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SPONTANEOUSLY ARISING DISEASE

Altered Expression of Anti-Müllerian Hormone during the Early Stage of Bovine Persistent Ovarian Follicles

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Summary

Anti-Müllerian hormone (AMH) is a homodimeric glycoprotein expressed exclusively in the gonads. This hormone is an important regulator of the early growth of follicles through inhibitory effects on the recruitment of primordial follicles into the pool of growing follicles and on granulosa cell proliferation. Cystic ovarian disease (COD) is an important disorder affecting the fertility of dairy cattle. In the present study, we evaluated the expression of AMH in granulosa cells and AMH secretion into follicular fluid in pre-ovulatory follicles from control cows, animals with spontaneously arising COD and during the development of the disease, at 5, 10 and 15 days of follicular persistence. To this end, after an oestrous synchronization protocol, low doses of progesterone was administered for 5, 10 and 15 days after the expected day of ovulation (day 0 of follicular persistence) in treated cows (groups P5, P10 and P15, respectively), using an intravaginal progesterone-releasing device. Results showed a decrease in the expression of AMH in granulosa cells throughout folliculogenesis ($P < 0.05$) and in the spontaneously arising follicular cysts and persistent follicles related to the control group ($P < 0.05$). There was also a higher concentration of AMH in the follicular fluid of persistent follicles at 10 and 15 days of follicular persistence ($P < 0.05$). Together, these results may indicate an alteration in AMH expression and secretion, which occurs early in folliculogenesis and incipiently during the development of COD, and which could contribute to the recurrence of this disease in cattle.

Keywords: anti-Müllerian hormone; cow; cystic ovarian disease; follicular persistence

Introduction

Cystic ovarian disease (COD) is an important disorder affecting the fertility of dairy cattle. It is characterized by follicles that fail to ovulate, persist for more than 6 days in the ovary and achieve a diameter of at least 20 mm (larger than the diameter of an ovulatory follicle), in the absence of a corpus luteum, with lack of uterine tonicity and interruption of the normal oestrous cycles (Silvia *et al.*, 2002; Bartolomé *et al.*, 2005; Ortega *et al.*, 2015). The incidence of COD ranges from 5% to 30%, depending on the herd where the problem appears. Although the main component of the aetiopathogenesis of COD is failure in the hypothalamic–pituitary–ovarian axis, there are important intraovarian components involved in the follicular persistence associated with the lack of ovulation (Matiller *et al.*, 2014; Ortega *et al.*, 2015, 2016).

At present, numerous ovarian extracellular cytokines have been identified and implicated in the autocrine/paracrine bidirectional communication between the oocyte and its surrounding somatic cells, as well as in the regulation of follicle survival, development and

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apoptosis. Members of the transforming growth factor-β superfamily are fundamental factors implicated in this dialogue (Knight and Glister, 2006; Field *et al*., 2014). One of the important members of this family is anti-Müllerian hormone (AMH), a homodimeric glycoprotein of 140 KDa expressed exclusively in the gonads. This hormone is an important factor in male sex differentiation, produced by Sertoli cells of the testis from fetal life until puberty, which promotes regression of Müllerian ducts during the differentiation of the male reproductive tract (Josso *et al*., 2001; Knight and Glister, 2006). In females, AMH is expressed in the granulosa cells of non-atretic pre-antral and small antral follicles and its expression is less evident in large antral and atretic follicles (Ueno *et al*., 1989; Durlinger *et al*., 1999). In the ovary, AMH has inhibitory effects on granulosa cell proliferation (Kim *et al*., 1992; Seifer *et al*., 1993), aromatase activity and luteinizing hormone (LH) receptor expression (di Clemente *et al*., 1994). AMH is also an important regulator of the early growth of follicles through inhibitory effects on the recruitment of primordial follicles into the pool of growing follicles (Durlinger *et al*., 1999, 2002a). Furthermore, AMH reduces the sensitivity of large pre-antral follicles and small antral follicles to follicle stimulating hormone (FSH) (Durlinger *et al*., 2001; Gruijters *et al*., 2003). In this way, the AMH secreted by pre-antral and small antral follicles is able to control the recruitment of primordial follicles and the number of follicles that can reach the pre-ovulatory stage (de Vet *et al*., 2002; Gruijters *et al*., 2003; Visser and Themmen, 2005).

In cows, AMH is used as an endocrine marker of the small antral gonadotropin responsive follicle reserve (Rico *et al*., 2009, 2011). Studies carried out in cows with COD have shown plasma and follicular fluid AMH concentrations similar to those of cows with normal ovarian cycles (Monniaux *et al*., 2008; Kitahara *et al*., 2012; El-Sheikh Ali *et al*., 2013).

Considering the multiple functions of AMH in ovarian physiology and taking into account the multiple alterations at ovarian level that exist in the animals with follicular persistence and cystic ovarian disease, we hypothesize that there is an alteration in the expression of this hormone that could affect these processes. The aim of the present study was to examine the ovarian expression and follicular fluid concentrations of AMH in cows with spontaneously arising COD and cows with ovarian follicles with 5, 10 and 15 days of persistence, developed in response to long-term administration of progesterone.

Materials and Methods

Animals

All the procedures were approved by the institutional ethics and security committee of Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Argentina (protocol numbers 44/10 and 131/12), and are consistent with the 'Guide for the Care and Use of Agricultural Animals in Research and Teaching' (2010). This study was performed in Argentinean Holstein cows with regular oestrous cycles, all of which had calved at least once. For the experimental protocol, the animals were obtained at the end of lactation from local commercial farms and housed outside in an open lot. The cows were fed a diet based on alfalfa pasture, oat or rye grass grazing, supplemented with corn and alfalfa silage, corn grain, soybean expeller and hay, following the recommendations of the Nutrient Requirements of Dairy Cattle (2001). For the spontaneously arising COD group, cows from dairy herds of the milk producing region of Santa Fe, Argentina, were used.

Experimental Model

Ovarian activity was synchronized starting with the procedure commonly referred to as 'G6G' (Bello *et al*., 2006), with some modifications (Díaz *et al*., 2015). Holstein cows with one or more corpora lutea identified by transrectal ovarian ultrasonography were enrolled to start the experiment. The synchronization protocol consisted of two doses of prostaglandin (PG) F2α (0.150 mg D-Cloprostenol, Enzaprost D-C; Biogénesis-Bagó, Argentina) administered 12 h apart on day 0 to induce luteolysis, followed by a dose of gonadotropin-releasing hormone (GnRH; 20 mg buserelin acetate, Gonaxal; Biogénesis-Bagó) 2 days later to stimulate ovulation of the pre-ovulatory follicles present. Six days after the first dose of GnRH, the cows started Ovsynch™ (Pursley *et al*., 1995) with an injection of GnRH (20 mg buserelin acetate, Gonaxal; Biogénesis-Bagó). Seven days later, cows received two doses of PGF2α (0.150 mg D-Cloprostenol, Enzaprost D-C; Biogénesis-Bagó), 12 h apart, to ensure luteolysis (completion of the modified synchronization protocol).

After synchronization, the cows were divided into four groups: control ($n = 10$), P5 (5 days of follicular persistence; $n = 10$), P10 (10 days of follicular persistence; $n = 10$) and P15 (15 days of follicular persistence; $n = 10$). Follicular aspiration was performed in five animals from each group and ovariectomy was carried out in the other five animals (both techniques