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Expression of bone morphogenetic proteins and receptors in porcine cumulus–oocyte complexes during *in vitro* maturation

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Abstract

In vitro oocyte growth is the essential technology which enables oocytes to achieve maturation and acquire the competence for subsequent manipulation. There is increasing evidence that members of the transforming growth factor-β (TGF-β) superfamily are expressed in a variety of cell types within the ovary in a developmental stage-related manner and function as crucial factors in oocyte growth and follicular development. However, the expression of TGF-β family members has been studied extensively in follicular compartment cells in the ovaries while poorly explored in the cumulus–oocytes complex (COC) within culture systems. Using semi-quantitative RT-PCR, we investigated the temporal and spatial expression patterns of several bone morphogenetic proteins (BMP-4, BMP-6, BMP-15 and GDF-9), as well as BMP receptors (BMPRIA, BMPRIB, BMPRII and ActRII), in porcine COCs throughout *in vitro* maturation (IVM). In oocytes, the transcription of BMP-6, BMP-15, GDF-9 and BMPRII were down-regulated, while BMP-4, BMPRIA and BMPRIB remained unchanged during IVM. In cumulus cells, BMP-4 mRNA expression increased significantly, while BMP-6 and ActRII was down-regulated during IVM. Nevertheless, mRNAs of BMPRIA, BMPRIB and BMPRII were constantly expressed in cumulus cells in the process. However, BMP-15 was absent in cumulus cells and ActRII was not detected in oocytes. In addition, hardly any transcription of BMP-2, BMP-5, BMP-7, ActRIA was found in porcine COCs throughout IVM. These data demonstrate a complex BMP-signaling system for member gene expression within porcine COCs during IVM and indicate the need for further functional characterization of these factors during oocyte maturation.

Keywords: Gene expression; BMP; BMPR; Porcine; Oocyte; Cumulus

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1. Introduction

The bone morphogenetic proteins (BMPs) belong to the transforming growth factor β (TGF-β) superfamily, a large family of signaling proteins that participate in the regulatory events of cell proliferation and differentiation in a number of organ systems, including the ovary. Several known BMPs (BMP-2, BMP-4, BMP-6, BMP-7, BMP-15 and GDF-9) are involved in the ovarian folliculogenesis, follicular growth and differentiation, cumulus expansion, ovulation and luteinization (Hunter et al., 2005; Monget et al., 2002). BMPs are synthesized as prepropeptides, which are processed to form mature, active disulfide-linked dimers and then exert their effects via two classes of transmembrane serine-threonine kinase receptors, BMP receptor type I and type II (Mazerbourg and Hsueh, 2006). These factors are expressed in the oocyte, granulosa and theca cells in a specific spatiotemporal-regulated manner and exhibit differences among species (Gilchrist et al., 2004).

Oocyte maturation is one of the most important stages for the successful production of embryos *in vitro*. When grown oocytes are removed from their follicles, they are arrested at the prophase of the first meiosis (MI); however, they can resume meiosis and progress to the metaphase of the second meiosis (MII) during IVM. Only fully matured oocytes possess the ability to undergo fertilization and subsequent zygotic development (Mrazek and Fulka, 2003). As the IVM of immature porcine oocytes is essential for subsequent manipulation, understanding of the maturation process and the molecular mechanisms regulating it, will contribute significantly to the optimization of IVM conditions. Because some BMPs are known to be regulators of follicle development *in vivo* (Brankin et al., 2005a; Shimizu et al., 2004; Knight and Glister, 2003), the role of these factors in oocyte growth *in vitro* requires investigation. Recent studies have shown that the exogenous administration of several BMP factors have profound effects on somatic follicular cell growth and function, which facilitate the maturation of oocytes (Shimizu, 2006; Brankin et al., 2005a; Hussein et al., 2005; Otsuka et al., 2001). BMP-6, BMP-15 and GDF-9, previously identified as oocyte-secreted paracrine factors, could exert an effect on neighboring granulosa cells, which in turn regulate oocyte development *in vitro* (Wu and Matzuk, 2002; Hussein et al., 2005; Senbon et al., 2003). These observations suggest that BMPs play an important role in intercellular signaling during IVM of oocytes.

There have been few reports, however, on the regulation of endogenous BMPs, such as GDF-9 and BMP-15, during the development of intact COCs *in vitro*. The primary objective of this study was to characterize mRNA expression of some BMPs and BMPRs in porcine COCs using RT-PCR. The second objective was to determine the time-dependent changes in BMP system expression in both oocyte and cumulus cells of COCs during maturation *in vitro*.

2. Materials and methods

2.1. Collection of oocytes and in vitro maturation

Porcine ovaries were obtained from prepubertal Landrace gilts at a local slaughterhouse and transported to the laboratory within 2 h at 37 °C. COCs were aspirated from antral follicles (3–5 mm in diameter) using an 18-gauge needle fixed to a 10-ml disposable syringe. COCs with intact, compact cumulus vestments were selected as suitable for IVM. After a brief wash in PBS, pooled COCs were transferred to NCSU23 medium supplemented with 10% (v/v) porcine follicular fluid, 10 IU/ml PMSG and 10 IU/ml hCG. The COCs were cultured for 24 h and then incubated in NCSU23 without hormonal supplements for 44 h at 38 °C in 5% CO_2 in air. At 0 and 6 h of