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BIOCHEMISTRY OF UTERINE AND OVIDUCT CONTENTS FOLLOWING COPULATION IN AN INDUCED OVULATOR, THE ALPACA

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Abstract: Concentrations of glucose, fructose, citrate, calcium, zinc, chloride, total proteins, albumin, phospholipids, acid phosphatase, and alpha-glucosidase were determined in the uterus, and oviduct contents recovered at 0, 12-, 24-, 36- and 48-hours following copulation in the female alpaca. Twenty adult female alpacas, divided into five groups, were used in this study. Analysis of variance was used to analyze data. Glucose and chloride changed throughout the study period (P<0.05) being 0.03, 0.02, 0.006, 0.02 y 0.03 mg/dL; and 0.12, 0.016, 0.02, 0.04, 0.02 nmol/L, by 0, 12, 24, 36 and 48 hours after copulation, respectively. Fructose was consistently four times higher (P<0.05) than glucose throughout the study. The rest of the analytes did not change (P>0.05) for the time of the study, nor by organ. The decrease in glucose concentrations by 24 hours may represent a concomitant decrease in spermatozoa motility. Chloride decreased by 12, 24, 36, and 48 hours may constitute a minimal or lack of usage during those times.
Keywords: Biochemistry analytes; Uterus; Oviducts; Female; Alpaca.

INTRODUCTION

The female alpaca is an induced ovulator, and ovulation occurs within 24 to 36 hours following copulation (San Martin et al., 1968; Adams et al., 1989). Semen is deposited by the male within the uterus and vagina because of continuous ejaculation and during a copulation period of 15 to 20 minutes (Franco et al., 1981; Bravo et al., 1996). Spermatozoa are entrapped within a gelatinous seminal plasma and remain in the uterus and oviducts (Bravo et al., 1997) until ovulation. Time from ejaculation to ovulation is longer if compared to sheep, a spontaneous ovulator, wherein spermatozoa are present in the oviduct within 3-5 minutes following breeding (Mattner and Braden, 1963). These rapid spermatozoa movement is due, in part, to a fluid seminal liquid plasma and progressive spermatozoa motility rather than a gelatinous seminal plasma and slow-motion motility in the alpaca spermatozoa (Sumar and Leyva, 1981). Thus, the time of waiting for an oocyte is different, and there are no reports on the biochemistry of contents in the uterus and oviducts of alpacas. In the ewe, from one to four after estrus, there is no variation in total protein, glucose, citrate, and lactate (Iritani et al., 1969). The objectives of the present study were twofold: First, to determine the concentration of 11 different analytes, glucose, fructose, citrate, alpha-glucosidase, chloride, calcium, zinc, phospholipids, acid phosphatase, total proteins, and albumin at five other times following copulation, 0, 12, 24, 36 and 48 hours. Second, to determine concentrations of those analytes in two different reproductive organs, the uterus and oviduct.

MATERIAL AND METHODS

ANIMALS

Twenty adult females with reproductive history were bred to an intact male and divided into five groups of four for slaughtering at 0, 12-, 24-, 36- and 48 hours following copulation. All animals have kept grazing native pastures at the La Raya research center in Cusco, Peru, at 4200 m sea level, 14 ^{0}S latitudes, and 70-^{0}W longitude.

EXPERIMENTAL DESIGN

Uteri and oviducts were dissected immediately after slaughtering, isolated from every female, and their contents were flushed with 500 µL of pre-warmed Tris buffer solution. Each flush was labeled and frozen at -20 ^{0}C until analyzed.

Eleven components, glucose (Golden and Sapir, 2012), fructose (Karkoven and Malm, 1995), citrate (Guber and Mollering, 1955), calcium (Robertson, 1976), zinc (Marrades

et al., 2008), chloride (Schales and Schales, 1941), proteins and albumin (Peterson, 1977), phospholipids (Folch et al., 1957), acid phosphatase (Connolly et al., 1986), and alpha-glucosidase (Umapathysivam et al., 2001), were determined by spectrometry and in duplicate. In all cases, an aliquot of Tris diluent was also selected in duplicate, considered as a background lecture, and then subtracted to give an accurate sample reading. Concentrations of analytes in the oviducts were grouped since there were no differences between the left and right oviducts.

DATA ANALYSIS

Data were analyzed using the analysis of variance and performed by the NCSS, Number Crunching Statistical System, a computer program. A *P* value of 0.05 was used to denote significance.

RESULTS

CONCENTRATIONS OF ANALYTES FOLLOWING COPULATION

The glucose concentration was higher ($P<0.05$) immediately after copulation, then decreased and maintained low for the remaining times except for 36 and 48 hours. Fructose was always four times as high as glucose and remained unchanged for the first 36 hours. It increased almost two-fold than previous times by 48 hours after copulation (Fig. 1). Citrate and alpha-glucosidase always remained intact with no differences ($P>0.05$) from zero through 48 hours (Table 1).

Chloride (Fig. 2) was elevated at the beginning and then decreased significantly during the remaining times ($P<0.05$). Calcium and zinc concentrations remained unchanged ($P>0.05$) at all times considered in this study (Table 1).

Phospholipid concentrations remained unchanged ($P>0.05$) throughout this study. Acid phosphatase was higher at time zero, almost twice as elevated ($P<0.05$) than the rest of the other times (Fig. 3).

Total proteins and albumin remained unchanged ($P>0.05$) from time zero through 48 hours after copulation (Fig. 4).

CONCENTRATIONS OF ANALYTES IN THE UTERUS AND OVIDUCTS

Glucose concentrations were four times higher in the oviducts than in the uterus ($P<0.05$) except for 24 hours (Fig. 5).

Fructose concentrations were twice as high ($P<0.05$) in the oviducts than in the uterus. Then, it remained similar ($P>0.05$) between the uterus and oviduct at 12, 24, and 36 hours after copulation (Fig. 5).

Chloride concentrations were elevated by 0 and 36 hours and with no difference between the uterus and oviducts at 12 and 24 hours ($P<0.05$)

Alfa glucosidase concentrations were higher in the oviduct than in the uterus at 12 hours after copulation ($P<0.05$). They then remained no apparent change in both organs (Fig. 6).

Concentrations of citrate, calcium, zinc, phospholipids, acid phosphatase, total protein, and albumin did not change ($P>0.05$) between the uterus and oviduct throughout the study period, Table 1.

DISCUSSION

This manuscript evaluated the dynamics of eleven analytes: glucose, fructose, citrate, alpha-glucosidase, chloride, calcium, zinc, phospholipids, acid phosphatase, total proteins, and albumin concentrations in the uterus and oviducts of an induced ovulator, the alpaca. All these analytes have been implicated in one way or another in the reproductive process. The alpaca was chosen because spermatozoa remain in the uterus and oviducts longer, waiting for the oocyte

following ovulation (Bravo et al., 1996).

Glucose concentrations were high at the time of copulation and then decreased to small amounts for the next 36 hours, at which time was elevated and comparable to the time of breeding. A high concentration during copulation indicates that average glucose concentration exists in the ejaculate (Garnica et al., 1993; Diaz, 1995). The fact that glucose is low until 36 hours might represent minimal use. Recently, a concomitantly decreased spermatozoa motility was determined in the alpaca (Ccanahuire, 2019). In addition, semen is deposited within the uterine horns and close to the oviduct, a short distance to travel for ovulation, and different from ewes and cows, where semen is deposited at the external os of the cervix. Naturally, the space to reach the oviduct is more than in the alpaca. Altogether, glucose is one of the carbohydrates that intervenes in nourishing spermatozoa within the oviduct, as demonstrated by Leese and Gray (1985). In addition, its low concentrations appear comparable to cows (Carlson et al., 1970).

Fructose concentrations remained unchanged from the time of copulation up to 36 hours, then increased significantly by 48 hours. This change may indicate a constant use from zero to 36 hours and no use by 48 hours. Fructose concentration was possibly related to increased demand by motility of spermatozoa. Taking together glucose and fructose within the uterus and oviduct, both were consistently higher in the oviduct than in the uterus (Fig. 5). This fact is substantiated because more spermatozoa are present in the oviduct (Bravo et al., 1996). Consequently, most of the activity occurs in the oviduct than in the uterus.

Furthermore, this is supported by two reasons: First, an increased spermatozoa motility because of a change of flagellum movement from oscillatory at the time of ejaculation to a rotational movement (Ccanahuire, 2019), and second, more usage of glucose than fructose demonstrated in vitro by alpaca spermatozoa (Garnica et al., 1997). In addition, the pathway of fructose utilization might be using the phosphorylated way, as suggested in the camel (Agarwal et al., 2004). In rams and bulls, semen is maintained in anaerobic conditions (Mann and Lutwak-Mann, 1981), like the uterine environment. Altogether, fructose concentrations were four times higher than glucose at all times considered in this study (Fig. 1). Although fructose concentrations appeared unchanged, a significant decrease was observed at 36 hours, which increased by 48 hours. Notably, glucose was depleted by 48 hours in the oviduct, and conversely, fructose was always at its highest. Thus, plenty of fructose was ready to be converted into glucose; theoretically, ovulation and, most likely, fertilization would be influenced by these two metabolites and remains to be elucidated.

Alpha-glucosidase remained unchanged from zero to 48 hours from copulation, and no change in the uterus and oviducts. Although its role in the alpaca has not been well established, in men, it might indicate a deficient maturation process in the epididymis, and consequently, human spermatozoa present low motility (Cooper et al., 1988). This may be the case in the alpaca, wherein low progressive motility might be associated with higher concentrations reported immediately following copulation in the uterus and oviduct. Citrate concentrations also remained unchanged during the five times considered in this study. In addition, no changes were observed within the uterus and oviducts.

Chloride concentrations were higher at time 0 and then decreased significantly for the remaining study period. Chloride is an anion in all ejaculates and from different

species (Mann, 1972) and helps with the appropriate pH balance (Mann and Lutwak-Mann, 1981). This could be the case in alpaca, where spermatozoa are subjected to anaerobic conditions within the uterus. Similar concentrations of chloride were also reported in rabbits (Hammer and Williams, 1965), ewes (Wales and Restall, 1973), and cows (Olds and VanDemark, 1957).

Calcium and zinc concentrations remained low and stable throughout the study period. Calcium is essential because of its involvement in acrosome reaction and sperm motility (Kaya et al., 2002). Low calcium concentrations are comparable to those reported in cows (Olds and Vandemark, 1957) and ewes (Restall and Wales, 1966). Zinc has protective antioxidant activity (Gavella and Lipovac, 1998) and may be involved in spermatozoa motility in controlling energy utilization through the adenosine triphosphate system (Hidiroglou and Knipfel, 1984). In the case of alpaca, its function might be diminished because of the low motility exhibited 24 hours after being deposited within the uterus (Ccanahuire, 2019). Likewise, phospholipids and acid phosphatase remained unchanged, suggesting minor or no changing conditions in the uterus. This differs from ram spermatozoa, wherein subtle changes have been demonstrated inside the uterus (Quinn and White, 1972). It may also represent adequate protection from the thick alpaca semen, as shown in cultured spermatozoa (Ccanahuire and Bravo, 2018). In addition, comparable chloride, calcium, and phospholipids concentrations have been reported in the uterine contents of ewes during estrus (Wales, 1973), similar to time 0 in the present study.

Total protein and albumin remained low and unchanged during the present study. It is worth mentioning that albumin was one-third of complete proteins. Generally, the percentage of proteins detected within the uterus and oviducts constituted one-fifth of serum concentrations (4 -5 g/L; Fowler, 2010). The origin of these two proteins is the seminal plasma as part of the ejaculate, as demonstrated previously in the alpaca (Garnica et al., 1993). In addition, minute amounts of these two proteins have been isolated in ewes (Wales, 1973) and cows at estrus (Martins et al., 2018).

Acid phosphatase was higher at time 0 and then decreased significantly during the remaining times in the present study. The elevated concentration at time zero indicated its origin, the prostate (Bravo et al., 2017), and since no more function could be attributed, its concentration decreased. Moreover, its role could be like those reported in mares, a species wherein ejaculation occurs in the uterus (Zavy et al., 1976), as in alpacas.

CONCLUSION

Glucose concentrations were low by 24 hours, suggesting a simultaneous decrease in spermatozoa motility. Glucose and fructose concentrations were two times higher within the oviduct than in the uterus, implying that most of their usage occurs in this organ. Chloride and acid phosphatase concentrations were elevated during copulation and then remained low during the times considered in this study. Calcium, zinc, phospholipids, total proteins, albumin, and alpha-glucosidase did not change throughout the study period. There was no additional change within the uterus and oviduct, suggesting minimal or no use by spermatozoa.

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AUTHOR CONTRIBUTION STATEMENT

MC performed data collection and sample analysis

JP supervised data collection and analyzed data.

WB conceived the study and wrote the paper.

DECLARATION OF INTEREST

No conflict of interest could be perceived as prejudicing the impartiality of the current manuscript.

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FIGURES CAPTIONS

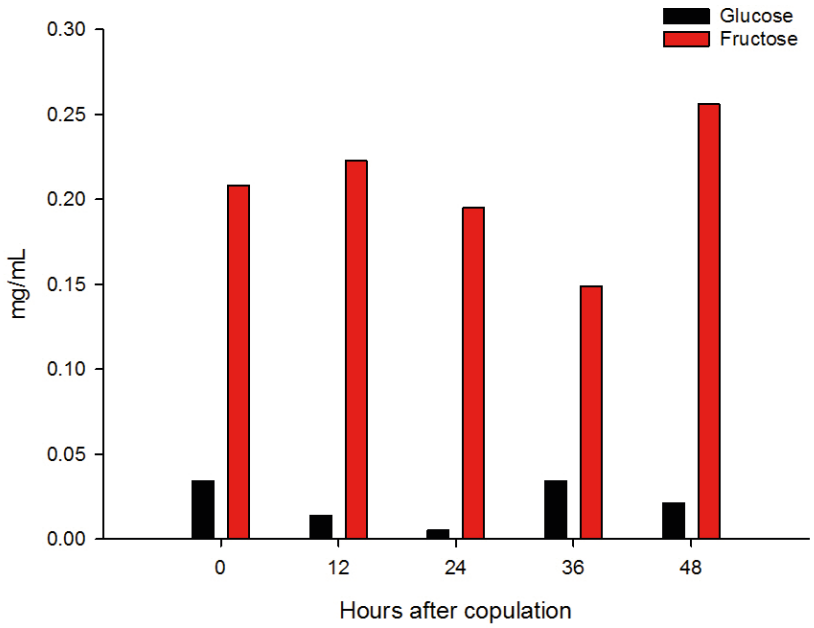

Fig. 1. Concentrations of glucose and fructose, in the reproductive tract of the female alpaca from time zero and through 48 hours after copulation.

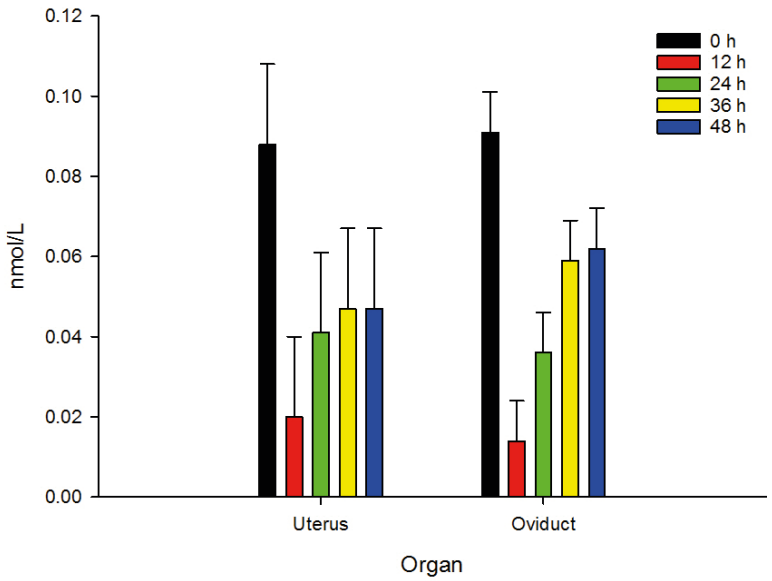

Fig. 2. Concentrations of chloride in the uterus and oviducts of female alpacas after copulation to an intact male from zero through 48 hours.

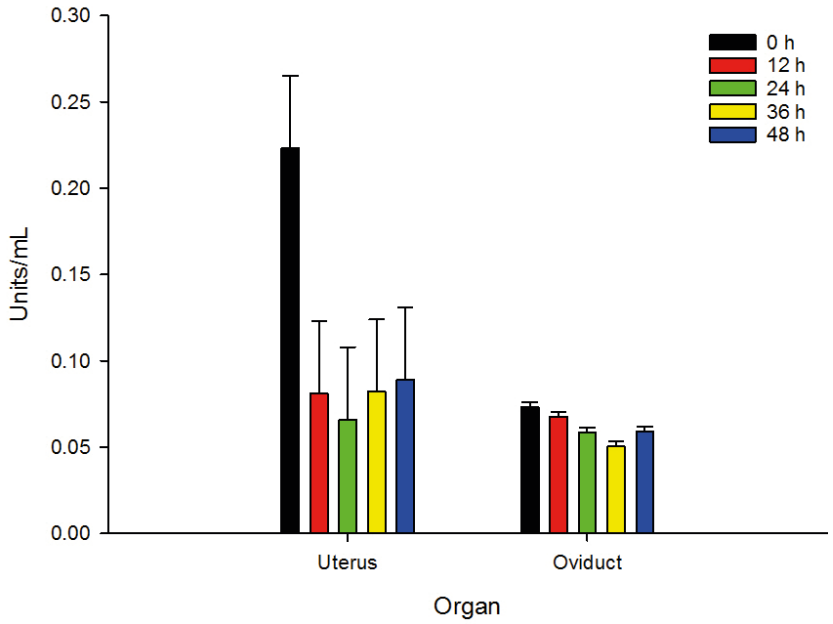

Figure 3. Concentrations of acid phosphatase in the uterus and oviducts from female alpacas bred to an intact male at time 0 and through 48 hours.

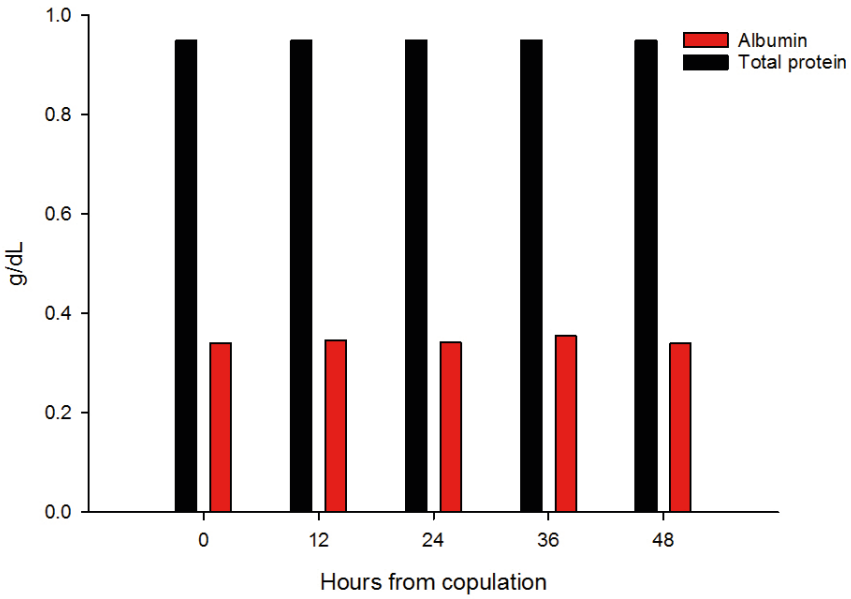

Figure 4. Concentrations of total proteins and albumin in the uterus and oviducts from female alpacas bred at time 0 and through 48 hours after copulation.

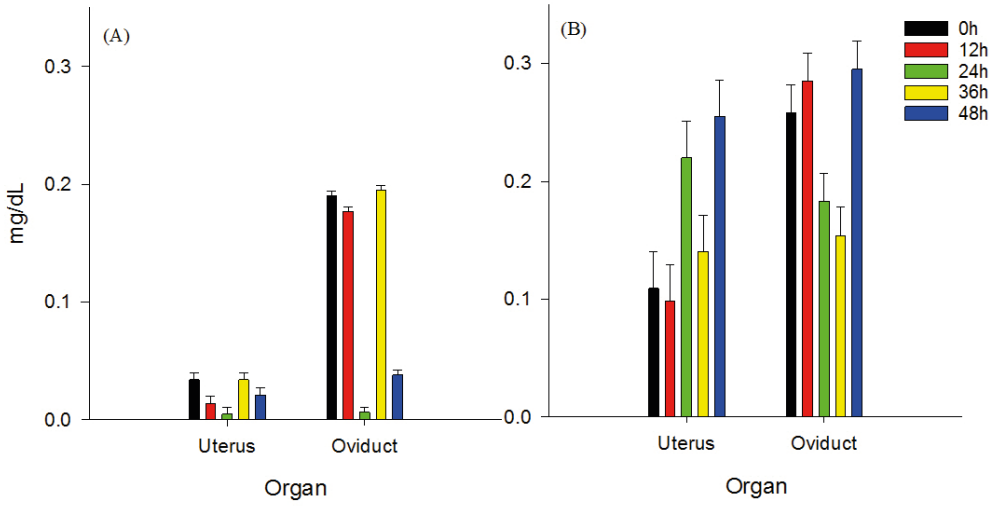

Figure 5. Concentrations of glucose (left) and fructose (right) in the uterus and oviduct of female alpacas bred to an intact male at time 0 and for 48 hours after copulation.

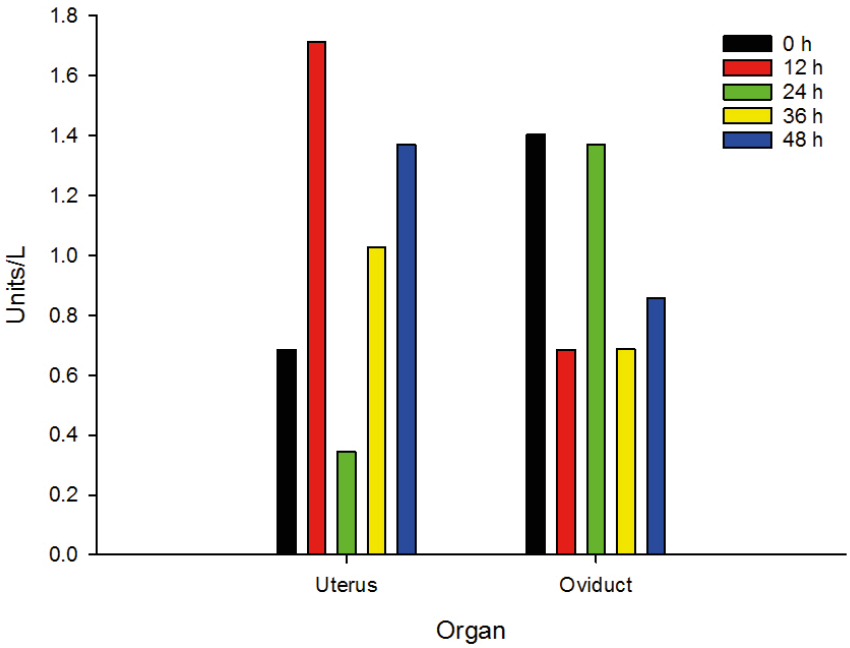

Figure 6. Concentrations of alpha-glucosidase within the uterus and oviduct of female alpacas from time of copulation (0) through 48 hours.