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Mating induces production of MMP2 in the llama oviduct: Analysis of MMP2 effect on semen

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ABSTRACT

Ovulation of South American Camelids is induced by mating. After copulation, sperm are stored into the oviduct to be released near ovulation time. To study whether copulation induces matrix metalloproteinase-2 (MMP2) secretion in the oviduct, the occurrence of MMP2 in oviductal tissue and oviductal fluid (OF) from 24 h post-mated was compared with non-mated llama females. There was an incremental increase of MMP2 in the oviductal epithelial cells, and MMP2 activity in OF after copulation. Additionally, MMP2 activator (MMP14), inducer (EMMPRIN) and inhibitor (TIMP2) were present in the oviductal epithelial cells of both non-mated and post-mated females. A post-mating segment-specific regulation occurred because relative abundance of TIMP2 mRNA was greater in the utero tubal-junction which was accompanied with a reduced amount of MMP14 in the ampulla in comparison with the non-mated females. To examine the effect of MMP2 on semen liquefaction and sperm physiology, the effects of addition of recombinant human MMP2 was evaluated. The MMP2 had no effect on semen thread formation and seminal plasma protein profile. Sperm viability and plasma membrane function were not influenced by the enzyme treatment either. In summary, in llamas the oviductal microenvironment changes in response to stimuli induced by copulation, increasing the production and secretion of MMP2.

1. Introduction

South American camelids (SACs) are induced-ovulating species. During mating, the male deposits semen deep inside the uterine horns of the female with ovulation occurring 26–42 h later (San-Martin et al., 1968; Ratto et al., 2006). A protein in the seminal plasma of llamas and alpacas, identified as β-Nerve Growth Factor (β-NGF), induces ovulation by stimulating pituitary LH secretion (Adams and Ratto, 2013; Berland et al., 2016). After insemination, sperm is stored in the utero-tubal-junction (UTJ) segment of the

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llama oviduct, and it is released 28 h post-mating and transport to the site of fertilization continues (Apichela et al., 2009). The oviduct environment is favourable for fertilization to occur (Hunter, 2005; Rodriguez-Martinez, 2007). There, however, is a lack of knowledge about the varying oviduct functions and timing of these functions, particularly in induced-ovulating species because the oviductal microenvironment likely changes in response to stimuli induced by copulation.

In previous studies, the two metalloproteinases (MMP2 and MMP9) were present in the llama oviduct and oviductal fluid (OF) of non-pregnant and non-mated females, and there was a greater amount of MMP2 activity than MMP9 in the OF (Zampini et al., 2014, 2017). The MMP2 is an important contributor to oocyte release from preovulatory follicles of rats, mice, sheep, and humans (Curry and Osteen, 2003). While the role of MMP2 in the ovary is well defined, its role in the oviduct remains unknown. In the present study, the hypothesis was that mating would induce MMP2 secretion in the oviduct. To assess this hypothesis, in the current study, the presence of MMP2 in oviductal epithelial cells and OF in 24 h post-mated was compared with non-mated llamas. In addition, to enhancing the understanding of the MMP2 function in the llama oviduct, the effect of addition of MMP2 on sperm and seminal plasma was evaluated, as well as presence of modulators of MMP2 activity in the oviduct after mating.

2. Materials and methods

2.1. Animals

Fertile, non-lactating, 5–8 year-old female llamas (*Lama glama*, $n = 11$) used in this study were provided by the Instituto Nacional de Tecnología Agropecuaria (INTA) Abra Pampa, located on the high Andean plateau in the Argentine northwest.

Fertile male llamas ($n = 3$), ranging between 5 and 8 years of age, used in the study belonged to the Faculty of Veterinary Sciences of the University of Buenos Aires, in Buenos Aires, Argentina.

2.2. Oviducts, oviductal epithelial cells (OECs) and oviductal fluid (OF)

Llama oviducts from 24 h post-mated females ($n = 3$) were obtained immediately after slaughtering, in accordance with protocols approved by the local institutional animal care committee. To minimize hormonal influences only the oviducts ipsilateral to dominant follicles and having dominant follicles smaller than 7 mm were used. Oviducts were dissected and OF was obtained by perfusion with 100 µl of 10 mM Tris-HCl, 150 mM NaCl (pH 7.4) at 4 °C. The perfused solution was subsequently centrifuged to remove cellular debris. Oviducts were subsequently separated into ampulla, isthmus and UTJ segments, and fixed with 4% formaldehyde in PBS (pH 7.4) (Zampini et al., 2017). Ipsilateral oviducts and OF from non-mated females were used as the control ($n = 3$). For RT-PCR assays, OECs from oviductal segments from five additional llamas, three non-mated and two post-mated, were used. Epithelial cells from ampulla, isthmus and UTJ segments were separately collected by gently scraping the mucosal epithelial layer with the blunt side of a sterile scalpel (Apichela et al., 2009). The OECs were lysed in RNA Lysis Buffer solution (SV total RNA isolation system, Promega, Madison, WI, USA), and stored in liquid nitrogen until RNA isolation.

2.3. Semen collection

Semen collections were conducted between the months of August and October by electroejaculation with animals being under general anaesthesia using a P-T Electronics 304 electroejaculator (Oregon, USA) with a #4 probe and three ventral electrodes. Electrical stimulation was performed as previously described by Director et al. (2007). All procedures were approved by the Committee for the Care and Use of Laboratory Animals (CICUAL) of the Faculty of Veterinary Sciences of the University of Buenos Aires (protocol 2010/24).

2.4. Experiment I – RNA relative abundance of MMP2 and related genes in OECs of mated females

2.4.1. RNA isolation and cDNA synthesis

Total RNA from OECs (ampulla, isthmus and UTJ) was isolated using the SV total RNA isolation system (Promega, Madison, WI, USA) according to the manufacturer's instructions. The RNA was quantified spectrophotometrically at 260 nm, and RNA integrity was examined by electrophoresis on 1.5% agarose gels, stained with SYBR Safe DNA Gel Stain (Invitrogen, Carlsbad, CA, USA).

Reverse transcription was performed using Moloney murine leukemia virus (M-MLV) reverse transcriptase (Promega, Madison, WI, USA) and oligo $(dT)_{15}$ primer. The reaction mixture (25 µl) consisted of 1 µg of RNA, 50 mM Tris–HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 0.5 mM of each dNTP, 25 pmol of oligo $(dT)_{15}$, 200 units of reverse transcriptase, and RNase-free water. Reactions were performed in a thermal cycler at 42 °C for 90 min, followed by enzyme inactivation at 94 °C for 5 min.

2.4.2. Semi-quantitative PCR

Relative abundance of MMP2, MMP14, TIMP2 and EMMPRIN mRNA was analyzed by semi-quantitative PCR in 24 h post-mated llama OECs (ampulla, isthmus and UTJ).

The MMP2 and TIMP2 primers were designed based on llama MMP2 and TIMP2 sequences previously identified (Zampini et al., 2014). The MMP14, EMMPRIN and ACTB (β-actin) primers were designed using predicted *Vicugna pacos* nucleotide sequences. Primer sequences are shown in Table 1.

Amplifications were conducted in a final volume of 10 µl containing 1 µl of cDNA, 2 µl of 5X Green GoTaq Reaction Buffer (pH