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# Bioactivity of ovulation inducing factor (or nerve growth factor) in bovine seminal plasma and its effects on ovarian function in cattle

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ABSTRACT

To understand the role of ovulation-inducing factor (or nerve growth factor) (OIF [NGF]) in bovine seminal plasma, we (1) used an *in vivo* llama bioassay to test the hypothesis that bovine seminal plasma induces ovulation and CL development in llamas similar to that of llama seminal plasma when the dose of seminal plasma is adjusted to ovulation-inducing factor content (experiment 1) and (2) determined the effect of bovine seminal plasma on the interval to ovulation and luteal development in heifers (experiment 2). Within species, seminal plasma was pooled (n = 160 bulls, n = 4 llamas), and the volume of seminal plasma used for treatment was adjusted to a total dose of 250 μg of ovulation-inducing factor. In experiment 1, mature female llamas were assigned randomly to four groups and treated intramuscularly with either 10 mL of PBS (negative control, n = 5), 50-μg GnRH (positive control, n = 5), 6-mL of llama seminal plasma (n = 6), or 12 mL of bull seminal plasma (n = 6). Ovulation and CL development were monitored by transrectal ultrasonography. In experiment 2, beef heifers were given a luteolytic dose of prostaglandin followed by 25-mg porcine LH (pLH) 12 hours later to induce ovulation. Heifers were assigned randomly to three groups and given 12 mL bovine seminal plasma intramuscularly 12 hours after pLH treatment (n = 10), within 4 hours after ovulation (n = 9), or no treatment (control, n = 10). Ovulation was monitored by ultrasonography every 4 hours, and the CL development was monitored daily until the next ovulation. In experiment 1, ovulation was detected in 0/5, 4/5, 4/6, 4/6 llamas in the PBS, GnRH, llama seminal plasma, and bovine seminal plasma groups, respectively ($P < 0.05$). Luteal development was not different among groups. In experiment 2, the interval to ovulation was more synchronous (range: 4 vs. 22 hours; $P < 0.0001$) in heifers treated with seminal plasma before ovulation compared with the other groups. Luteal development was not different among groups; however, plasma progesterone concentrations tended to be greater in the postovulation treatment group compared with other groups. In summary, results confirmed the presence of bioactive ovulation-inducing factor in bull seminal plasma and supported the hypothesis that bovine and llama seminal plasma have similar ovulatory effects, using a llama bioassay. Treatment with bovine seminal plasma resulted in greater synchrony of ovulation in heifers pretreated with pLH. Plasma progesterone concentration tended to be higher in heifers given bovine seminal plasma within 4 hours after ovulation, suggesting that bovine ovulation-inducing factor is luteotrophic.



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## 1. Introduction

The presence of an ovulation-inducing factor has been documented in the seminal plasma of induced ovulators such as llamas, alpacas [1], and Bactrian camels [2]. This substance is a protein that provokes ovulation by triggering LH release and has luteotrophic properties in llamas and alpacas [3]. The results of recent studies provide evidence for the presence of ovulation-inducing factor (OIF) in the seminal plasma of spontaneous ovulators such as cattle [1], horses, and pigs [4]. In a previous study [1], bovine seminal plasma induced ovulation in 26% (5 of 19) of llamas compared with 0% (0 of 19) in the placebo-treated group, but proportionately less than llamas treated with alpaca or llama seminal plasma (19 of 19, 100%). Treatments, however, were based on the volume of seminal plasma; the actual dose of OIF was unknown. In a more recent study [5], OIF from llama seminal plasma had a dose-dependent effect on ovulation rate, CL diameter, and progesterone production in llamas. Recently, OIF in seminal plasma has been shown to be identical to nerve growth factor β (βNGF) [6] and will be hereafter referred to as OIF (NGF).

The role of seminal plasma as the trigger for ovulation has been documented in some induced ovulators; i.e., camelids [3,7–9] and koalas [10]. Although spontaneous ovulators do not require a copulatory stimulus to ovulate, the importance of seminal plasma and its components has been investigated. In pigs, intrauterine infusion of seminal plasma near the onset of estrus advanced ovulations [11]. In another study in pigs, no effect on ovulation was detected, but an increase in CL size and progesterone secretion was documented after intrauterine infusion of porcine seminal plasma [12]. Although uterine exposure to seminal plasma in mice did not alter CL development or progesterone secretion in one study [13], intramuscular treatment of prepubertal mice with llama seminal plasma induced ovulation at a rate similar to treatment with GnRH [4]. In an early study in dairy cattle, ovulations occurred earlier in heifers mated to a vasectomized bull compared with those not mated [14]. Authors of a more recent study concluded that intracervical deposition of seminal plasma improved pregnancy rates marginally in cows with compromised fertility (i.e., conception rates below 50%) [15]. Although pure llama OIF (NGF) did not induce ovulation in prepubertal heifers, it was associated with a rise in systemic FSH concentrations and early follicular wave emergence [16]. In the same study, OIF (NGF) treatment was also associated with modifications in follicular dynamics and apparent luteotrophic effects in sexually mature heifers [16].

The objectives of the present study were to test the bioactivity of OIF (NGF) present in bovine seminal plasma and determine its effect on ovulation and luteal development in cattle. In experiment 1, we tested the hypothesis that bovine seminal plasma induces ovulation and CL development in llamas in a manner similar to that of llama seminal plasma when the dose of seminal plasma is adjusted to the concentration of OIF (NGF). In experiment 2, we tested the hypothesis that OIF (NGF) in bovine seminal plasma enhances LH-induced ovulation and causes luteotrophic changes in the ovulatory follicle; i.e., enhances the form and function of the incipient CL.

## 2. Materials and methods

### 2.1. Experiment 1

#### 2.1.1. Seminal plasma

Ejaculates were collected from four mature male llamas (5–7 years old) two to three times a week over a period of 2 months using an artificial vagina inserted into a wooden phantom [17]. Semen samples were processed according to procedures previously described [3]. Briefly, the ejaculates were diluted 1:1 (v:v) with PBS (Invitrogen, Grand Island, NY, USA), drawn back-and-forth through an 18-ga needle attached to a 10-mL syringe to reduce viscosity, and centrifuged at 1500× *g* for 30 minutes. The supernatant was transferred to a new tube, and the pellet was discarded. A drop of the supernatant was evaluated by microscopy to confirm the absence of cells. If spermatozoa were detected, the sample was centrifuged and evaluated again until no spermatozoa were detected. The seminal plasma was stored at −80 °C.

Ejaculates (2–4 mL each) were collected from 160 bulls by electroejaculation (Pulsator III; Lane Manufacturing, Denver, CO, USA) using a 75-mm diameter rectal probe with three ventrally oriented electrodes. Ejaculates collected on the same day (n = 10–50) were pooled and kept at 4 °C until transport to the laboratory. The pooled semen was centrifuged for 15 minutes successively at 500, 1000, and 1500× *g*. After each centrifugation, the supernatant was transferred to a new tube, and the pellet was discarded. A drop of seminal plasma was examined by microscopy to confirm the absence of spermatozoa. If cells were detected, the sample was centrifuged again. The seminal plasma was stored at −80 °C. To obtain a sufficient volume for treatments, pools of seminal plasma from different days were thawed and combined to form a single large pool of bovine seminal plasma which was filtered through a 0.22-μm syringe filter (Millipore, Bradford, MA, USA). The filtered seminal plasma was stored frozen at −80 °C.

Total protein was estimated in two aliquots of each of the pooled llama and bovine seminal plasma by spectrometry (Bradford method; Bio-Rad Laboratories, Philadelphia, PA, USA). The concentration of OIF (NGF) was estimated in two aliquots of each of the pooled llama and bovine seminal plasma using a double-antibody RIA [18]. A standard curve was made using known concentrations of purified OIF (NGF) of 0, 1, 10, 25, 50, and 100 ng/mL in PBS. Purified OIF (NGF) was iodinated with iodine-125. The first antibody used in the double-antibody RIA was rabbit polyclonal against the NGF (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The second antibody was goat anti-rabbit. The minimum detectable limit of the assay was 10 ng/mL. Samples were analyzed in one assay, and low- and high-reference samples (1 and 200 ng/mL) were distributed throughout the assay. The intra-assay coefficients of variation were 1.1% and 3.5% for low and high reference concentrations, respectively.

#### 2.1.2. Animals, treatments, and ultrasonography

Mature, nonlactating female llamas (n = 30), 4 years or elder of age and weighing between 90 and 120 kg were