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SPONTANEOUSLY ARISING DISEASE

Role of Components of the Insulin-like Growth Factor System in the Early Stages of Ovarian Follicular Persistence in Cattle

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Summary

Cystic ovarian disease (COD) is one of the main causes of infertility in dairy cattle. It has been postulated that the insulin-like growth factor (IGF) system may contribute to follicular persistence and development of COD. The initiation of the IGF response is a result of interactions between IGF-binding proteins (IGFBPs) and IGFBP proteases, mainly pregnancy-associated plasma protein A (PAPP-A). IGFBPs bind IGFs with high affinity and consequently regulate their access to IGF receptors (IGFRs). The aim of this research was to determine variations in components of the IGF system in the ovaries of cows with persistent follicles induced by long-term administration of progesterone. Proteins of the IGF system were evaluated at 0 (expected day of ovulation), 5, 10 and 15 days of follicular persistence to determine whether the changes occur early in the development of COD. The concentrations of IGF1 and IGFBP4 in follicular fluid were similar in all groups with follicular persistence and in control antral follicles. IGFR1 and IGFBP4 expression *in situ* were higher in granulose cells in persistent follicles than in control follicles. No differences were found in PAPP-A concentration within follicular fluid in persistent follicles relative to control antral follicles. These data support the hypothesis that the IGF system is altered in the initial stages of development of follicular persistence and has a determinant role in ovarian function in cattle.

Keywords: cattle; follicular persistence; insulin-like growth factor system; progesterone

Introduction

In recent years, reproductive disorders during the early postpartum period have led to great economic losses (Lucy, 2008). Cystic ovarian disease (COD) is a major disorder contributing to poor reproductive efficiency of lactating dairy cows (Kesler and Garverick, 1982). COD is characterized by the presence of large follicular structures that persist in the ovary for 10 days or more in the absence of a corpus luteum, with interruption of the normal oestrous cycle (Silvia *et al*., 2002; Thomas *et al*., 2007). The most widely accepted hypothesis postulates that COD is the result of a 'hormonal imbalance' within the hypothalamic–pituitary–gonadal axis (Vanholder *et al*., 2006); however, experimental evidence also suggests that follicular persistence may be caused by intra-ovarian components (Ortega *et al*., 2015). In this sense, different physiological alterations, such as a delay in the first ovulation and/or in the pre-ovulatory luteinizing hormone (LH) surge, with alterations in LH pulses, have been demonstrated (Opsomer *et al*., 1999; Díaz *et al*., 2015).

Although the exact mechanism by which COD occurs is not defined, the influence of various metabolic and hormonal factors is suggested. Of these factors, gonadotropins and the insulin-like growth factor (IGF) system have been proposed as key mediators

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of ovarian dysfunction and development of COD (Spicer and Chamberlain, 2000; Vanholder *et al.*, 2006; Thomas *et al.*, 2007; Ortega *et al.*, 2008; Rey *et al.*, 2010; Rodríguez *et al.*, 2011, 2013, 2015). The IGF system is an important regulator of follicular development and selection, cell differentiation, steroidogenesis and oocyte maturation (Giudice, 1992; Spicer and Echternkamp, 1995; Brogan *et al.*, 2010). This system is composed of two ligands (IGF1 and IGF2), specific receptors (IGFR1 and IGFR2), six IGF-binding proteins (IGFBP1 to 6) and IGFBP proteases (Spicer and Echternkamp, 1995; Silva *et al.*, 2009; Sanchez *et al.*, 2014). IGFs are involved in proliferation and follicular development, while IGFBPs are responsible for increasing the average life of these ligands, forming inactive complexes, as well as for transport to the site of action (Monget *et al.*, 2002; Brogan *et al.*, 2010). IGFR1 is mainly involved in the actions of IGF1 (Monget *et al.*, 2002) and IGFBP specific proteases cleave IGFBPs, releasing IGFs (Spicer, 2004; Conover, 2012; Oxvig, 2015).

The main protease detected in cattle is pregnancy-associated plasma protein A (PAPP-A) (Mazerbourg *et al.*, 2001; Spicer, 2004; Conover, 2012). PAPP-A is responsible for cleavage of IGFBP4 in ovarian follicular fluid (FF) (Conover *et al.*, 1999) and may contribute to the bioavailability of free IGF1 for follicle development to the pre-ovulatory phase (Monget *et al.*, 2003; Spicer, 2004; Aad *et al.*, 2009; Sudo *et al.*, 2007).

In previous studies, we observed modifications in the IGF system in cows with COD (Ortega *et al.*, 2008; Rey *et al.*, 2010; Rodríguez *et al.*, 2011, 2013, 2015). Therefore, the aim of the present study was to determine the concentrations of IGF1, IGFBP4 and PAPP-A in FF, and IGFBP4 and IGFR1 protein expression in bovine ovarian follicles at different stages of persistence. Since these members of the IGF system have been suggested to be the main components modified in cows with COD, we aimed to determine the critical moment of altered expression and gain insights into the role of these members of the IGF system in COD pathogenesis, by using a previously optimized model of bovine follicular persistence, proven to be useful to study the early stages of cyst formation in cattle (Díaz *et al.*, 2015, 2016).

Materials and Methods

Ethical Approval

All procedures were carried out according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Federation of Animal Science Societies, 2010) and the protocol was approved by the Ethics and Safety Committee of the Facultad de Ciencias Veterinarias of the Universidad Nacional del Litoral, Santa Fe, Argentina, under protocol number 131/12.

Animals and Experimental Design

The study was performed using non-lactating Holstein cows ($n = 25$) with regular oestrous cycles (Díaz *et al.*, 2015). Ovarian activity was synchronized starting with the procedure commonly referred to as 'G6G' (Bello *et al.*, 2006), with some modifications (Fig. 1; Díaz *et al.*, 2015). Briefly, the synchronization protocol consisted of two doses of prostaglandin F2α (PGF2α; 150 μg D + cloprostenol; Enzaprost DC, Biogénesis-Bagó, Garín, Buenos Aires, Argentina) administered 12 h apart on day 0 to induce luteolysis (Hatler *et al.*, 2008), followed by a dose of GnRH (20 μg buserelin acetate; Gonaxal, Biogénesis-Bagó, Argentina) 2 days later to stimulate ovulation of the pre-ovulatory follicles present. Six days after the first dose of GnRH, the cows were given another injection of GnRH. Seven days later, the cows received two doses of PGF2α, 12 h apart, to ensure luteolysis (completion of the modified synchronization protocol). After synchronization, cows were divided into five groups: control (C; $n = 5$), cows receiving no additional hormonal treatment; P0 group ($n = 5$), cows treated with progesterone from day 1 after the final doses of PGF2α until sampling on the expected day of ovulation; P5 group ($n = 5$), cows with 5 days of follicular persistence after the expected day of ovulation; P10 group ($n = 5$), cows with 10 days of follicular persistence after the expected day of ovulation and; P15 group ($n = 5$), cows with 15 days of follicular persistence after the expected day of ovulation (Fig. 1). To obtain the persistence groups, cows were given a low dose of progesterone by inserting an intravaginal progesterone-releasing device (750 mg of micronized progesterone; Pro-Ciclar P4-Zoovet®; Santa Fe, Argentina) one day after the first PGF2α injection of the Ovsynch protocol (Bello *et al.*, 2006). In the last two groups (P10 and P15), a new intravaginal progesterone-releasing device was inserted 1 day before removal of the first one in order to maintain a more consistent concentration of progesterone throughout the treatment period. In group P15, a third intravaginal progesterone-releasing device was inserted on day 11 of persistence, 1 day before removal of the second one.

Collection and Preparation of Tissues

Bilateral ovariectomy was performed 2 days after completion of the synchronization protocol in control