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Impaired insulin signaling pathway in ovarian follicles of cows with cystic ovarian disease

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ABSTRACT

Cystic ovarian disease (COD) is an important cause of infertility in dairy cattle. Follicular cell steroidogenesis and proliferation in ovulatory follicles is stimulated by hormones such as insulin and its necessary post-receptor response. The aim of this study was to determine the expression of insulin receptor (IR), IR substrate-1 (IRS1) and phosphatidylinositol 3-kinase (PI3K), key intermediates in the insulin pathway, in control cows and cows with spontaneous COD and ACTH-induced COD. *IR* and *IRS1* mRNA levels were greater in granulosa cells and lower in follicular cysts than in control tertiary follicles. *PI3K* mRNA levels were similar in all follicles evaluated, whereas the expression of *IR*, *IRS1* and *PI3K* was similar in theca cells. Protein expression of IR was higher in control tertiary follicles than in the same structures in animals with COD and with cysts. IRS1 and PI3K protein expression showed the same pattern in tertiary and cystic follicles. However, the protein expression of subunit alpha p85 of PI3K was greater in theca cells from tertiary follicles than in cystic follicles. These results provide new insights into the insulin response in cows with COD. The lower gene and protein expressions of some insulin downstream effectors at an early stage of the signaling pathway could negatively influence the functionality of ovaries and contribute to follicle persistence.

1. Introduction

Milk production in dairy cows has increased over the past decades. Unfortunately, infertility associated with metabolic and reproductive disorders during the early postpartum period in high-yielding dairy cows has increased, causing considerable economic loss (Opsomer et al., 1999; Royal et al., 2000; Lucy, 2007). An important cause of infertility in dairy cattle is cystic ovarian disease (COD), which has been defined as the presence of one or more follicular structures of at least 20 mm in diameter in the ovaries, which persist in the absence of luteal tissue, interrupting the normal reproductive cycle (Silvia et al., 2002; Peter, 2004; Vanholder et al., 2006). Cystic ovarian disease pathogenesis is not fully understood, mainly because different factors contribute to cyst formation in bovines (Vanholder et al., 2006). However, a hypothalamic unresponsiveness to estradiol appears to be one of the underlying causes of follicular cyst development (Gümen et al., 2002).

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Follicular cell steroidogenesis and proliferation in ovulatory follicles are stimulated by metabolic hormones such as insulin and insulin-like growth factor-1 (IGF1). IGF1 is essential for the growth of the dominant follicle, stimulating estradiol-17B secretion, whereas insulin is needed to promote follicular maturation (Bossis et al., 1999; Spicer et al., 2002; Silva et al., 2006; Kawashima et al., 2007). Therefore, estradiol-17B enhanced by IGF1 and modulated by insulin levels induces an LH surge with the consequent ovulation of the dominant follicle (Butler et al., 2004; Kawashima et al., 2012; Walsh et al., 2012). Insulin and IGF1 concentrations are reduced by the dietary restriction and negative energy balance of the peripartum period, (Spicer et al., 1990; Lucy et al., 1992; Beam and Butler, 1998; Diskin et al., 2003; Francisco et al., 2003). Lower insulin concentrations and a lack of LH response have been reported in postpartum anestrous cows (Sinclair et al., 2002; Lucy, 2007). Probably, an absence of LH receptors in granulosa cells, which are dependent on the combined actions of FSH and estradiol-17B, could contribute to this lack of responsiveness (Bao and Garverick, 1998; Webb et al., 1999; Marelli et al., 2014). Follicular estradiol-17B is dependent on LH-stimulated production of androgens from theca cells, which in turn appears to be enhanced by insulin and IGF1 (Diskin et al., 2003). Therefore, low plasma insulin concentrations could not only reduce androgen and estradiol production, but also compromise the ability of follicles to acquire LH receptors, all of which finally alters the ovarian function.

Insulin exerts its biological activities by binding to the extracellular portion of its receptor complex (IR). In women, IRs are widely distributed throughout all ovarian compartments (el-Roeiy et al., 1994; Willis and Franks, 1995; Shimizu et al., 2008), whereas in cattle, they are mainly expressed in granulosa cells (Shimizu et al., 2008; Bossaert et al., 2010).

Insulin signaling is mediated by a complex, highly integrated network that regulates distinct biological effects. In the presence of insulin, the substrate tyrosine kinase activity of the IR initiates a cascade of cellular phosphorylation reactions that regulate protein interactions and enzyme activities. Substrates of the IR include the insulin receptor substrate proteins-1 and 2 (IRS1, IRS2), which serve as docking molecules binding to and activating cellular kinases involved in signaling pathways. The metabolic effects of insulin are regulated by activation of the phosphatidylinositol 3-kinase (PI3K) pathway, in which the regulatory PI3K subunit binds to phosphotyrosine residues on IRS1 (Taniguchi et al., 2006; Youngren, 2007). Insulin-mediated activation of PI3K increases production of 3-phosphorylated phosphoinositide lipids (PIP3), which serve as second messengers to recruit Akt to the plasma membrane (Datta et al., 1999). Once properly localized in the membrane, Akt becomes activated by phosphorylation and in turn phosphorylates a number of downstream targets which finally regulate transcription factors and cell growth (Puig et al., 2003). The adequate activation of insulin signaling pathway is determinant both in steroidogenesis and in multiple metabolic pathways. Insulin can enhance FSH-stimulated estradiol production in bovine granulosa cells (Spicer et al., 1993; Gutiérrez et al., 1997) and modulate the expression of the enzyme cytochrome P450 aromatase and the secretion of estradiol in the absence of FSH (Gutiérrez et al., 1997; Silva et al., 2000). FSH stimulates PI3K and other downstream kinases with potential modulation of aromatase activity in bovine cells (Silva et al., 2006). Therefore, considering the relevance of several targets in the insulin action for an adequate ovarian function, in the present study, we aimed to evaluate the expression of downstream effectors in the insulin pathway in cows with COD.

2. Materials and methods

2.1. Induced cystic follicles and controls

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, Universidad Nacional del Litoral (Santa Fe, Argentina). The model of ACTH-induced ovarian follicular cysts used in the present study has been previously described (Dobson and Smith, 2000; Ortega et al., 2008; Salvetti et al., 2010; Salvetti et al., 2012; Amweg et al., 2013) and has been optimized to evaluate the target proposed with a time controlled cyst formation. Briefly, ten nulliparous Argentine Holstein heifers (18–24 mo old; maintained under standard husbandry conditions) with regular estrous cycles according to prior detection of estrus, rectal palpation and ultrasonography were used. Their estrous cycles were synchronized using the Ovsynch protocol, as described previously (Ortega et al., 2008; Rodríguez et al., 2011). 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, as described by Pursley et al. (1995). Beginning on day 15 of a synchronized estrous cycle, five heifers received subcutaneous injections of tetracosactrin hexaacetate (1 mg/mL/animal; Synacthen Depot, Novartis, Basel, Switzerland), a synthetic polypeptide with ACTH activity, every 12 h for 7 d (Ortega et al., 2008). Five control animals received saline (1 mL) (Dobson and Smith, 2000; Ortega et al., 2008). Ultrasonographic ovarian examinations were performed in all animals, as previously described, using a real-time, B-mode scanner equipped with a 7.5 MHz, linear-array, transrectal transducer (Aloka, SSD 500; Wallingford, CT, USA) (Sirois and Fortune, 1988; Rodríguez et al., 2011). Daily ovarian ultrasonography was performed throughout one complete estrous cycle (21 d in the control group) and from day 14 (day 0 = day of estrus) until day 48 (treated group). Cysts detected by ultrasonography were defined as any follicular structure equal to or greater than 20 mm in diameter that was present for 10 d without forming a corpus luteum (Dobson and Smith, 2000; Silvia et al., 2002). All heifers used in this study developed follicular cysts. The first day of follicular cyst formation was the day on which a follicle attained 20 mm or more in diameter. The ovaries were removed 10 d later (approximately day 48) by ovariectomy, following surgical protocols described previously (Marelli et al., 2014). Control heifers were ovariectomized, to obtain normal growing follicles (approximately day 18), when the