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Short communication

Stage-specific expression of bone morphogenetic protein type I and type II receptor genes: Effects of follicle-stimulating hormone on ovine antral follicles

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Abstract

We investigated the mRNA expression patterns of receptor genes for bone morphogenetic proteins-15 (BMP15) and growth differentiation factor-9 (GDF-9) in granulosa cells of sheep treated with FSH. The effects of FSH and estradiol (E2) on the regulation of BMPRII, BMPRIB and ALK-5 in ovine granulosa cells were also examined. Ovaries were collected on day 16 of the estrous cycle and granulose cells were harvested from follicles of two sizes (3–5 and >5 mm in diameter). For *in vitro* studies, granulosa cells were obtained from follicles of 3–5 mm in diameter and cultured in serum-free McCoy's 5A medium supplemented with different doses of FSH (0, 1, 5, 10 ng/ml) or a combination of 5 ng/ml FSH with 1 ng/ml E2. Expression of BMPRII, BMPRIB and ALK-5 mRNA was estimated by quantitative real-time PCR. Our results demonstrated that BMPRII, BMPRIB and ALK-5 expression was significantly higher in the granulosa cells of large follicles than of small follicles. Treatment of granulose cells with FSH (1–10 ng/ml) alone down-regulated the expression of BMPRIB ($P < 0.05$). BMPRII and ALK-5 mRNA expression was not significantly different at an FSH concentration of 5 ng/ml compared to control. A further increase in FSH (10 ng/ml) down-regulated the expression of BMPRII and ALK-5 ($P < 0.05$). The combination of FSH (5 ng/ml) and E2 (1 ng/ml) up-regulated the expression of BMPRII, BMPRIB and ALK-5 in granulose cells ($P < 0.05$). Therefore, the present study establishes the expression levels of the receptor genes of BMP15

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and GDF-9 and suggests that the expression of BMPRII, BMPRIB and ALK-5 may be regulated by FSH and E2 in ovine granulosa cells.

Keywords: Gene expression; BMPRII; BMPRIB; ALK-5; Ovine granulosa cells

1. Introduction

Evidence from *in vivo* and *in vitro* studies suggests that the transforming growth factor-β (TGF-β) superfamily is crucial for normal folliculogenesis in the ovary. Two members of the family, BMP15 and GDF-9, have been identified as essential for normal follicular development and maturation of preovulatory follicles, and, in sheep, as playing a central role in the determination of ovulation quota and litter size (Shimasaki et al., 2004). BMP 15 and GDF-9 act together to form a high-affinity complex with BMP type II receptors and the appropriate type I receptors (Shimasaki et al., 2004). BMP15 exhibits the highest affinity for the BMPRIB and BMPRII receptors (Moore et al., 2003). In contrast, GDF-9 preferentially binds to ALK-5 and BMPRII (Vitt et al., 2002; Mazerbourg et al., 2004). In sheep ovary, BMPRIB, ALK-5 and BMPRII are expressed mainly in the granulosa cells of primary to late antral follicles (Souza et al., 2002). However, there are no reports on the quantitative analysis of BMPRII, BMPRIB and ALK-5 expression in ovine granulose cells.

Several studies have demonstrated the mutual regulation of the hormone and BMP system in the mammalian ovary, where BMP receptors are precisely controlled in a stage- and hormone-dependent manner during follicular development. Previous studies in humans (Miyoshi et al., 2006), mice (Miyoshi et al., 2007) and cattle (Jayawardana et al., 2006) have indicated that FSH alone, or in synergy with other factors, regulate the ovarian BMP system. For example, FSH administration unregulated expression of BMPIA, BMPRIB and BMPRII in the human granulosa-like tumor cell line, KGN (Miyoshi et al., 2006). In the bovine follicle selection process, FSH and estradiol (E2) regulated the expression of BMPRII and ALK-5 (Jayawardana et al., 2006). However, there are no reports on the role of FSH or E2 in regulatoffing the expression of BMP receptor genes in sheep.

The aim of the present study was to assess mRNA expression levels of BMPRII, BMRIB and ALK-5 in ovine granulosa cells of two follicle sizes (3–5 and >5 mm) in sheep after treatment with FSH. We also investigated, through *in vitro* experiments, the effects of FSH and E2 on the expression of these genes in ovine granulosa cells.

2. Materials and methods

Unless specified otherwise, all regents were purchased from Sigma Chemical Co. (St. Louis, MO, USA). This experiment was approved by the Institutional Animal Care and Use Committee at Zhejiang University and was conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publ. No. 85–23, 1985).

2.1. Animals and follicle collection

The experiments were conducted during the breeding season in Zhejiang Province, China. Ten-adult non-pregnant, cycling ewes of Hu breed at 2–3 years of age were used for the experiments.