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Role of activin, inhibin, and follistatin in the pathogenesis of bovine cystic ovarian disease

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ABSTRACT

Cystic ovarian disease (COD) is an important cause of infertility in dairy cattle. Although many researchers have focused their work on the endocrine changes related to this disease, evidence indicates that intraovarian components play an important role in follicular persistence. Activin, inhibin, and follistatin participate as intraovarian regulatory molecules involved in follicular cell proliferation, differentiation, steroidogenesis, oocyte maturation, and corpus luteum function. Given the importance of these factors in folliculogenesis, we examined the expression and immunolocalization of activin/inhibin βA-subunit, inhibin α-subunit, and follistatin in the ovaries of healthy estrus-synchronized cows and in those of cows with spontaneous or adrenocorticotropic hormone (ACTH)-induced COD. We also studied inhibin B (α βB) levels in serum and follicular fluid. We found an increased expression of the βA-subunit of activin A/inhibin A, the α-subunit of inhibin, and follistatin in granulosa cells of spontaneous follicular cysts by immunohistochemistry, and decreased concentrations of inhibin B (α βB) in the follicular fluid of spontaneous follicular cysts. These results, together with those previously obtained, indicate that the expression of the components of the activin–inhibin–follistatin system is altered. This could lead to multiple alterations in important functions in the ovary like the balance between pro- and anti-apoptotic factors, follicular proliferation/apoptosis, and steroidogenesis, which may contribute to the follicular persistence and endocrine changes found in cattle with COD.

1. Introduction

Cystic ovarian disease (COD) is an important cause of infertility in dairy cattle and has been defined as the presence of one or more follicular structures in the ovaries, of at least 20 mm in diameter, which persist in the absence of luteal tissue, interrupting the normal reproductive cycle (Silvia et al., 2002; Peter, 2004; Vanholder et al., 2006). Many factors such as stress, nutritional management, and infectious diseases can cause COD in cattle. However, the primary cause of this disease has not yet been elucidated. It is accepted that the main component of the ethio-pathogenesis of COD is related to the hypothalamus–pituitary–ovarian axis. However, the

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persistence of the follicles in the absence of ovulation is related to an intraovarian component (Silvia et al., 2002).

Multiple intraovarian factors participate in the autocrine/paracrine signaling between theca interna cells, granulosa cells, and oocytes and contribute to a coordinated program of follicular cell proliferation and differentiation. Particularly in the later stages of follicular development, intraovarian factors also modulate the sensitivity of follicular cells to gonadotropins and other extraovarian factors (Glister et al., 2010). Locally produced regulatory factors include various members of the transforming growth factor beta (TGFB) superfamily, which in turn includes bone morphogenetic proteins, activins, and inhibins (Mihm and Austin, 2002).

Much evidence indicates that activins, follistatin and, to a lesser extent, inhibins synthesized by follicular cells exert local autocrine–paracrine actions to modulate follicular growth, gonadotropin responsiveness, steroidogenesis, oocyte maturation, ovulation, and corpus luteum function (Nishimori and Matzuk, 1996).

Inhibins and activins are composed of three subunits, α, βA, and βB, derived from three different precursor polypeptides encoded by distinct genes (Ying, 1988). The subunits combine to make the different forms of activins and inhibins. The dimerization of the β-subunits gives rise to three forms of activin referred to as activin A (βAβA), activin AB (βAβB), and activin B (βBβB). Dimers of an α-subunit and either a βA- or βB-subunit generate inhibin A (αβA) or inhibin B (α βB) (Knight, 1996; Knight and Glister, 2001, 2003, 2006). Follistatin is a cysteine-rich monomeric glycoprotein encoded by a single gene, structurally unrelated to the TGFB superfamily, but functionally linked through its role as a high-affinity binding protein for activins. Follistatin has several different isoforms due to alternative mRNA splicing and post-translational modifications (Knight, 1996; Knight and Glister, 2001, 2003, 2006).

The genes for both inhibin subunits are expressed in granulosa cells and affect follicle growth and steroidogenesis directly in vivo (Woodruff et al., 1990) and in vitro (Wrathall and Knight, 1995). Activin and inhibin have opposite effects, both at the pituitary and ovarian levels, because both compete for the type II activin receptor (Gray et al., 2001). Activin also enhances FSH receptor expression and inhibin synthesis by granulosa cells and promotes granulosa cell proliferation and steroidogenesis during early follicular development (Xiao et al., 1992). However, activin interaction with its receptors is regulated by follistatin levels. Follistatin specifically neutralizes activin functions in the pituitary and the ovary (Robertson et al., 1987) and has, therefore, inhibin-like activity.

Considering the importance of these factors in folliculogenesis, we hypothesized that an imbalance in the activin–inhibin–follistatin system may result in ovarian alterations such as follicular persistence that could contribute to the pathogenesis of COD. Therefore, in the present study, we examined the immunolocalization and expression of the activin/inhibin βA-subunit, inhibin α-subunit, and follistatin in the ovaries of healthy cows and animals with spontaneous or adrenocorticotropic hormone (ACTH)-induced COD. The concentration of inhibin B in follicular fluid (FF) and serum was measured in controls and animals with spontaneous COD.

2. Materials and methods

2.1. Induction and detection of cysts

All procedures were evaluated and approved by the Institutional Ethics and Security Committee (Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Santa Fe, Argentina; Protocol number: 44/10) and are consistent with the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Federation of Animal Science Societies, 2010).

2.1.1. Induction of COD by adrenocorticotropic administration

Ten nulliparous Argentinean Holstein heifers (18–24 months old; 400–450 kg body weight; maintained under standard husbandry conditions) with regular estrous cycles were used. The stages of the estrous cycles were synchronized using the Ovsynch protocol: the animals were injected with a gonadotropin-releasing hormone (GnRH) analog (Buserelin acetate, Gonaxal®, Biogénesis-Bagó, Buenos Aires, Argentina, 10 μg/animal) on day 9, a Prostaglandin $F_{2\alpha}$ analog (D+ Cloprostenol, Enzaprost D-C®, Biogénesis-Bagó, Argentina, 150 μg/animal) on day 2, and a GnRH analog (Buserelin acetate, Gonaxal®, Biogénesis-Bagó, Buenos Aires, Argentina, 10 μg/animal) on day 0. The time of ovulation was monitored by transrectal ultrasonography and designated as day 1 of the estrous cycle, because ovulation occurs 24–32 h after the second injection of GnRH (Pursley et al., 1995).

The model of ACTH-induced ovarian follicular cysts used in the present study has been previously described and characterized (Dobson et al., 2000; Ortega et al., 2008; Salvetti et al., 2010; Amweg et al., 2013). Briefly, beginning on day 15 of a synchronized estrous cycle, five heifers received subcutaneous injections of 1 mg of a synthetic polypeptide with ACTH activity (Synacthen Depot, Novartis, Basel, Switzerland), every 12 h for 7 days (ACTH-treated group). The other five animals received saline solution (1 ml) (control group).

Ovarian ultrasonographic examinations were performed in all animals, using a real-time, B-mode scanner equipped with a 5 MHz, linear-array, transrectal transducer (Honda HS101V, Japan) (Sirois and Fortune, 1988). The growth and regression of follicles >5 mm, corpora lutea, and follicular cysts were monitored. Daily ovarian ultrasonography was performed throughout a complete estrous cycle in control heifers (21–23 days) and from day 14 (day 0 = day of ovulation) until ovariectomy approximately on day 48 in ACTH-treated heifers. Follicular cysts detected by ultrasonography were defined as any follicular structure with a diameter equal to or greater than 20 mm present for 10 days or more, without ovulation or corpus luteum formation (Dobson et al., 2000). The first day of follicular cyst formation was the day a follicle attained 20 mm or more in diameter and the ovaries were removed 10 days later by flank laparotomy (approximately day 48). In five heifers of the control group, ovariectomy was conducted