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Is an ovulation-inducing factor (OIF) present in the seminal plasma of rabbits?

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ABSTRACT

The objectives of this study were (1) to determine the effect of rabbit seminal plasma on LH secretion and ovulation using the llama animal model as an *in vivo* ovulation bioassay and (2) to determine the effect of llama or rabbit seminal plasma on ovulation induction in the rabbit model. In Experiment 1, llamas with a growing follicle ≥8 mm in diameter were assigned randomly to one of three groups ($n = 5$ per group) and given an intramuscular dose of 1 mL of: (a) llama seminal plasma, (b) rabbit seminal plasma, or (c) phosphate buffered saline (PBS; negative control). Blood samples for LH measurement were taken every 15 min from 1.5 h before to 8 h after treatment (Day 0: starting of treatment). Llamas were examined by ultrasonography every 12 h from treatment to ovulation, and then every other day until Day 16 after treatment to evaluate corpus luteum (CL) development. Blood samples for progesterone measurement were taken every other day from Day 0 to Day 16. Ovulation was detected in 4 of 5, 5 of 5, and 0 of 0 llamas treated with llama or rabbit seminal plasma and PBS, respectively ($P < 0.001$). After treatment, plasma LH concentration increased and decreased ($P < 0.01$) in the llama and rabbit seminal plasma group but not in the PBS-treated group. No differences were observed on CL development ($P \geq 0.3$) and progesterone secretion ($P > 0.05$) between both seminal plasma treated groups. In Experiment 2, receptive female rabbits ($n = 5$–7 per group) were given an intramuscular dose of: (a) 0.5, (b) 1.0 and (c) 2.0 mL of either rabbit or llama seminal plasma, (d) 0.5 mL PBS (negative control), or (e) 25 μg of gonadoreline acetate (GnRH; positive control). Does were submitted to laparotomy 24–36 h after treatment to determine the ovulatory response and the presence of antral and hemorrhagic anovulatory follicles. Ovulation sites (7.0 ± 0.6) were only detected in GnRH-treated does ($P < 0.01$). There was an increase ($P < 0.01$), in the total number of follicles (antral plus hemorraghic follicles) in those females treated with 1 mL of rabbit seminal plasma and there was a tendency ($P = 0.08$) for more hemorrhagic anovulatory follicles in does treated with 1.0 and 2.0 mL of either rabbit or llama seminal plasma. Results document the presence of OIF in the seminal plasma of rabbits. The differential ovulatory response between species, however, requires further investigation.

1. Introduction

Mammalian species have been classified as either spontaneous or induced ovulators based on the type of stimulus responsible for eliciting GnRH release from the mediobasal

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hypothalamus (Bakker and Baum, 2000; Spies et al., 1997). In induced or reflex ovulators (e.g. rabbit, Bactrian camel, llama, alpaca, cat, ferret) neural signals from copulatory stimulation trigger hypothalamic GnRH secretion, followed by the preovulatory release of LH from the pituitary gland (Bakker and Baum, 2000) which increases systemic LH concentration, a requisite for ovulation. Although different stimuli (tactile, olfactory and visual) have been associated with eliciting/facilitating ovulation in reflex ovulators, the physical stimulation of penile intromission or cervical stimulation has been ascribed the pivotal role on triggering the preovulatory LH surge and subsequent ovulation (Bakker and Baum, 2000).

Mating-induced ovulation has been documented in several species of the Camelidae, such as, llamas (Bravo et al., 1990, 1991, 1992; England et al., 1969), alpacas (Bravo et al., 1991, 1992; Fernandez-Baca et al., 1970; San Martin et al., 1968) and Bactrian camel (Chen et al., 1980). However, the use of vasectomized males in studies specifically designed to determine factors controlling ovulation in South American camelids (Fernandez-Baca et al., 1970) did not allow discriminate between the mechanical stimulus and the seminal plasma effect to determine the roles that each component may have on ovulation induction. In this regard, recent studies in llamas and alpacas (Adams et al., 2005; Ratto et al., 2005, 2006, 2010) as well as in Bactrian camel (Zhao et al., 2001; Li and Zhao, 2004; Pan et al., 2001) have documented the presence of a potent ovulation-inducing factor (OIF) in the seminal plasma of these species. The biochemical characterization and purification of llama OIF has established that this factor is a protein, with a molecular mass equal or greater than 30 kDa, which presents high resistant properties to heat and enzymatic digestion (Ratto et al., 2010). However, a recent study (Ratto et al., 2011) identified llama OIF as a 14 kDa protein, suggesting that this molecule could be part of a larger protein complex or be a bioactive pro-hormone form.

An alpaca and llama model was developed to determine the presence of an ovulation inducing factor(s) in the seminal plasma from species with both induced and spontaneous ovulation (Adams et al., 2005; Ratto et al., 2005, 2006; Bogle et al., 2009). Llama and alpaca have emerged as an excellent biological model to study the presence of these factors when seminal plasma of different species is given by intramuscular administration (Adams et al., 2005; Ratto et al., 2006; Bogle et al., 2009) or intrauterine deposition (Ratto et al., 2005). As induced ovulators, the effect of these factors on pituitary LH secretion is not diminished by the endogenous LH, because the occurrence of spontaneous ovulation in these species is an unusual phenomenon (Adams et al., 1989; Fernandez-Baca et al., 1970). Moreover a recent study (Ratto et al., 2005), documented that physical stimulation of the uterus, including prolonged uterine curettage, did not induce ovulation in alpacas. Therefore mechanical stimulation is not a confounding variable for the study of ovulation on this animal model.

The intramuscular administration of homologous seminal plasma in llamas elicits a significant increase of systemic LH concentration (Adams et al., 2005; Tanco et al., 2007) which closely resembles that observed after natural mating (Bravo et al., 1990), and is also similar to that observed in llamas treated with GnRH, although more sustained (Adams et al., 2005). Ovulations have also been efficiently induced (100%) in llamas after intramuscular administration of seminal plasma from a related induced ovulating species, the alpaca (Ratto et al., 2006), and to a lesser extent (26%) after intramuscular administration of seminal plasma from spontaneous ovulating species, such as cattle (Ratto et al., 2006), horse (29%) and swine (18%) (Bogle et al., 2009). Semen-induced ovulations also have been described in another induced ovulator, the marsupial koala (Johnston et al., 2004) and a recent study has determined that llama seminal plasma is able to induce ovulation in a prepubertal mouse model (Bogle et al., 2008). The sum of the evidence suggests that the presence of one or several ovulation inducing factor(s) in the semen could be a trait conserved among induced or spontaneous ovulating species.

Rabbits have been frequently employed as an animal model, in investigations of the mechanism of induced ovulation; however, to date no study has been conducted to determine the presence of such factor(s) in the seminal plasma of this species. In female rabbits a short mating bout, including ejaculation, induces a rapid release of LH from the pituitary and, consequently, ovulation (Jones et al., 1976). However, several studies have demonstrated that mechanical stimulation of the genital tract in the doe by different means (glass rods and vibrating probes) is only partially effective in ovulation induction (Sawyer and Markee, 1959; Vincent et al., 1970). These findings suggest a facilitative role for additional mating-related stimuli/cues on the ovulatory response, and semen factors could comprise one of these cues.

Collectively, these observations suggest that an OIF could be present in the seminal plasma of rabbits and may support the notion for an evolutionary conservation of the ovulatory mechanism among species. Hence, the purposes of this work were (1) to determine the effect of rabbit seminal plasma on LH secretion, ovulation and CL formation using the llama animal model as an *in vivo* ovulation bioassay and (2) to determine the effect of llama or rabbit seminal plasma on ovulation induction in the rabbit model.

2. Materials and methods

All procedures were performed in accordance with the animal care protocols established by the Universidad Austral de Chile and were revised and approved by the Bioethics Committee from the same institution.

2.1. Semen collection and seminal plasma preparation

Semen from three adult male llamas and four adult male rabbits was collected once per week for two months prior to the beginning of the experiment. Llamas were maintained on pasture supplemented with hay and water *ad libitum* and were housed indoors at nights. Semen was collected with the use of an artificial vagina designed for sheep that was fitted into a phantom mount built of wood and covered with a llama hide (Bravo et al., 1997). Adult rabbit males were housed in individual wire cages under a constant photoperiod of 16 h of light/day in a traditional building under