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Review article

Ovulation-inducing factor in seminal plasma: A review☆

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ABSTRACT

Ovulation in mammals involves pulsatile release of GnRH from the hypothalamus into the hypophyseal portal system with subsequent release of LH from the anterior pituitary into systemic circulation. Elevated circulating concentrations of LH induce a cascade of events within the mature follicle, culminating in follicle rupture and evacuation. The broad classification of species as either *spontaneous* or *induced* ovulators is based on the type of stimulus responsible for eliciting GnRH release from the hypothalamus. In spontaneously ovulating species (e.g., human, sheep, cattle, horse, pigs), release of GnRH from the hypothalamus is triggered when, in the absence of progesterone, systemic estradiol concentrations exceed a threshold. In induced ovulators (e.g., rabbits, ferrets, cats, camelids), release of GnRH is contingent upon copulatory stimuli; hence, ovulation is not a regular cyclic event. Since a classic 1970 Peruvian study, dogma has maintained that physical stimulation of the genitalia during copulation is the primary trigger for inducing ovulation in alpacas and llamas. Exciting results of recent studies, however, provide direct evidence for the existence of an ovulation-inducing factor (OIF) in semen, and compel us to re-examine the mechanism of ovulation in both induced and spontaneous ovulators. Ovulation-inducing factor in seminal plasma is a potent stimulant of LH secretion, ovulation and luteal gland development, and acts via a systemic rather than a local route. OIF is a protein molecule that is resistant to heat and enzymatic digestion with proteinase K. It has a molecular mass of 14 kDa, and may be part of a larger protein complex or pro-hormone. The effect of OIF is dose-related and evident at physiologically relevant doses (i.e., as little as 1/100th that present in the ejaculate), and is mediated, in whole or in part, at the level of the hypothalamus in vivo. The factor exists in the seminal plasma of every species in which it has been examined thus far, including Bactrian camels, alpacas, llamas, cattle, horses, pigs, and koalas. Seminal plasma OIF does not appear to be a phylogenetic vestige in spontaneous ovulators since it (1) induced ovulation in pre-pubertal mice, (2) altered ovarian follicular wave dynamics in cows, and (3) elicited LH release in vitro from primary pituitary cell cultures of rats, mice, guinea pigs, rabbits, llamas and cows.

Contents

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1. Introduction

Ovulation in mammals is regulated by a complex neuroendocrine mechanism that involves signaling pathways between the reproductive organs and the brain. In spontaneous ovulators (e.g., woman, sheep, cattle, rat), estrogen produced by a mature ovarian follicle triggers a surge release of GnRH from the hypothalamus. However, in induced ovulators (e.g., rabbits, cats, llamas, camels), factors associated with coitus are responsible for triggering GnRH secretion.

In the newly emerging field of semen biochemistry at the time, Thaddeus Mann (1964) stated that he made every attempt to "...refrain from the tendency, currently prevalent among workers in this field, to assign to every newly discovered chemical constituent of semen a major role in the process of fertilization." Notwithstanding this note of caution, recent findings about systemic effects in the female suggest a new role of seminal plasma – as an inducer of ovulation. The discovery of an ovulation inducing-factor (OIF) in the seminal plasma of camelids, and subsequently in several other species, challenges the long-established concept of what controls the ovulatory cascade as well as the categorical distinction between species considered to be either induced or spontaneous ovulators. Uncovering the biological role of OIF in mammalian semen may have important implications in the diagnosis of male and female infertility, and holds potential for development of new pharmaceuticals for the treatment of infertility.

The following is intended as a brief review of what is known about ovulation-inducing factor in seminal plasma, with particular emphasis on recent studies from the author's laboratory. In sequence, the discourse covers the discovery of OIF, its effects and route of action, biochemical isolation and purification, evidence for a dose-related response and mechanism of action, and its existence among species.

2. Discovery of OIF

The first direct evidence of an ovulation-inducing factor (OIF) in semen came from workers in China who concluded that some factor in the semen was responsible for eliciting ovulation in Bactrian camels, rather than the mechanical stimulation of copulation. Ovulation occurred after intravaginal (Chen et al., 1985; Xu et al., 1985) or intramuscular/intrauterine (Pan et al., 1992) administration of Bactrian seminal plasma to female Bactrian camels. In early studies of alpacas and llamas, New World relatives of camels, ovulation occurred in >95% of the females subsequent to mounting and penile intromission compared to <14% of the females in which intromission was not allowed (England et al., 1969; Fernandez-Baca et al., 1970; San-Martin et al., 1968). This led to the concept that physical stimulation of the genitalia during copulation is the primary trigger for inducing ovulation in alpacas and llamas. However, early studies were not designed to control potential confounding factors that may influence ovulation (e.g., physical stimulation of the genitalia during artificial insemination). In this regard, artificial insemination (intravaginal deposition of alpaca semen) was associated with ovulation in 6/10 alpacas and 5/8 llamas (cited in Sumar, 1994), in apparent contradiction to earlier studies (Fernandez-Baca et al., 1970). Later studies were designed to more carefully control other potential factors, and have involved intramuscular administration of seminal plasma to circumvent the issue of physical stimulation of the vagina, cervix and uterus.

The original discovery of OIF in Bactrian camels went largely unnoticed for 20 years, until it was confirmed in a series of studies involving llamas and alpacas (Adams et al., 2005) wherein the ovulatory effect of seminal plasma was startlingly clear. A single intramuscular dose of seminal plasma (representing <1/4 of an ejaculate) of alpacas and llamas induced ovulation in >90% of females of the respective species (Table 1; Adams et al., 2005). The discovery of OIF in seminal plasma was made in species categorized as induced ovulators since factors influencing the occurrence of ovulation can be studied without the confounding effects of spontaneous ovulation. However, it appears that OIF in seminal plasma is conserved among both induced and spontaneously ovulating species (discussed below).

3. Effects of OIF and route of action

The effects of intramuscular and intrauterine administration of seminal plasma were examined in a series of experiments in alpacas and llamas to document the role of seminal plasma on ovulation in females of the same species and to determine the route of action; i.e., local versus systemic (Adams et al., 2005; Ratto et al., 2005; Table 1). In all experiments, treatment was given when a growing follicle $\geq$8 mm was detected; i.e., ovulatory capability existed. Collectively over 4 separate experiments, intramuscular administration of seminal plasma (equivalent to <1/4 of an ejaculate) resulted in ovulation in 33/35 (94%) females compared to 0/35 (0%) given saline. Intrauterine administration of seminal plasma resulted in ovulation 17/44 (39%) females compared to 0/42 (0%) females given saline (Table 1). By ultrasonographic examination every 4 h, ovulations were detected 29.3 ± 0.7 h after treatment with seminal plasma (Adams et al., 2005), similar to the

Table 1
Effect of seminal plasma administered intramuscularly or by intrauterine infusion with or without endometrial curettage on ovulation.

Ovulation rate	Intramuscular		Intrauterine		Intrauterine with curettage	
	Seminal plasma	Saline	Seminal plasma	Saline	Seminal plasma	Saline
Alpacas (Adams et al., 2005 – OIF)	13/14[a] (93%)	0/14[b] (0%)	0/12[b] (0%)	0/12[b] (0%)	–	–
Alpacas (Ratto et al., 2005)	14/15[a] (93%)	0/15[c] (0%)	7/17[b] (41%)	0/15[c] (0%)	10/15[ab] (67%)	0/15[c] (0%)
Llamas (Adams et al., 2005–OIF)	6/6[a] (100%)	0/6[b] (0%)	–	–	–	–
Total	33/35[a] (94%)	0/35[d] (0%)	7/29[b] (24%)	0/27[d] (0%)	10/15[c] (67%)	0/15[d] (0%)

Adapted from Adams et al. (2005), Ratto et al. (2005).
[a,b,c,d]Within rows, proportions with different superscripts are different ($P < 0.05$).

interval after natural mating or treatment with GnRH or LH (30.0 ± 0.5, 29.3 ± 0.6, 29.3 ± 0.7 h, respectively; Ratto et al., 2006a).

An unexpected finding in the initial study (Adams et al., 2005), however, was that 0/12 alpacas ovulated after being given seminal plasma by transcervical intrauterine deposition. This led to the subsequent study to test the hypothesis that differences are due to attenuated absorption of OIF from the genital mucosa compared to intramuscular administration (Ratto et al., 2005). In this regard, copulation in alpacas and llamas is a prolonged event (30–50 min; Bravo et al., 1990; San-Martin et al., 1968) and ejaculation is intrauterine (Bravo et al., 1996). A normal sequela of copulation in these species is acute, transient inflammation of the endometrium as a result of repeated abrasion by the penis (Bravo et al., 1996). Collectively, results are consistent with the hypothesis that OIF exerts its effect via a systemic rather than a local route and that endometrial curettage enhances the ovulatory response to intrauterine deposition of seminal plasma in alpacas (Table 1). Ovulation rate was highest after intramuscular administration of seminal plasma, intermediate after intrauterine treatment with endometrial curettage, and lowest after intrauterine administration without curettage (93%, 67%, 24%, respectively; Table 1). We interpret these findings to suggest that under natural conditions, absorption of OIF in seminal plasma subsequent to natural mating is facilitated by the hyperemia of the excoriated endometrium.

To determine if ovulation induced by treatment with seminal plasma is associated with a preovulatory surge in circulating concentrations of LH, blood samples were collected frequently from female llamas for 8 h after treatment (Adams et al., 2005; Fig. 1). The timing of the LH surge in response to seminal plasma treatment was similar to that reported after natural mating (i.e., it began within 30 min of treatment and was maximal by 2 h; Bravo et al., 1990, 1991) and consistent with that reported in Bactrian camels (Xu et al., 1985). Interestingly, the duration of the LH surge was prolonged after treatment with seminal plasma compared to GnRH treatment; LH concentrations had not yet returned to basal levels by 8 h (Fig. 1).

The observed effects of seminal plasma on the function of the ensuing corpus luteum (CL) are equally surprising and no less significant than the effects on ovulation. The CL that developed after ovulation induced by seminal plasma treatment tended to be larger and regress later than CL resulting from GnRH-induced ovulation, and produced more than twice as much progesterone (Fig. 2; Adams et al., 2005). The positive relationship between the magnitude of LH release and subsequent luteal form and function in females treated with seminal plasma vs GnRH provides rationale for the hypothesis that the luteotrophic effect of OIF in seminal plasma is mediated by LH. The LH-releasing and luteotrophic effects of seminal plasma have been confirmed in 3 subsequent studies using OIF isolated and purified from the seminal plasma of llamas (Figs. 3, 4 and 5; Ratto et al., 2011; Silva et al., 2011a; Tanco et al., 2011).

4. Biochemical isolation and purification

Attempts have been made to isolate and purify OIF in camel seminal plasma using a combination of anion exchange and hydrophobic chromatography (Li and Zhao, 2004; Pan et al., 2001; Xilong and Zhao, 2004; Zhao et al., 2001); however, interpretation of the results is limited because of the lack of a validated bioassay to quantitatively test the effects of various fractions. Authors of one of the studies (Pan et al., 2001) suggested that OIF consists of a large folded complex of glycoprotein layers with bioactive forms composed of different molecules ranging from 16 to 54 kDa. Authors of another (Xilong and Zhao, 2004) suggested that at least 2 fractions of camel seminal plasma were able to elicit LH secretion from in vitro culture of rat pituitary cells. The initial supposition that OIF is related to the GnRH peptide is reasonable based on LH-releasing effects on pituitary cells and the presence of GnRH immuno-reactivity in human seminal plasma (Izumi et al., 1985; Sokol et al., 1985). However, the addition of GnRH antibodies to in vitro rat pituitary cell culture did not block the LH-releasing effect of alpaca seminal plasma (Paolicchi et al., 1999), suggesting that OIF has a different chemical structure than GnRH.

Using a systematic approach to ablate the bioactivity of seminal plasma, three experiments were conducted involving (1) molecular mass cut-off filtration, (2) treatment with proteinase K, charcoal, or heat, and (3) treatment with

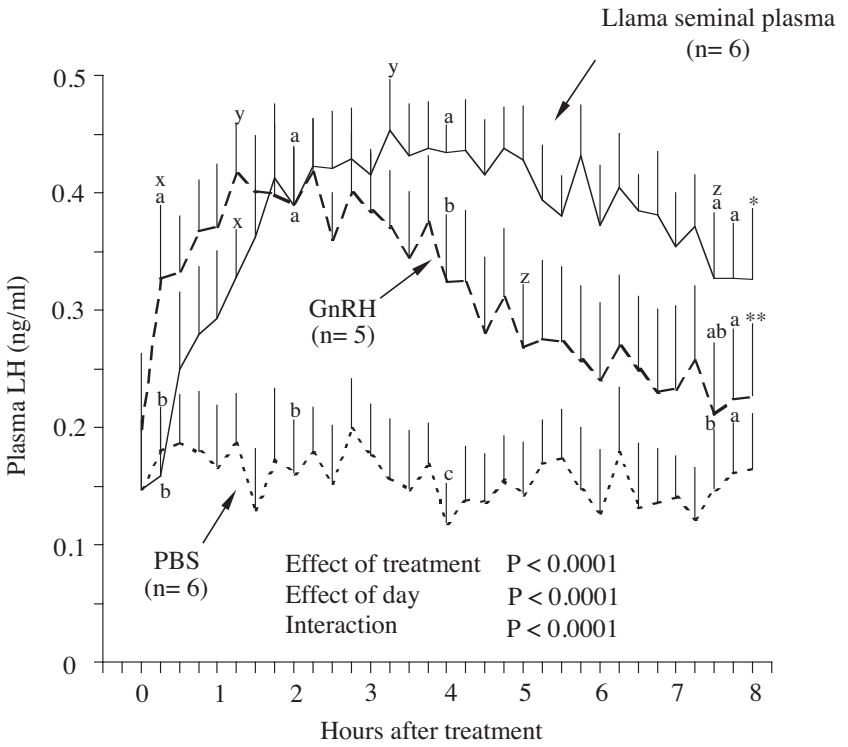

Fig. 1. Plasma LH concentrations (mean + SEM) in female llamas after intramuscular treatment with llama seminal plasma, GnRH or phosphate buffered saline (PBS; from Adams et al., 2005). [abc]On a given day, values with no common superscript are different among groups ($P < 0.05$). [x]Within group, the first increase from pre-treatment (Time 0) concentration ($P < 0.05$). [y]Within group, the maximum concentration ($P < 0.05$). [z]Within group, the first decrease from maximum concentration ($P < 0.05$). *Within group, the last value is higher than the pre-treatment value ($P < 0.05$). **Within group, the last value is not different from the pre-treatment value ($P = 0.9$).
From Adams et al. (2005).

pronase E (Ratto et al., 2010). An in vivo llama ovulation bioassay was used to test the various fractions of seminal plasma produced by the treatments. Results document that OIF is not a steroid, prostaglandin, or GnRH; it is a protein molecule that is resistant to heat and enzymatic digestion with proteinase K, and has a molecular mass of more than about 30 kDa (Ratto et al., 2010; Table 2).

In a follow-up study (Ratto et al., 2011), protein fractions of llama seminal plasma were isolated and purified using liquid chromatography, and tested for ovulation-inducing bioactivity using the in vivo llama ovulation bioassay. Three protein fractions were identified clearly using hydroxylapatite column chromatography (Fractions A, B, and C). A prominent protein band with a mass of 14 kDa was identified by SDS PAGE of Fraction C. Fraction C was loaded into a sephacryl gel filtration column for further purification using fast protein liquid chromatography, resulting in 2 distinct sub-fractions, C_1 and C_2, of which the latter was more prominent. The purified protein (Fraction C_2) elicited a preovulatory LH surge (Fig. 3) followed by ovulation and corpus luteum formation in llamas after intramuscular administration (Table 3, Fig. 4).

Interestingly, the molecular mass of the protein isolated in the follow-up study (based on the band pattern on denatured SDS PAGE) represents about half that found in the previous study in which only the seminal plasma fraction >30 kDa (nominal molecular mass cut-off using centrifugal filtration devices) elicited ovulation in llamas.

Table 2
Ovulation rate in llamas treated with different fractions of llama seminal plasma based on molecular mass, or after exposing seminal plasma to various treatments.

Whole seminal plasma	≥30 kDa	10–30 kDa	5–10 kDa	<5 kDa
9/9[a] (100%)	9/9[a] (100%)	0/9[b] (0%)	0/9[b] (0%)	0/9[b] (0%)
Untreated seminal plasma	**Charcoal**	**Heat (65 °C)**	**Proteinase K**	**Pronase E**
16/17[a] (94%)	7/7[a] (100%)	7/7[a] (100%)	7/7[a] (100%)	0/10[b] (0%)

Modified from Ratto et al. (2010).
[a,b]Within rows, values with different superscripts are different ($P < 0.01$).

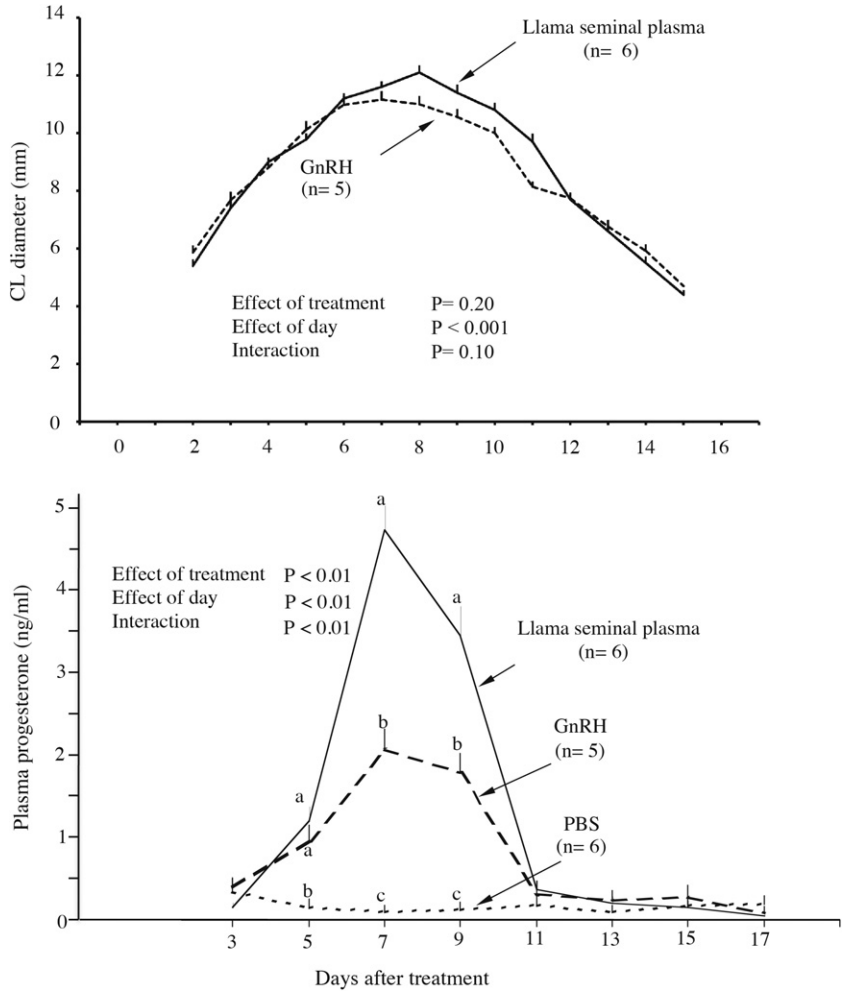

Fig. 2. Corpus luteum diameter and plasma progesterone concentrations (mean + SEM) in female llamas after intramuscular treatment with llama seminal plasma, GnRH, or phosphate buffered saline (PBS). [abc]On a given day, values with no common superscript are different ($P < 0.05$).
Modified from Adams et al. (2005).

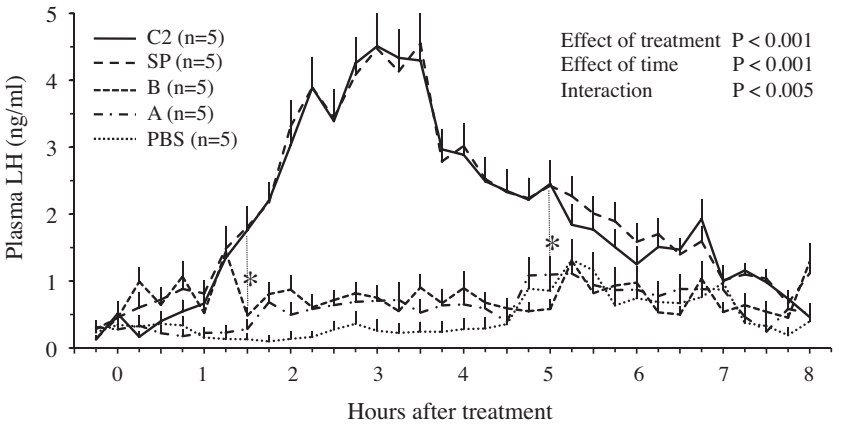

Fig. 3. Effect of different protein fractions of llama seminal plasma on circulating LH concentration in llamas. Female llamas were given whole seminal plasma (SP, positive control), Fractions A or B (isolated by hydroxylapatite column chromatography), Fraction C_2 (isolated by gel filtration chromatography), or phosphate buffered saline (PBS, negative control). *Interval during which values in SP and C_2 were higher ($P < 0.05$) than in other groups (Ratto et al., 2011).

Table 3
Effect of protein fractions of llama seminal plasma, isolated by column chromatography, on ovulation and corpus luteum development in llamas (mean ± SEM).

	Saline	Whole SP	Fraction A	Fraction B	Fraction C_2
Ovulation rate	0/14[a]	14/15[b]	0/14[a]	2/15[a]	14/15[b]
(%)	(0%)	(93%)	(0%)	(13%)	(93%)
Day CL detected (Day 0 = treatment)	–	2.9 ± 0.1[a]	–	2.5 ± 05[ab]	2.1 ± 0.2[b]
Maximum CL diameter (mm)	–	11.0 ± 0.4[a]	–	12.0 ± 1.0[ab]	13.3 ± 0.4[b]
CL diameter on Day 15 (mm)	–	4.9 ± 0.2[a]	–	4.5 ± 0.5[a]	6.4 ± 0.5[b]

Modified from Ratto et al. (2011).
[a,b]Within rows, values with different superscripts are different ($P < 0.01$).

However, partial enzymatic digestion with proteinase K did not ablate bioactivity despite rendering all proteins to ≤19 kDa (Ratto et al., 2010). We interpret these findings to suggest that the 14 kDa protein identified in the latter study is part of a larger protein complex or represents a bioactive pro-hormone form.

5. Dose–response and mechanism of action

In a recent study (Tanco et al., 2011) designed to determine if the dose of purified OIF from llama seminal plasma required to provoke an ovulatory response is physiologically relevant in terms of the proportion present in a normal ejaculate, female llamas were given a single intramuscular dose of 500 μg, 250 μg, 125 μg, or 60 μg of purified OIF (representative of the amount present in 1/25th to 1/200th of a normal ejaculate). A clear dose–response relationship

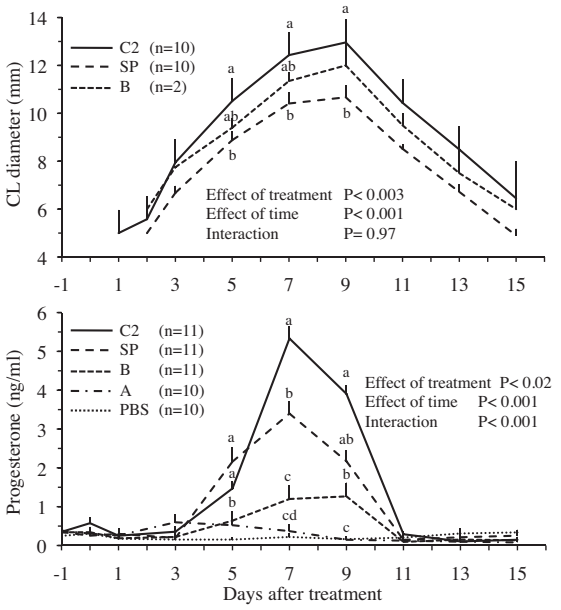

Fig. 4. Effect of protein fractions of llama seminal plasma on CL diameter and plasma progesterone concentrations in llamas. Female llamas were given whole seminal plasma (SP, positive control), Fractions A or B (isolated by hydroxylapatite column chromatography), Fraction C_2 (isolated by gel filtration chromatography), or phosphate buffered saline (PBS, negative control). [abcd]Within days, values with no common superscript are different ($P < 0.05$).
From Ratto et al. (2011).

was observed in circulating LH concentration (Fig. 5), the incidence of ovulation, maximum CL diameter (Table 4), and day-to-day profiles of CL diameter and plasma progesterone concentrations (Fig. 6). We conclude that OIF from seminal plasma has a dose-dependent effect on ovulation and CL form and function, and that the biological effect of OIF is evident at physiologically relevant doses (i.e., as little as 1/100th that present in an ejaculate).

Classically, ovulation in mammals implies pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the medio-basal nuclei of the hypothalamus into the hypophyseal portal system, followed by the release of LH from the anterior pituitary into systemic circulation (Karsch, 1987). Elevated circulating concentrations of LH elicit a cascade of events within the mature follicle culminating in follicle wall rupture and evacuation of its fluid and cellular contents (Richards et al., 2002). While it is clear that the ovulatory effect of OIF in seminal plasma is mediated through a surge release of LH into circulation, it is not clear whether the site of action is at the level of the pituitary, hypothalamus, or both. In a recent study designed to test the hypothesis that OIF elicits LH secretion directly at the level of the pituitary (Bogle et al., 2012), cells from the anterior pituitary of llamas were cultured in vitro and LH concentration was measured in the culture medium after treatment. Treatment with OIF and GnRH induced more LH secretion than untreated controls, and LH concentrations were greater in wells treated with higher doses of OIF or GnRH compared to wells treated with a lower dose. This is consistent with the dose–response effect of OIF observed in vivo (Tanco et al., 2011), and an earlier study in which alpaca seminal plasma stimulated LH secretion from rat anterior pituitary cells in vitro (Paolicchi et al., 1999). Although these observations do not exclude a possible effect at the level of the hypothalamus, they document that OIF has a direct effect on pituitary gonadotrophs independent of hypothalamic input (i.e., GnRH). In an elegant study to determine the site of action on OIF in vivo, pretreatment of llamas with a GnRH antagonist (Cetrorelix) ablated the effects of OIF (i.e., blocked LH release and ovulation), suggesting a direct or indirect effect of OIF on GnRH neurons in the hypothalamus (Silva et al., 2011b).

6. Conserved among species

As previously mentioned, results of recent studies support the hypothesis that OIF in seminal plasma is conserved among species, including those considered to be

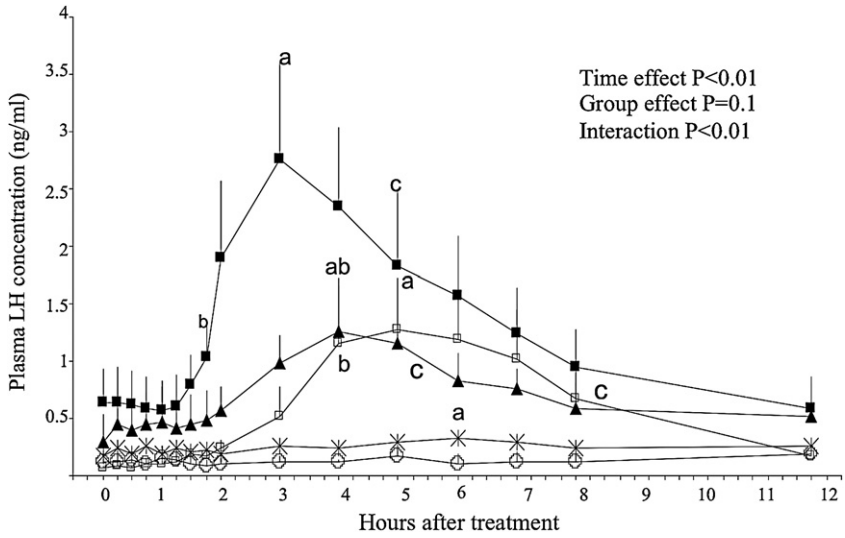

Fig. 5. Plasma LH concentrations (mean ± SEM) in female llamas that ovulated following treatment with OIF (500 μg ■; 250 μg ▲, 125 μg □, 60 μg * and PBS ○). [a]Within group, the maximum concentration ($P < 0.05$). [b]Within group, the first increase ($P < 0.05$). [c]Within group, the first decrease from maximum ($P < 0.05$).
From Tanco et al. (2011).

Table 4
Effect of dose of purified OIF on ovulation and CL development in llamas (mean ± SEM).

Group	Saline	60 μg	125 μg	250 μg	500 μg
Proportion that ovulated	0/10[a] (0%)	3/10[a] (30%)	7/10[b] (70%)	9/10[b] (90%)	9/10[b] (90%)
Day of 1st detection of CL	–	3.3 ± 0.3[a]	2.3 ± 0.2[b]	2.5 ± 0.2[b]	2.1 ± 0.1[b]
Maximum CL diameter (mm)	–	10.9 ± 1.0[a]	11.6 ± 0.7[ab]	10.8 ± 0.7[a]	13.5 ± 0.5[b]
CL diameter on Day 8 (mm)	–	8.5 ± 2.0[a]	11.3 ± 0.8[ab]	10.4 ± 0.7[ab]	12.8 ± 0.6[b]

Modified from Tanco et al. (2011).
[a,b]Within rows, values with different superscripts are different ($P < 0.05$).

spontaneous ovulators (e.g., bovine, equine and porcine; Bogle et al., 2011; Johnston et al., 2004; Ratto et al., 2006b). The broad classification of species as either spontaneous or induced ovulators is based on the type of stimulus responsible for eliciting GnRH release from the hypothalamus (Bakker and Baum, 2000). In spontaneously ovulating species (e.g., human, sheep, goats, cattle, horse, pigs), release of GnRH from the hypothalamus is triggered when, in the absence of progesterone, systemic estradiol concentrations exceed a certain threshold (Chenault et al., 1975; Jaffe and Keys, 1974; Kelly et al., 1988; Knobil, 1980; Turzillo and Nett, 1999). As a consequence of regularly occurring luteolysis and development of one or more estrogen-producing follicles, a preovulatory surge in circulating concentrations of LH occurs at regular intervals. In induced ovulators (e.g., rabbits, ferrets, cats, camelids),

Table 5
Ovulatory effect of treatment with the con-specific and hetero-specific seminal plasma among species.

Ovulation in females	Control		Seminal plasma						
	Neg.	Pos.	Bactrian	Llama	Alpaca	Rabbit	Bull	Stallion	Boar
Bactrian (Chen et al., 1985)	0/7	–	6/8	–	–		3/7	–	0/3
Bactrian (Pan et al., 1992)	–	–	9/10	–	–		1/3	–	–
Alpacas (in Sumar, 1994)	–	–	–	–	6/10		4/11	–	–
Llamas (Ratto et al., 2006)	0/19[a]	–	–	19/19[b]	19/19[b]		5/19[c]	–	–
Llamas (Bogle et al., 2011)	0/16[a]	–	–	18/18[c]	–		–	5/17[b]	3/17[ab]
Cows (Tanco et al., 2012)	1/11	9/11	–	1/11	–		–	–	–
Mice	6/36[a]	31/36[b]	–	28/36[b]	–		–	–	–
Ov. per mouse (Bogle et al., 2011)	6.2 ± 1.3[a]	27.4 ± 2.7[b]		19.2 ± 2.8[c]					
Rabbits	0/5	6/6	–	0/7	–	0/7	–	–	–
Ov. per rabbit	0 ± 0[a]	7.0 ± 0.6[b]		0 ± 0[a]		0 ± 0[a]			
Llamas (Silva et al., 2010)	0/5			4/5		5/5			

[a,b,c]Within rows, values with different superscripts are different ($P < 0.05$).

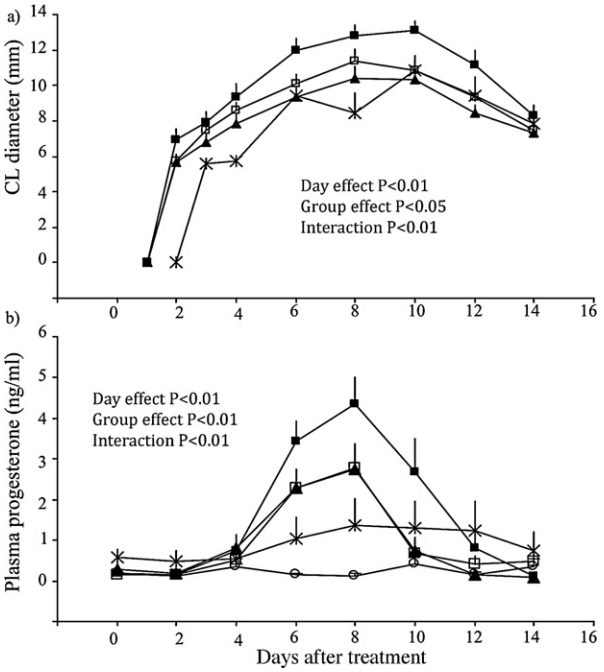

Fig. 6. CL diameter (a) and plasma progesterone concentrations (b) in llamas given a single intramuscular dose of OIF (60 μg *, 125 μg □, 250 μg ▲, 500 μg ■) or PBS (○); $n = 11$ per group (mean ± SEM).
Modified from Tanco et al. (2011).

however, neural signals from copulatory stimulation trigger GnRH secretion from the hypothalamus, followed by the preovulatory release of LH from the pituitary (Bakker and Baum, 2000). Similar to spontaneous ovulators, a surge in the circulating concentration of LH appears to be requisite for ovulation in induced ovulators, but its occurrence is contingent upon copulatory stimuli; hence, ovulation is not a regular cyclic event.

Though numbers were low, results of early studies were suggestive of an ovulation-inducing capability of bovine seminal plasma in Bactrian camels (Chen et al., 1985; Pan et al., 1992) and alpacas (cited in Sumar, 1994; Table 5). In a more recent and controlled study to determine the effect of seminal plasma of con-specific versus hetero-specific males, the ovulation-inducing effect of seminal plasma from alpacas and cattle was compared with that of the llama using female llamas as a bioassay (Ratto et al., 2006a). Ovulation was induced by seminal plasma of all three species, providing rationale for the hypothesis that OIF is a conserved constituent of seminal plasma among mammals, and has an effect on ovarian function in females of unrelated species (Table 5). The existence of OIF has also recently been documented in equine and porcine seminal plasma (Bogle et al., 2011; Table 5), but the incidence of ovulation was lower in llamas treated with seminal plasma from stallions and boars (similar to bull seminal plasma), leading authors to conclude that OIF in the seminal plasma of these species is in lower concentration or is a different and perhaps species-specific isoform. Interestingly, seminal plasma of rabbits (also an induced ovulator) induced ovulation in llamas, but not in rabbits (Silva et al., 2011a; Table 5). Seminal plasma treatment in rabbits was, however, associated with a significant increase in the total number of antral follicles and hemorrhagic anovulatory follicles detected at laparotomy (Silva et al., 2011a).

The corollary to examining the effects of seminal plasma from other species (bull, stallion, boar) on an induced ovulator (llama, Bactrian) is examining the effect of seminal plasma from an induced ovulator on a spontaneous ovulator. To determine the functional role of OIF in spontaneous ovulators, authors of a recent study examined the effects of llama seminal plasma on female mice (Bogle et al., 2011). The experiment involved the use of a superstimulated prepubertal mouse model, and results showed that llama seminal plasma induced not only more mice to ovulate, but more ovulations per mouse than in negative controls, and that the effect was nearly as potent as the positive controls given hCG (Table 5). To test whether purified OIF from llama seminal plasma will induce ovulation in cattle, peri-pubertal heifers were used for the same reason prepubertal mice were used in the previous experiment – to minimize the confounding effect of spontaneous ovulation (Tanco et al., 2012). Contrary to the effect seen in mice, purified OIF did not induce ovulation in heifers (Table 5). It did, however, hasten both the regression of the extant dominant follicle and the emergence of a new follicular wave, suggesting that the role of OIF in spontaneously ovulating species (e.g. Bos taurus) involves controlling follicular wave dynamics through a suppressive effect on the dominant follicle.

7. Conclusion

The findings of studies done in camelids have implications that extend beyond camelid species. Identification of the amino acid sequence and structural form of the OIF protein will be important in developing tools to examine the mechanism of action of OIF, including the tissue source within the male and the tissue targets within the female. Development of tools to measure OIF and OIF receptors will also permit test of the hypothesis that some as yet unexplained causes of infertility are based on alterations in the sensitivity to, or abundance of, this molecule. Recent documentation of the presence of OIF in the seminal plasma of several mammalian species suggests an evolutionary link between species classified as induced or spontaneous ovulators. Further characterization of OIF is needed to determine the relative prevalence and functional role of OIF among species.

Conflict of interest

None.

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