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Identification of the ovulation-inducing factor in alpaca seminal plasma

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The viscous nature of camelid seminal plasma hinders the handling and dilution of semen, but its composition is poorly understood. To date, non-specific enzymes have been used in various camelid species to assist with liquefying seminal plasma, but these enzymes have detrimental effects on sperm function and ultimately fertilisation ability due to their non-selective digestion of sperm and seminal plasma proteins (Morton et al., 2008). Dilution or removal of seminal plasma in other domestic livestock species may also improve or reduce sperm function (Kershaw-Young and Maxwell, 2011). Moreover, timing of artificial insemination in relation to induction of ovulation is also poorly defined. Therefore, the commercial viability of artificial insemination in alpacas, with or without cryopreservation, depends on a better understanding of the components and functions of seminal plasma.

During the separation and identification of camelid seminal plasma components, some major proteins have been isolated including enzymes, growth factors and an ovulation-inducing factor (OIF; Adams et al., 2005; Xilong et al., 2004). When OIF extracted from camelid seminal plasma is injected into camelid females with an ovarian follicle considered capable of ovulation, there is an LH surge that peaks 2-4 hours after administration and lasts approximately 6-8 hours. Ovulation is induced approximately 30 hours after treatment in a majority of females, and CL formation is first detected 2-3 days after treatment (Adams et al. 2005; Tanco et al., 2011).

Ovulation-inducing factor has also been isolated from other induced-ovulating species such as the rabbit and spontaneous ovulators such as cattle, horses and pigs indicating that it is a conserved constituent of mammalian seminal plasma (Bogle, et al., 2011; Silva et al., 2011).

Using one-dimensional gel electrophoresis, a 14 kDa protein (under reducing conditions) appears abundantly in seminal plasma (Kershaw-Young et al., 2012; Ratto et al., 2011). It has been isolated and identified as \( \beta \)-nerve growth factor by liquid chromatography.
mass spectrometry, and shown to induce ovulation in female alpacas in a similar manner to the GnRH analogue buserelin and seminal plasma (Table 1; Kershaw-Young et al., 2012).

**Table 1:** Follicle diameter before treatment, and corpus luteum (CL) diameter and plasma progesterone concentrations on Day 8 after treatment in female alpacas injected i.m. with 1 mL 0.9 % saline, 4 μg buserelin, 2 mL alpaca seminal plasma or 1 mg human β-nerve growth factor (β-NGF) in 1 mL 0.9 % saline (mean ± SEM; adapted from Kershaw-Young et al., 2012).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Follicle diameter (mm)</th>
<th>Number of females ovulating</th>
<th>CL diameter (mm)</th>
<th>Progesterone* (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>8.0 ± 0.6a</td>
<td>0/5</td>
<td>None present</td>
<td>0.12 ± 0.01a</td>
</tr>
<tr>
<td>Buserelin</td>
<td>8.8 ± 0.7a</td>
<td>4/5</td>
<td>9.3 ± 1.5a</td>
<td>4.01 ± 0.90b</td>
</tr>
<tr>
<td>Seminal plasma</td>
<td>8.2 ± 0.8a</td>
<td>4/5</td>
<td>9.3 ± 1.3a</td>
<td>2.44 ± 0.72b</td>
</tr>
<tr>
<td>β-NGF</td>
<td>8.0 ± 1.2a</td>
<td>4/5</td>
<td>10.3 ± 1.0a</td>
<td>3.28 ± 0.68b</td>
</tr>
</tbody>
</table>

*a,b* Values within a column with different superscript letters differ significantly (P < 0.05).

*Mean values for plasma progesterone concentrations do not include data from animals that did not ovulate, except in the case of saline-treated animals.

β-nerve growth factor is the most abundant protein in seminal plasma (Kershaw-Young et al., 2012), a finding that correlates well with findings on ovulation-inducing factor. On average, a llama ejaculate contains approximately 12 mg of ovulation-inducing factor (Tanco et al., 2011), the total protein content of alpaca seminal plasma is 40 mg/mL (Garnica et al., 1993) and if an average alpaca ejaculate is 1 mL (Vaughan et al., 2003), then the ovulation-inducing factor contributes 30 % of the total protein in camelid seminal plasma.

Nerve growth factors have been identified in the seminal plasma of camelid and non-camelid species, other than alpacas, and findings suggest that the source of β-nerve growth factor is likely to be from the accessory sex glands rather than the testes, and that β-nerve growth factor is likely to work at the level of the hypothalamo-pituitary axis, inducing ovulation by stimulating the secretion of LH (Kershaw-Young et al., 2012).

The identification of β-nerve growth factor as an abundant protein in alpaca seminal plasma, that induces ovulation in alpacas may assist with the development of protocols to induce ovulation.
References


