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Animal Reproduction Science 106 (2008) 232–240

ANIMAL REPRODUCTION SCIENCE

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In vivo evidence of role of bone morphogenetic protein-4 in the mouse ovary

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Received 29 August 2006; received in revised form 17 April 2007; accepted 24 April 2007
Available online 3 May 2007

Abstract

The transition of a primordial follicle to a primary follicle is an early step in folliculogenesis. All female mammals are born with a fixed stock of primordial follicles, and exhaustion of that stock leads to menopause or infertility. Recently, several *in vitro* studies have indicated that BMP-4, BMP-7, and several other growth factors affect the transition of primordial to primary follicles. The aim of our present study was to investigate role of BMP-4 in this process using passive immunization to investigate the role of BMP-4 in a prepubertal mouse model. After seven days of treatment, the weight of antiBMP-4 treated ovaries was significantly lower than the ovaries from mice treated with nonimmune Ig. The number of primary follicles was lower, and the numbers of primordial follicles were higher in antiBMP-4 treated ovaries compared to control ovaries. Treatment with equine chorionic gonadotrophin (eCG) showed no influence on the effects of antiBMP-4 in the mouse ovary. Thus, the results of our study indicate that *in vivo* BMP-4 acts as transition factor in transition of primordial to primary follicle.

Keywords: BMP-4; Mouse; Ovary; Primordial follicle; Primary follicle

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doi:10.1016/j.anireprosci.2007.04.015

1. Introduction

The primary function of the ovary is to release a fertilizable egg each estrous cycle, and to prepare the accessory organs for pregnancy by secreting endocrine, autocrine, and paracrine factors (Gougeon, 1996; McGee and Hsueh, 2000). Folliculogenesis is a continuous process of follicle development, in which a few resting primordial follicles are converted to primary follicles, which grow in orderly progression until finally becoming ovulatory or atrectic follicles (Peters et al., 1975). While much is known about the final stages of this process relatively little is known about the initiation of follicular development in a primordial follicle. The development of a primary follicle from a primordial follicle has been considered independent of gonadotrophins because these follicles have been shown to develop in FSH β subunit (Kumar et al., 1997) and FSH receptor (Dierich et al., 1998) knockout mice. In contrast, some studies have indicated that the development of a primary follicle is influenced by gonadotrophin dependent as chronically elevated levels of LH has been shown to increase the depletion of the primordial follicle reserve in mice (Flaws et al., 1997). In addition, increased concentrations of FSH induced by unilateral ovariectomy in rats is associated with an increased loss of primordial follicles (Meredith et al., 1992). On the basis of above studies it could be hypothesized that the local regulatory factors produced by ovary or the follicles are responsible for the development of primary follicles, and that gonadotrophins may modulate this process.

Bone morphogenetic proteins (BMPs) are the members of transforming growth factor-β (TGF-β) superfamily (Shimasaki et al., 2004). Recently, several BMPs have been implicated for their role in follicular development (Vitt et al., 2000; Lee et al., 2001). *In situ* hybridization and immunolocalization studies have shown that BMP receptors (ALK-2, BMPRIA, BMPRIB, ActRII, and ActRIIB) are present in rat primordial follicles (Drummond et al., 2002; Erickson and Shimasaki, 2003). Whereas rat primordial follicles do not express BMP-2, BMP-4, BMP-3, BMP-3b, and BMP-6 (Erickson and Shimasaki, 2003) suggesting that these proteins are secreted by other cell types within the ovary, and may promote primordial follicle development. The expression of BMP-4 has been localized to the basement membrane and stromal cells surrounding follicles in neonatal rat ovaries while in theca, ovarian surface epithelium, and sex cords of adult rat ovaries (Erickson and Shimasaki, 2003; Nilsson and Skinner, 2003). BMP-4 has been shown to stimulate production of estradiol, and inhibit production of progesterone in the presence of FSH in rat granulosa cell culture (Shimasaki et al., 1999). BMP-4 is also found to increase both basal and IGF stimulated production of estradiol, inhibin-A, activin-A, and follistatin, and inhibited both basal and IGF stimulated progesterone production in bovine granulosa cell culture (Glister et al., 2004). The addition of BMP-4 was found to decrease the production of androgens from human ovarian theca-like tumor cells (HOTT) by modulating CYP 17 and 3β HSD ratio in these cells, which ultimately leads to increase in production of progesterone with concomitant decrease in production of androstenedione (Dooley et al., 2000). Apart from these paracrine and autocrine effects of BMP-4 in granulosa and theca cell culture, BMP-4 null mouse embryos are devoid of primordial germ cells (Lawson et al., 1999). Recently, BMP-4 has been shown to promotes transition of primordial follicle to primary follicle in whole rat ovary culture (Nilsson and Skinner, 2003).

The biological activities of BMPs are regulated at multiple levels by various extracellular and intracellular factors (Miyazono, 2000). The data from BMP-4 null mice and *in vitro* studies strongly suggest a role for BMP-4 in folliculogenesis, subsequent recruitment and follicular functions. The aim of our study was to elucidate the biological role of BMP-4 in transition of primordial follicle to primary follicle, and to observe the effect of gonadotrophin on the biological actions of BMP-4 *in vivo*.