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Caprine luteinizing hormone isoforms during the follicular phase and anestrus

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Abstract

The relative proportion of the circulating luteinizing hormone isoforms in goats during follicular phase (pre-ovulatory peak; F) and anestrus (A) was investigated. Estrus was synchronized in six goats with a prostaglandin analogue. After estrus was detected, blood samples were taken at 1 h intervals for 24 h. Four anestrous goats received 100 μg i.v. of GnRH and blood samples were collected every 15 min for 5 h. Samples with the greatest LH concentration in follicular phase and after GnRH administration (anestrus) were analyzed by chromatofocusing and eluted with a pH gradient from 10.5 to 3.5. For quantification purposes eluted LH was grouped into basic (pH ≥ 7.5), neutral (pH 7.4–6.5) and acidic isoforms (pH ≤ 6.4) as well as by pH unit. In both physiological conditions (PC), basic and acidic isoforms were greater than the neutral. With this grouping criteria, there was an interaction between PC and pH group, with the proportion of neutral isoforms being greater ($p < 0.05$) in A ($12.0 \pm 0.8\%$) as compared with F ($5 \pm 2\%$). Analysis by pH unit showed a very basic group of eluted isoforms (pH ≥ 10), which amounted to a percentage of $6.0 \pm 0.4\%$ of the total observed during A, and $3 \pm 1\%$ during F ($p < 0.05$). Predominant isoforms in A eluted in the pH range 9.99–9.0 ($42 \pm 3\%$) as compared to $7 \pm 3\%$ ($p < 0.01$) in that pH range in F. In contrast, the predominant isoforms in F eluted in the pH range 8.99–8.0, representing $55 \pm 8\%$, while in A the proportion was $11 \pm 2\%$ ($p < 0.01$). Isoforms eluted at the pH range 7.9–7 represented a significantly greater proportion during A ($5.0 \pm 0.6\%$) as compared with F ($3 \pm 1\%$). This is the first report on goat LH circulating isoforms. During A the LH isoforms secreted by the pituitary are more basic than during F.

Keywords: Heterogeneity; Gonadotropin-releasing hormone; Seasonal reproduction; Secretion

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1. Introduction

The secretion pattern of luteinizing hormone (LH) changes in accordance with the different reproductive stages (Chemineau et al., 1988; Tanaka et al., 1995). During the follicular phase, LH pulse frequency increases in response to increments of circulating estradiol (Caraty et al., 1989; Caraty and Skinner, 1999). In anestrus, LH secretion is reduced as a response to the increment in sensitivity of the hypothalamic–hypophyseal axis to the negative feedback imposed by estradiol (Legan and Karsch, 1979).

Luteinizing hormone is a heterogeneous glycoprotein due to the structure of its oligosaccharides (Combarnous, 1988; Baenzinger and Green, 1988; Manzella et al., 1996). This heterogeneity is reflected in its physicochemical properties and its biological and immunological activity (Burgon et al., 1996; Creus et al., 2001; Barrios-De-Tomasi et al., 2002; Mi et al., 2002; Perera et al., 2004).

Luteinizing hormone heterogeneity has been studied in ovine (Keel et al., 1987; Zalesky et al., 1992, 1993; Padmanabhan et al., 1992; Hassing et al., 1993; Christianson et al., 1998; Arrieta et al., 2006), bovine (Stumpf et al., 1992; Kojima et al., 1995; Cupp et al., 1995; Perera et al., 2004), caprine (Perera et al., 1996) pituitaries, and recently in bovine (Perera-Marín et al., 2005) and ovine serum (Arrieta et al., 2006).

The relative proportion of each isoform group depends on the physiological status (Ulloa-Aguirre et al., 1995, 1999; Cooke et al., 1996, 1997; Padmanabhan et al., 1998). For example, an endocrine environment with high concentrations of circulating estradiol (Stumpf et al., 1992; Kojima et al., 1995; Cooke et al., 1997) is correlated with a higher percentage of acidic LH isoforms and basic FSH isoforms in the pituitary gland and serum. In contrast, the decrease in circulating estradiol concentration (Kojima et al., 1995; Christianson et al., 1998) and increased of the progesterone (Perera-Marín et al., 2005; Arrieta et al., 2006); after gonadectomy (Christianson et al., 1998; Kojima et al., 1995) and an inhibition with GnRH antagonists (Zalesky et al., 1993), favors an increase in the percentage of more basic LH isoforms and more acidic FSH isoforms in pituitary and serum. These findings indicate that gonadal factors and GnRH participate in the regulation of the LH isoforms distribution pattern.

Most studies on LH heterogeneity in domestic animals relate to sheep and cattle; in goats, however, the information is limited to the obtention and purification of the two adenohyphophysis proteins with heterogeneous charge, as well as biological and immunological LH activity (Perera et al., 1996), but the regulation of heterogeneity in circulation has not been studied in this specie.

The aim of the present study was to examine the proportion relative of circulating LH isoforms in goats, during different stages of the ovarian cycle.

2. Materials and methods

2.1. Experimental protocol and serum collection

The study was carried out at an experimental station of the Facultad de Medicina Veterinaria y Zootecnia of the Universidad Nacional Autónoma de México, located in the central highland of México, at 1800 m, with a semi-dry temperature climate.

Group F: Six cycling crossbred goats (during October, confirmed by serum progesterone concentrations >1 ng/ml and ultrasonography) of age between 2 and 6 years, mean ± S.D. weight 45 ± 5 kg and a body condition score of 3.0 (Delavaud et al., 2000) were used. Estrus was synchronized using two intramuscular injections 0.075 mg prostaglandin (PG) analogue (Preloban, Intervet, México, Santiago Tianguistenco, México; 0.075 mg/ml prostanglandin) 9 days apart.