

Steroidogenic changes and steady state amount of messenger RNA encoding steroidogenic enzymes, gonadotropin receptors and cell-death signalling in the dominant ovarian follicle during estradiol-induced atresia in cattle[☆]

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Abstract

Changes in steroidogenic function and associated gene expression were characterized in dominant ovarian follicles (DF) of cattle where follicles were induced to become atretic by systemic administration of estradiol benzoate (EB). In experiment 1, follicular fluid (FF) steroid concentrations in the DF were measured at 12-hourly time points for 48 h in heifers treated with 1 mg EB i.m./500 kg body weight (EB; $n = 20$) as compared with untreated controls (C; $n = 19$). Treatment with EB promoted a transient reduction in circulating FSH, a rapid (12 h) and sustained reduction in FF estradiol, a rapid (12 h) but transient reduction in FF progesterone and a delayed (36 h) increase in FF testosterone concentrations. In experiment 2, whole follicular wall tissue was collected from DF of mature non-lactating cows allocated to a 0 h control group (OHC; $n = 7$), a 24 h control group (24HC; $n = 7$) or an EB-treated group where tissue was collected 24 h after administration of 1 mg EB i.m./500 kg body weight (EB; $n = 8$). As for experiment 1, EB promoted a transient reduction in circulating FSH, a pronounced reduction in FF estradiol and a smaller but significant reduction in FF progesterone concentrations. Semi-quantitative RT-PCR on follicular wall tissue revealed that the loss in estrogen activity at 24 h after EB was associated with two-fold reduction in aromatase mRNA, with an apparent acceleration in loss of 17 α -hydroxylase mRNA. Expression of genes for gonadotropin receptors (LHR and FSHR) and a cell-death signalling pathway (Fas antigen and Fas ligand) were unchanged during the

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initial 24 h of EB-induced atresia. These results suggest that EB initiates atresia in dominant ovarian follicles through a rapid suppression of follicular estradiol synthesis, an effect associated with down-regulation of the aromatase gene. A transient suppression in circulating FSH following administration of EB appears to have initiated these events, and it is suggested that subsequent processes involved in atresia follow this loss in estrogenic function.

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1. Introduction

Strategic regulation of ovarian follicular development during estrous synchronization treatments in cattle is an essential requirement for optimizing the degree of synchronization and fertility to AI (Macmillan and Burke, 1996; Day and Burke, 2002; Bo et al., 2002). Administration of estradiol benzoate (EB) in conjunction with a progestin is one method for inducing a new wave of ovarian follicular development to promote a synchronous establishment of the mature pre-ovulatory follicle at treatment termination (Burke et al., 2000); an effect associated with improved fertility in dairy cattle (Day et al., 2000). Emergence of a new wave of follicular development is necessarily preceded by atresia of the current DF.

Knowledge about ovarian follicular atresia in cattle is largely based on comparative differences between follicles that become dominant compared with those that become subordinate, or those that are retrospectively classified as healthy (estrogenic) compared with those that become atretic (non-estrogenic). Some studies have characterized atresia by measuring various functional changes in the first DF of the estrous cycle of cattle at progressive time points in its development (Badinga et al., 1992; Xu et al., 1995a,b; Bao and Garverick, 1998; Valdez et al., 2005). From this body of literature, it could be surmised that a reduction in estradiol synthesis is observed in the early stages of atresia (Price et al., 1995) without substantial changes to gonadotropin receptor numbers on granulosa cells (Bodensteiner et al., 1996). Loss of estrogen activity may (Badinga et al., 1992; Lucy et al., 1992; Burke et al., 2005) or may not (Xu et al., 1995a; McNatty et al., 1984; Valdez et al., 2005) be associated with a reduction in androgen precursors, but is commonly a consequence of reduced aromatase activity (Price et al., 1995; Valdez et al., 2005). Progression of atresia follows the process of programmed-cell death referred to as apoptosis (Hughes and Gorospe, 1991; Tilly et al., 1991; Kaipia and Hsueh, 1997). Binding of Fas ligand (FasL) to membrane-bound receptors (Fas antigen/APO-1/CD95; Fas) is a known cell-death signalling pathway for apoptosis in bovine follicles (Hu et al., 2001; Porter et al., 2001), and also in corpora lutea (Taniguchi et al., 2002).

Exogenous estradiol is not consistently effective in promoting atresia of the DF and the interval to new emergence can be variable (Burke et al., 2001; Diskin et al., 2002). Only limited information (Manikkam and Rajamahendran, 1997) is available about the intra-follicular events that occur following exogenous induction of atresia in follicles that have previously achieved a state of dominance. A more precise understanding of the mechanistic processes during estradiol-induced atresia of DFs may lead to the development of more effective means for regulating this event. The objectives of the present studies were to characterize the temporal path of perturbed steroidogenic function in DF during estradiol-induced atresia (experiment 1), and subsequently measure changes in the expression of several genes (experiment 2) that regulate key intra-follicular processes.

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