244^{a}**	**0^{a}**	**0.643 ± 0.099^{b}**
23	31	0.285 ± 0.049	0.115 ± 0.088	0.194 ± 0.166	0.226 ± 0.199	0.307 ± 0.039
24	29	0.024 ± 0.026	0.059 ± 0.099	0.342 ± 0.330	0	0.822 ± 0.734
25	28	0.098 ± 0.032	0.314 ± 0.517	0.158 ± 0.258	0.227 ± 0.085	0.641 ± 0.167
26	27	0.017 ± 0.016	0.465 ± 0.261	0.458 ± 0.264	0.515 ± 0.474	0.304 ± 0.151
27	**25**	**0.745 ± 0.167^{c}**	**0.281 ± 0.157^{ab}**	**0.256 ± 0.21^{ab}**	**0.486 ± 0.211^{bc}**	**0.101 ± 0.024^{a}**
28	**24**	**0.280 ± 0.031^{b}**	**0.014 ± 0.014^{a}**	**0.044 ± 0.044^{a}**	**0.151 ± 0.218^{ab}**	**0.033 ± 0.038^{a}**
29	23	0.112 ± 0.099	0	0	0.036 ± 0.033	0.491 ± 0.848
30	**22**	**0.083 ± 0.077^{a}**	**0^{a}**	**0^{a}**	**0.016 ± 0.015^{a}**	**0.581 ± 0.310^{b}**
31	**21**	**0.077 ± 0.019^{a}**	**0.355 ± 0.294^{ab}**	**0.4917 ± 0.0865^{bc}**	**0.797 ± 0.208^{c}**	**0.154 ± 0.212^{ab}**
32	**19**	**0.541 ± 0.051^{c}**	**0.203 ± 0.189^{b}**	**0^{a}**	**0.233 ± 0.022^{b}**	**0.091 ± 0.079^{ab}**
33	16	0.190 ± 0.021	0.182 ± 0.062	0.202 ± 0.178	0.105 ± 0.100	0.092 ± 0.091
34	15	0.063 ± 0.043	0.157 ± 0.034	0.096 ± 0.086	0.099 ± 0.088	0.171 ± 0.088
35	14	0.094 ± 0.083	0.078 ± 0.068	0.034 ± 0.030	0.087 ± 0.076	0.173 ± 0.044

Table 5
Identity of differentially expressed proteins in the alpaca oviductal fluid.

Protein band	Identity	Theoretical protein MW (kDa)	Experimental protein MW (kDa)	Accession no.	No. of peptides matched	Coverage (%)	MASCOT score	ProFound expectation score
8	PREDICTED: moesin [*Loxodonta africana*]	70	71	gi|344281953	17	24	119	1.2e−06
15	Actin, cytoplasmic 2 [*Camelus ferus*]	40	42	gi|528769986	13	52	468	1.6e−41
27	PREDICTED: putative hydroxypyruvate isomerase isoform 1 [*Tursiops truncatus*]	30	25	gi|470652752	6	42	131	7.8e−08
28	PREDICTED: peroxiredoxin-6 [*Vicugna pacos*]	25	24	gi|560977841	12	57	231	7.8e−18
32	PREDICTED: ferritin light chain [*Vicugna pacos*]	20	19	gi|560972398	10	71	139	1.2e−08

5. Conclusion

This is the first analysis of the biochemical composition and protein profile of oviductal fluid is assayed in a camelid. This study also revealed differences regarding the physiological state. Further understanding of these physiological conditions probably provides greater insight into manipulation or control of reproductive processes at the oviductal level.

Conflict of interest statement

Authors declare that do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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