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Biochemical and Protein Profile of Alpaca (*Vicugna pacos*) Uterine Horn Fluid During Early Pregnancy

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Contents
South American camelids show high embryo loss rate, during the first 60 days of pregnancy. One of the factors which may be related to this situation is that over 98% of the embryos implant in the left uterine horn (LUH) even though both ovaries contribute similarly to ovulation. There is scarce information about the uterine environment of female camelids at any physiological state that could explain the capability of the LUH to attract the embryo and maintain pregnancy. We describe, for the first time, the biochemical and protein profile of uterine fluid (UF), addressing the right and LUH environment in non-pregnant and pregnant alpacas. Different substrates, electrolytes and metabolites were assayed in both uterine horn fluids. Small changes were observed in glucose and total protein levels, which were more noticeable during pregnancy. In addition, 10 specific proteins were found in the left horn fluid in 5-week-pregnant alpacas, and two protein bands were identified in non-pregnant alpaca right horn fluid. These results would provide basic information for identification of possible markers for pregnancy diagnosis, reproductive diseases and hormone-treated animals evaluation and hence contributing to improve the pregnancy rate.

Introduction

South American camelids (SAC) display complex reproductive characteristics: induced ovulation, short half-life of the corpus luteum, different luteolytic activity between the two uterine horns, maternal recognition of pregnancy (MRP) beginning before day 10 after mating, pregnancies only in left uterine horn (LUH), highly viscous semen with low sperm concentration, etc. (Vaughan and Tibary 2006). As the knowledge about the reproductive physiology of SACs advances, more clear becomes the concept that processes assumed in other domestic livestock cannot be extrapolated to camelids.

Certain basic reproductive physiology, such as MRP signalling, remains still unclear. The lack of this knowledge limits the efficiency of embryo transfer protocols, development of early markers for pregnancy diagnosis, reproductive diseases and evaluation of hormone-treated animals.

Early embryonic development and MRP require a specific well-timed uterine environment. This environment is created in synergy between the developing embryo and the uterus, which inhibits luteolysis, offers the embryo immunological protection and provides nutrition for the unattached developing embryo (Roberts 1989). According to Sumar et al. (1988), the pregnancy rate in SACs approximately 30 days post-mating is <50%, and this loss increases to 60–80% in the first 90 days; however, stressful conditions due to harsh climate and nutritional constraints have been also suggested responsible for the greater rates of embryo loss in alpacas (Sumar and Adams 2006). Because fertilization rates are similar in all ruminant species, it seems that embryo loss rates are much higher in SACs than in sheep or goats (Diskin and Morris 2008). One of the factors that may be responsible for the higher embryo loss rate is the fact that over 98% of the foetuses in camelids implant in the LUH although both ovaries contribute almost equally to ovulation (Fernandez-Baca et al. 1979; Vaughan et al. 2013). In addition, the right uterine horn (RUH) would be unable to sustain a pregnancy beyond 95 days meaning that the embryo has to migrate from the right to the LUH to implant and survive (Fernandez-Baca et al. 1970). Nevertheless, when the embryo was deposited directly into the LUH in the presence of a CL in the ipsilateral ovary (the ideal location to avoid embryo migration), pregnancy rate was not as great as could be expected (Trasorras et al. 2010). At present, it has been established that neither location (right or left ovary) nor stage of development of the dominant follicle has an influence on ovulation and embryo survival rate in alpacas (Ratto et al. 2011). There would seem to be some other factor/s responsible for embryo viability into the uterine environment.

There is scarce information about the uterine environment of SAC females at any physiological state that could explain the capability of the LUH to attract the embryo and maintain pregnancy. It has been proposed that differences in vascular anatomy between the left and right ovaries, oviducts and uterine horns in camelids are related to the site-specific implantation (LUH) of the embryo (Del Campo et al. 1996). However, the molecular mechanism of embryo-maternal crosstalk during attachment, implantation and pregnancy recognition between the embryo and the LUH in SACs has not been described yet.

This is the first study that attempts a broad characterization of the endometrial secretome in non-pregnant and pregnant alpacas in the left and right UH. Therefore, the biochemical composition and protein profile of UF were assayed. In addition, MALDI-MS technology was used to identify for the first time proteins with differential secretion patterns during early pregnancy in alpacas.

Materials and Methods

Animals and sampling

Reproductive systems of adult female *Vicugna pacos* (var. Huacaya) were obtained from a slaughterhouse at Huancavelica (12°S, 74°W, 3676 MASL), Peru, during the first fortnight of May 2011. The samples were transported at 0°C to the laboratory of reproductive biotechnology, Universidad Nacional de Huancavelica where they were processed. A total of 11 animals were used in this experiment, three of which were non-pregnant (NP) with ovarian follicles smaller than 7 mm; four were pregnant females with 34–37 days of gestation or 5 weeks of pregnancy (5WP) and the remaining four animals with 60–64 days or 9 weeks of gestation (9WP). The gestational age of the animals was calculated by measuring the crump-rump length or total foetal length of the foetuses according to Olivera et al. (2003) and Catone et al. (2006).

To obtain the UF, embryos and membranes were carefully removed and then a blunted needle attached to a syringe was inserted into the uterine horn through the uterine tubal junction. The distal end of the horn was clamped, and 5 ml of phosphate-buffered saline solution (PBS), pH 7.4, was flushed separately into each horn (right and left) and then aspirated. Each flushing was centrifuged at $5000 \times \boldsymbol{g}$ (10 min, 4°C) to pellet any cellular debris.

All samples were preserved and transported in dry ice, then stored at −80°C until further analysis.

Biochemical analyses

An automated analyser (912 Automatic Analyzer; Hitachi Boehering Mannheim, Mannheim, Germany) was used to measure concentrations of the following parameters in uterine flushings: triglycerides, cholesterol, total bilirubin, glucose, creatinine, urea, total protein, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (γ-GT), creatinine kinase (CK), lactate dehydrogenase (LDH), magnesium (Mg) and calcium (Ca). The enzymatic parameter values were normalized using total protein concentration (Juyena et al. 2013).

1D electrophoresis assays

The total protein concentration in UF was determined, and then samples were pooled in six groups according to the UH (right or left) and the gestational stage (NP, 5WP and 9WP). The same amount of total proteins was used to pool the samples. The pools were concentrated and separated into two fractions: >50 kDa and <50 kDa, using first 50 000 molecular weight cut-offs (MWCO) Amicon® Ultra-2 and then 3000 MWCO Amicon® Ultra-0.5 filters (Millipore, Milan, Italy).

One-dimensional (1D) PAGE of UF was performed under denaturing conditions with methods previously described by Laemmli (1970), using a Mini-PROTEAN Tetra Cell slab gel (Bio-Rad Laboratories, Milan, Italy). A 4% stacking gel was used in all runs; the concentrated samples (35 μg) were loaded onto 10% (>50 kDa fractions) and 20% SDS-polyacrylamide gels (<50 kDa fractions). Molecular mass markers covering the range of 10–245 kDa (prestained protein marker VI; AppliChem, Darmstadt, Germany) were applied to each gel. Gels were run at 150 V using a Bio-Rad power supply unit (PowerPac™ Universal Power Supply; Bio-Rad Laboratories).

After the runs, gels were stained with Colloidal Coomassie Blue G-250 (Bio-Rad Laboratories) and destained with a solution of 10% methanol and 10% glacial acetic acid. Gel images were acquired using a Pentax Optio M90 camera (Pentax, Milan, Italy). The apparent molecular mass of the bands on SDS-PAGE was estimated by comparison of the mobility of proteins in the gel with that of the molecular mass markers using GelAnalyzer version 2010a freeware software (Copyright 2010 by Istvan Lazar and Dr. Istvan Lazar, Hungary). The same software was used to determine the intensity of the detected bands on the digitalized gel images.

Protein analysis

Three different images from three different electrophoresis assays were analysed and compared. Statistical analysis was carried out using ANOVA, followed by Fisher's test using the InfoStat programme (Infostat Group, Facultad de Ciencias Agrarias, Universidad Nacional de Córdoba, Argentina) with a significance level of $p < 0.001$. Bands with $p < 0.001$ in protein abundance between the LUH and RUH at different stages were digested with trypsin and analysed using matrix-assisted laser desorption–ionization mass spectrometry (MALDI-MS) on an Ultraflex II TOF/TOF Bruker Daltonics mass spectrometer (CEQUIBIEN 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 (MatrixScience, London, UK). Fragmentation was carried out with more intense MS peaks (MS/MS). Where 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. When the same peaks were observed in different samples, they were considered as contamination and not included in the final analysis. In some cases, when the score obtained was not significant, the higher score, the number of matched peptides and the molecular weight of the predicted proteins were taken into account to assign the putative identity of the band.

Results

Biochemical parameters of UF during early pregnancy

Different substrates, electrolytes and metabolites were assayed in the fluid of the LUH and RUH in NP, 5WP and 9WP females (Tables 1 and 2).

The concentration of glucose was significantly different between the UF of non-pregnant and pregnant animals, even differences were found between the horns

Table 1. Substrates and metabolites assayed in the uterine horn fluid of alpaca

	Substrates and metabolites						
Parameter	Glucose mg/dl	Cholesterol mg/dl	Triglycerides mg/dl	Total protein µg/l	Creatinine µg/µg Prot	Urea µg/µg Prot	Total bilirubin mg/dl
Sample							
NPr	0.67 ± 0.58^{ABC}	0.0	1.67 ± 1.15	616.67 ± 678.85	2.75 ± 2.09	371.4 ± 150	0.0
NPl	0.33 ± 0.58^{AB}	0.0	0.50 ± 0.65	433.33 ± 152.75	2.67 ± 1.61	388.9 ± 100	0.0
5WPr	0.50 ± 0.09^{A}	0.0	0.33 ± 0.58	439.33 ± 115.41	1.27 ± 0.50	216.2 ± 100	0.0
5WPl	1.00 ± 0.00^{BC}	0.0	2.50 ± 2.38	853.61 ± 175.37	0.99 ± 0.14	309.9 ± 160	0.0
9WPr	1.50 ± 0.71^{C}	0.0	0.50 ± 0.71	600.00 ± 141.42	1.17 ± 0.04	242.9 ± 60	0.0
9WPl	3.00 ± 1.41^{D}	0.0	1.50 ± 0.71	1125.00 ± 530.33	0.93 ± 0.00	533.3 ± 370	0.0

NPr, non-pregnant right uterine horn; NPi, non-pregnant left uterine horn; 5WPr, 5 weeks of pregnancy right uterine horn; 5WPl, 5 weeks of pregnancy left uterine horn; 9WPr, 9 weeks of pregnancy right uterine horn; 9WPl, 9 weeks of pregnancy right uterine horn. Glucose $p < 0.001$, dissimilar letters indicate significant differences.

Table 2. Enzymes and electrolytes assayed in the uterine horn fluid of alpaca

	Enzymes						Electrolytes	
Parameter	Ɣ-GT µ/µg Prot	AST µ/µg Prot (E^{-03})	ALT µ/µg Prot (E^{-03})	ALP µ/µg Prot	CK µ/µg Prot	LDH µ/µg Prot	Mg mg/dl	Ca mg/dl
Sample								
NPr	0.0123 ± 0.01^{B}	0.33 ± 0.06	0.1 ± 0.1	0.074 ± 0.060	0.253 ± 0.16	0.020 ± 0.020	0.15 ± 0.12	0.17 ± 0.06
NPl	0.0125 ± 0.01^{B}	1.0 ± 1.70	0.1 ± 0.1	0.095 ± 0.080	0.182 ± 0.09	0.014 ± 0.020	0.16 ± 0.08	0.13 ± 0.15
5WPr	0.0048 ± 0.002^{AB}	20.0 ± 3.00	0.3 ± 0.2	0.098 ± 0.100	0.072 ± 0.03	0.001 ± 0.001	0.14 ± 0.14	0.18 ± 0.17
5WPl	0.0020 ± 0.002^{A}	15.0 ± 10.0	0.2 ± 0.1	0.095 ± 0.080	0.127 ± 0.09	0.012 ± 0.020	0.09 ± 0.04	0.30 ± 0.24
9WPr	0.0028 ± 0.007^{A}	4.5 ± 10.0	0.1 ± 01	0.028 ± 0.010	0.034 ± 0.04	0.000 ± 0.000	0.08 ± 0.03	0.10 ± 0.10
9WPl	0.0029 ± 0.001^{AB}	20.0 ± 30.0	0.1 ± 0.1	0.074 ± 0.001	0.034 ± 0.04	0.011 ± 0.010	0.10 ± 0.09	0.10 ± 0.14

NPr, non-pregnant right uterine horn; NPl, non-pregnant left uterine horn; 5WPr, 5 weeks of pregnancy right uterine horn; 5WPl, 5 weeks of pregnancy left uterine horn; 9WPr, 9 weeks of pregnancy right uterine horn; 9WPl, 9 weeks of pregnancy right uterine horn. Ɣ-GT $p < 0.048$, dissimilar letters indicate significant differences.

in the 5WPr and 9WPr fluids. Among the enzymes analysed, γ-GT decreased significantly in pregnant animals. The other parameters did not show significant differences.

Tables 1 and 2 show average values, SD and statistical significance of the parameters assayed under the different experimental conditions (NPr, NPl, 5WPr, 5WPl, 9WPr and 9WPl).

1D electrophoresis

The protein composition of UF of alpaca LUH and RUH was determined by SDS-PAGE (Figs 1–3). Differences in band patterns were observed among the physiological states assayed as well as between the right and left UH. In NP animals, a total of 21 protein bands were identified in the RUH and 25 protein bands in the LUH. Two protein bands, of 45.33 and 28.75 kDa, showed significant higher intensity ($p < 0.001$) in the LUH and in the RUH, respectively (Table 3).

Animals of 5WP showed 21 in the RUH and 23 proteins bands in LUH, respectively. Two protein bands (43.0 and 30.07 kDa) were found exclusively in LUH, and five of them displayed higher intensity levels (47.78, 25.57, 16.8, 13.00 and 12.61 kDa). In RUH 2, protein bands (89.56 and 73.56 kDa) were exclusive to this horn, and 1 band of 38.35 kDa was more intense (Table 4).

In 9WP animals, 23 protein bands were identified in both the RUH and the LUH. None of the protein bands identified showed statistical difference ($p < 0.001$) in intensity between both uterine horns (Table 5).

Protein identification

After 1D SDS-PAGE, protein bands were assayed with UV-MALDI-MS to characterize differentially expressed proteins between the LUH and RUH in pregnant and non-pregnant animals. A search of the 12 selected protein bands against the MASCOT database yielded eight positive identifications, five of them with statistically significant scores. As some of the matches were not significant, the identity was assigned based on the best scores and molecular weights (Table 6).

Discussion

In the current study, we described the UH environment during the pregnancy, noticing the particular characteristics of the RUH and LUH milieu. Among the biochemical parameters examined, glucose increased from non-pregnant to 9WP animals; furthermore, significant differences between the LUH and RUH of pregnant animals were observed, with highest values in the LUH, the gravid horn. Glucose is a major metabolic fuel source for conceptus (Peters et al. 1990) that

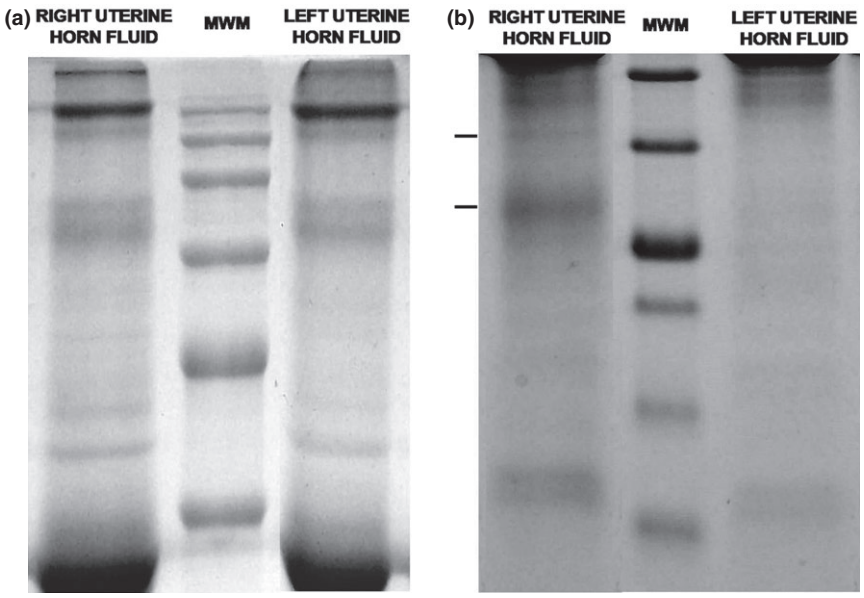

Fig. 1. 1D SDS-PAGE of the uterine fluid from non-pregnant alpacas. (a) >50 kDa fraction, (b) <50 kDa fraction. The bands that showed statistically different ($p < 0.001$) were cut and analysed by MALDI-MS

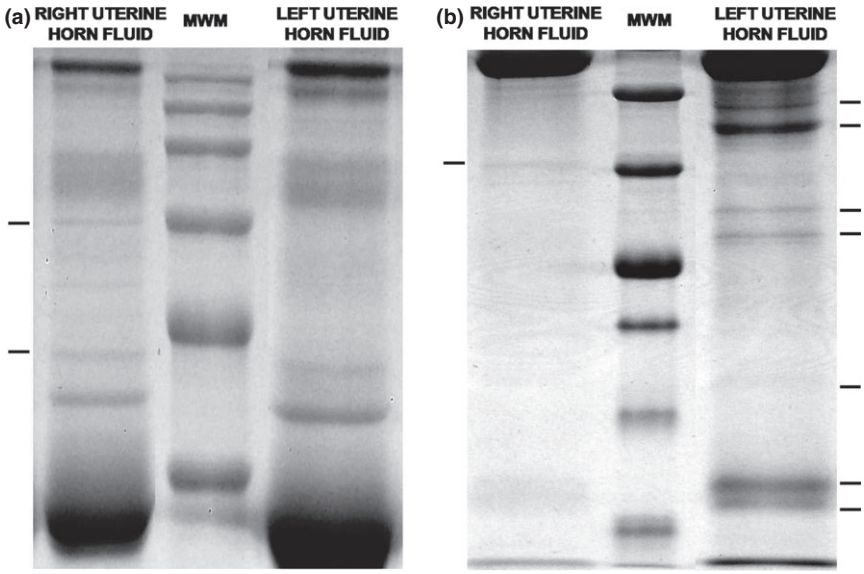

Fig. 2. 1D SDS-PAGE of the uterine fluid from 1-month-pregnant alpacas. (a) >50 kDa fraction, (b) <50 kDa fraction. The bands that showed statistically different ($p < 0.001$) were cut and analysed by MALDI-MS

increases in the uterine lumen during early pregnancy to support conceptus demands for energy (Lane and Gardner 2007). In ewes (Gao et al. 2009), glucose levels of uterine flushings were higher in pregnant than in cyclic females.

Other energy substrates assayed did not show any changes during the stages analysed (triglycerides), or were not detected in UF (cholesterol). The rabbit dam uses plasma lipids during the second half of pregnancy when energy requirements increase enormously. In contrast, tissue and plasma cholesterol exchange is generally reported to be very low (Ozegbe 2005), which could support our observations in the UF of alpaca.

The enzymes assayed (CK, LDH, AST, ALT and ALP) showed no variation between the two UH and between pregnant and non-pregnant animals, except for γ-GT, which decreased significantly in pregnant animals. As the uterus does not produce many of these enzymes and because the UF composition is a result of active endometrial secretion and diffusion from the blood, these enzymes could have a plasmatic source as happened with the amniotic fluid (Ozegbe 2005).

In general, minerals serve as osmolytes, but they also participate in the transport of amino acids and glucose into cells, in enzyme-catalysed metabolic pathways, and cell signalling events during pregnancy. Therefore, they are crucial to blastocyst expansion and implantation (Gao et al. 2009). Calcium and magnesium were the only ions determined as PBS was used to flush the uterine horns. No statistically significant fluctuations were found between the horns or the stages studied. In ewes, the total amount of calcium was higher in uterine fluids of pregnant than cyclic animals (Gao et al. 2009). This difference with our findings may be due to differences between species and/or the technique used to obtain the uterine fluid.

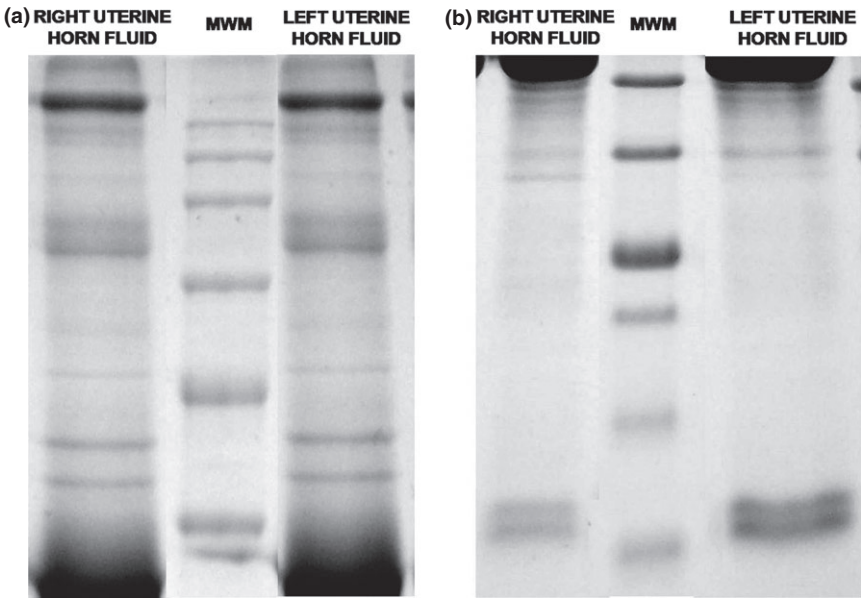

Fig. 3. 1D SDS-PAGE of the uterine fluid from 2-month-pregnant alpacas. (a) >50 kDa fraction, (b) <50 kDa fraction. The bands that showed statistically different ($p < 0.001$) were cut and analysed by MALDI-MS

Table 3. Identified protein bands of 1D SDS-PAGE of the uterine fluid from non-pregnant alpacas

	Intensity	SD	Intensity	SD	
MW	Right uterine horn		Left uterine horn		Positive statistic significancy
354.44	1578.00	635.60	912.00	701.70	
310.88	60.56	46.33	21.44	12.67	
254.44	2249.00	585.60	2236.00	716.00	
202.11	40.78	55.42	61.44	42.70	
122.44	202.2	84.81	254.40	172.10	
106.22	502.9	258.00	620.40	462.70	
80.11	99.33	40.84	78.00	46.45	
72.78	51.33	25.63	103.10	43.13	
69.57	–	–	25.00	20.54	
66.89	58.89	35.32	131.70	64.56	
64.67	173.00	102.50	596.90	418.30	
62.22	708.70	321.40	1050.00	663.80	
60.13	47.22	37.13	35.56	38.08	
58.89	11301	3392.00	12572.00	3858.00	
51.78	–	–	21.33	18.09	
49.56	51.11	35.46	207.80	173.10	
45.33	–	–	52.22	28.79	*
42.56	65.78	41.51	33.22	23.99	
35.22	38.44	27.06	34.44	17.11	
28.75	1062.00	729.30	37.11	26.28	*
24.11	–	–	70.22	66.31	
21.33	70.78	49.73	115.00	50.20	
16.67	90.44	54.35	126.00	44.00	
12.78	65.44	47.45	81.78	44.59	
12.33	87.00	38.43	133.90	122.10	

*Protein bands with statistically significant differences, $p < 0.001$.

The endometrium changes from a proliferative architecture to a secretory one, with maximal secretion during the period of embryonic receptivity. These structural and functional changes are produced by progesterone, which regulates expression of numerous proteins. Examination of the human endometrial secretome has demonstrated differences in the protein content of mid-proliferative UF compared with mid-secretory UF (Hannan et al. 2010). Moreover, Roy et al. (2006) found that buffalo UF contained uterus-specific and luteal phase-specific proteins, which varied during the stages of the oestrous cycle.

Preparation of the uterine luminal epithelium for attachment of trophectoderm and their implantation in all mammals that have been studied, including ruminants, involves carefully orchestrated spatio-temporal alterations in the gene expression within the endometrium. Actually, changes in mRNA endometrium expression profiles in cows during the oestrous cycle as well as in pregnant and non-pregnant sows at the time of initial placentation have been determined by several authors (Bauersachs et al. 2005; Østrup et al. 2010; Argañaraz et al. 2013). In our study, total proteins in female camelids between 5- and 9-week pregnant showed to be slightly higher in the LUH compared with the RUH. Coincidently, Iritani et al. (1974) observed that the protein content of uterine flushing of many mammalian species increases significantly under progesterone dominance. These data would indicate that there are proteins that are differentially produced according to the needs of the foetuses, moreover the endometrium transcriptome response specifically to the sex of the embryos (Gómez et al. 2013). Even more, studies on bovine endometrium indicate that there could be differences in gene expression between the contra- and ipsilateral UH (Bauersachs et al. 2005). In camelids, embryo transfer experiments have shown that the embryos deposited in the RUH migrated to the LUH. According to these data, Tibary and Anouassi (1997) suggested the presence of luteolytic factors and/or synthesis and secretion of specific proteins that allow receptivity in the RUH and/or migration factors in the LUH of camelids.

We have detected differences between RUH and LUH of non-pregnant and pregnant alpacas. From the four proteins that displayed higher expression in the RUH, we identified a 38.35 kDa protein band differentially expressed in 5WP RUH coincides with the **Aldolase reductase isoform X2.** The bovine embryo growth and development is accompanied by changes in energy utilization and protein synthesis, and a number of

Table 4. Identified protein bands of 1D SDS-PAGE of the uterine fluid from 1-month-pregnant alpacas

MW	Intensity	SD	Intensity	SD	Positive statistic significancy
	Right uterine horn		Left uterine horn		
364.06	279.00	218.30	93.33	77.73	
323.09	20.22	27.77	32.56	14.59	
261.67	1969.00	339.00	2964.00	747.00	
218.67	87.78	65.35	307.10	164.20	
176.67	23.56	10.41	97.22	81.34	
124.94	109.70	78.64	171.80	100.60	
109.50	208.00	110.90	252.30	93.19	
89.56	126.80	59.06	–	–	*
80.58	54.50	27.65	129.10	153.50	
73.56	86.33	57.80	–	–	*
64.83	210.70	119.40	272.70	125.70	
62.33	706.60	279.10	1467.00	538.30	
60.44	94.44	82.92	38.22	54.32	
58.78	12770.00	4818.00	14502.00	4723.00	
50.61	44.44	21.70	48.89	67.20	
47.78	49.11	25.8	326.60	131.80	*
43.00	–	–	2447.00	902.30	*
38.35	83.67	34.75	8.88	6.79	*
34.83	52.00	19.02	33.00	20.86	
30.00	–	–	226.80	122.20	*
25.57	113.60	51.07	392.40	215.40	*
22.13	–	–	80.13	55.24	
16.78	–	–	126.00	52.44	*
13.00	87.33	84.03	1024.00	318.40	*
12.61	185.90	112.70	602.7	374.6	*

*Protein bands with statistically significant differences, $p < 0.001$.

Table 5. Identified protein bands of 1D SDS-PAGE of the uterine fluid from 2-month-pregnant alpacas

Intensity	SD	Intensity	SD	Positive statistic significancy
Right uterine horn		Left uterine horn		
466.56	186.43	236.22	186.51	
6.77	3.56	6.66	4.89	
2624.60	768.67	2338.70	678.50	
156.78	62.70	171.00	95.56	
38.71	13.37	19.11	15.30	
38.88	17.56	43.00	46.24	
94.11	76.46	92.44	90.72	
317.78	138.28	279.22	140.89	
–	–	9.33	8.97	
69.00	39.10	46.22	45.13	
111.22	43.31	92.56	50.68	
408.67	138.52	415.22	73.79	
242.56	90.38	266.78	42.16	
35.66	42.38	–	–	
16408.00	4352.00	14581.00	3439.70	
29.77	24.40	–	–	
188.56	140.44	216.78	171.05	
–	–	14.25	8.98	
42.77	27.95	57.22	44.08	
126.89	56.75	197.00	82.31	
97.22	63.24	43.00	18.89	
57.77	34.734	102.00	59.18	
55.33	20.45	132.25	65.09	
496.89	372.91	677.00	568.41	
530.89	390.75	790.00	625.14	

enzymes involved in carbohydrate metabolism were identified as predominant in UF (Khurana and Niemann 2000). Aldose reductase converts glucose to sorbitol and markedly increases in pregnancy (Ledgard et al. 2012). This enzyme has a role in progesterone metabolism, PGF2α synthesis and apoptotic pathways all of which may influence embryo development and survival (Madore et al. 2003). However, the presence of glycolytic enzymes in UF should be treated with caution as metabolic proteins may be a reflection of proliferative changes in the uterine endometrium.

In the LUH of 5WP animals, eight proteins that seem to be positively induced were found. Four of them have positively matched with the following proteins.

Immunoglobulin heavy-chain variable region

Of the three immunoglobulin G (IgG) isotypes described in camelids, IgG2 and IgG3, unlike their conventional counterpart IgG1, do not associate with light chains and are called heavy-chain antibodies, because are only constituted by the heavy chains. Two IgG2 subisotypes of 43 and 40.9 kDa have been detected in alpaca (Daley et al. 2005). Jones et al. (1988) found increased levels of IgG in the uterine flushing of 6-day-pregnant rabbits. These data support the findings of two bands of different, but quite similar, molecular weight during pregnancy in SACs.

Retinol-binding protein (RBP) 4 precursor

Retinol is required for normal foetal development and successful gestation, circulates in blood bound to a specific protein, the RBP, which together with its nuclear receptors is expressed in the reproductive tissues of different species, and their expression is hormonally regulated. In the uterus, the expression of the mature RBP (20 kDa) is influenced by progesterone in a time- and concentration-dependent manner (Mullen et al. 2012). At the second week of pregnancy and coincident with elongation of conceptuses, retinol and retinol-binding protein (RBP) increased in the porcine UF, and RBP mRNA increases in the endometrium (Groothuis et al. 2002).

Haemoglobin subunit beta

It was found higher in UF compared to plasma from beef heifers on diestrous (day 7 of the oestrous cycle) (Faulkner et al. 2012). It was also described in the capsule and yolk sac of the equine conceptus and in equine UF (Quinn et al. 2004). The ubiquity of haemoglobin suggests that it is a non-specific contaminant.

Although the MASCOT score was not significant for some of the protein bands isolated, the coincidences between the molecular weights and their possible role in the reproductive processes may become the results compelling and interesting for further research. The lack of higher scores could be due to the species-specific protein sequence, or the fact that only few proteins and/or genes of camelids have been identified and sequenced to date. Nevertheless, it should be acknowledged that, although the origin of these proteins has not yet been elucidated, some of them might be produced by the endometrium, whereas others might originate in serum.

Table 6. Identity of differentially expressed proteins bands in the right uterine horn and left uterine horn of alpaca fluid

Samples	Protein identity	Protein ID	No. of matched peptides	Score	MW (kDa)		MALDI-TOF protein coverage (%)	Species	Band intensity RUH	Band intensity LUH
					D	E				
5WP	Immunoglobulin heavy-chain variable region (partial)	gi|169238707	3	98	10.51	47.77	50	*Vicugna pacos*	49.11	326.56
	Immunoglobulin heavy-chain variable region (partial)	gi|169238707	3	108	10.51	43.00	50	*V. pacos*	0.00	2447.22
	Aldose reductase isoform X2	gi|560989366	5	104	35.30	38.35	18	*V. pacos*	83.67	8.88
	Retinol-binding protein 4 precursor	gi|528762361	5	105	26.27	16.78	34	*Camelus ferus*	0.00	126.00
	Haemoglobin subunit beta	gi|560962104	6	102	16.24	13.00	40	*V. pacos*	87.00	1024.00

5WP, 5 weeks of pregnancy; RUH, right uterine horn; LUH, left uterine horn; D, deduced molecular weight; E, experimental molecular weight. Protein scores >73 are significant ($p < 0.05$). The band intensity shown is significantly different, $p < 0.001$.

Conclusions

The endometrium undergoes secretory changes in preparation for embryo implantation and development, but the identity of most of the endometrial secretory products remains unknown in most mammals, particularly SACs. This is the first report on differences in the biochemical composition and the protein profile of alpaca UF during the different gestational stages and between the left and right uterine horn. We observed specific variations in the protein pattern between the right and the left UH, differences that would be due to the different capacity of the UH to maintain pregnancy. The proteins identified have been reported to play a role in signal translation, immunology, glycolysis, angiogenesis and protection against oxidative stress. These results will form the basis for future, more thorough studies to unravel the embryo-maternal crosstalk in the SACs during early pregnancy.

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Conflict of interest

The authors have declared no conflicts of interest.

Author contributions

M Argañaraz contributed with experimental design, experimental assays, data analysis and drafting the article. S Apichela contributed with experimental design, analysis and interpretation of data and revising paper. R Zampini contributed with statistical analysis, interpretation of data and revising paper. J Vencatto contributed with experimental design and interpretation of results. C Stelletta contributed with experimental design, analysis and interpretation of data and revising paper.

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