

The role of TGF superfamily gene expression in the regulation of folliculogenesis and oogenesis in mammals: a review

H. PIOTROWSKA¹, B. KEMPISTY^{2,3}, P. SOSINSKA⁴, S. CIESIOLKA², D. BUKOWSKA⁵, P. ANTOSIK⁵, M. RYBSKA⁵, K.P. BRUSSOW⁶, M. NOWICKI², M. ZABEL²

¹Department of Toxicology, Poznan University of Medical Sciences, Poznan, Poland

²Department of Histology and Embryology, Poznan University of Medical Sciences, Poznan, Poland

³Department of Anatomy, Poznan University of Medical Sciences, Poznan, Poland

⁴Department of Pathophysiology, Laboratory of Gerontology, Poznan University of Medical Sciences, Poznan, Poland

⁵Department of Veterinary, Poznan University of Life Sciences, Poznan, Poland

⁶Institute of Reproductive Biology, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany

ABSTRACT: The normal differentiation of follicles from the preantral to the antral stage is regulated by the synthesis and secretion of several important growth factors. Moreover, the proper growth and development of the oocyte and its surrounding somatic granulosa-cumulus cells is accomplished through the activation of paracrine pathways that form a specific cross-talk between the gamete and somatic cells. It has been shown that several growth factors produced by the ovary are responsible for the proper growth and development of follicles. The developmental competence of mammalian oocytes (also termed developmental potency) is defined as the ability of female gametes to reach maturation (the MII stage) and achieve successful monospermic fertilisation. Proper oocyte development during folliculo- and oogenesis also plays a critical role in normal zygote and blastocyst formation, as well as implantation and the birth of healthy offspring. Several molecular markers have been used to determine the developmental potency both of oocytes and follicles. The most important markers include transforming growth factor beta superfamily genes (TGFB), and the genes in this family have been found to play a crucial role in oocyte differentiation during oogenesis and folliculogenesis. In the present review, we summarise several molecular aspects concerning the assessment of mammalian oocyte developmental competence. In addition, we present the molecular mechanisms which activate important growth factors within the TGFB superfamily that have been shown to regulate not only follicle development but also oocyte maturation.

Keywords: TGFs; oogenesis; folliculogenesis; regulation of gene expression

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1. Introduction

In all mammalian species, the proper course of folliculogenesis and oogenesis is the main factor that influences the growth and development of cumulus-oocytes complexes, the maturation of oocytes to reach the MII stage and subsequently monospermic fertilisation (Chaves et al. 2012; Songsasen et al. 2012; Jovanovic et al. 2013; Linke et al. 2013; Sobinoff et al. 2013). Developing from the preantral into the antral stage, the follicle is finally morphologically modified to three separate populations of somatic cell: theca cells, granulosa cells and cumulus oophorus (Figure 1). In the periovulatory period, the cumulus cells surrounding the oocyte expand. The process of cumulus expansion is associated with the proliferation of cumulus cells and of granulosa to cumulus differentiation. It has been demonstrated that the surrounding oocyte-granulosa-cumulus cells can proliferate and differentiate *in vitro* (Kempisty et al. 2013). However, the *in vitro* proliferation of cumulus cells may only

mimic *in vivo* conditions, and how the proliferation of granulosa-cumulus cells proceeds in a follicular environment is still speculative.

Since Derynck et al. (1985), first identified a partial amino acid sequence from transforming growth factor beta 1 (TGFB1) purified from blood platelets, numerous studies have highlighted the role of this peptide as a modulator of cell growth, proliferation and differentiation in many types of cells and tissues (Gilchrist and Ritter 2011; Nagamatsu et al. 2012). It is also thought that TGF proteins are involved in the regulation of oocyte and embryonic growth and development.

Although the expansion process in cumulus cells has been well documented, the molecular markers of this phenomenon are still not entirely known. Moreover, there is not much evidence on the role of TGFs as regulatory proteins during folliculo- and oogenesis in mammals. Therefore, in the present review, recent findings regarding the role of TGF superfamily genes and proteins during folliculogenesis and oogenesis will be presented and discussed.

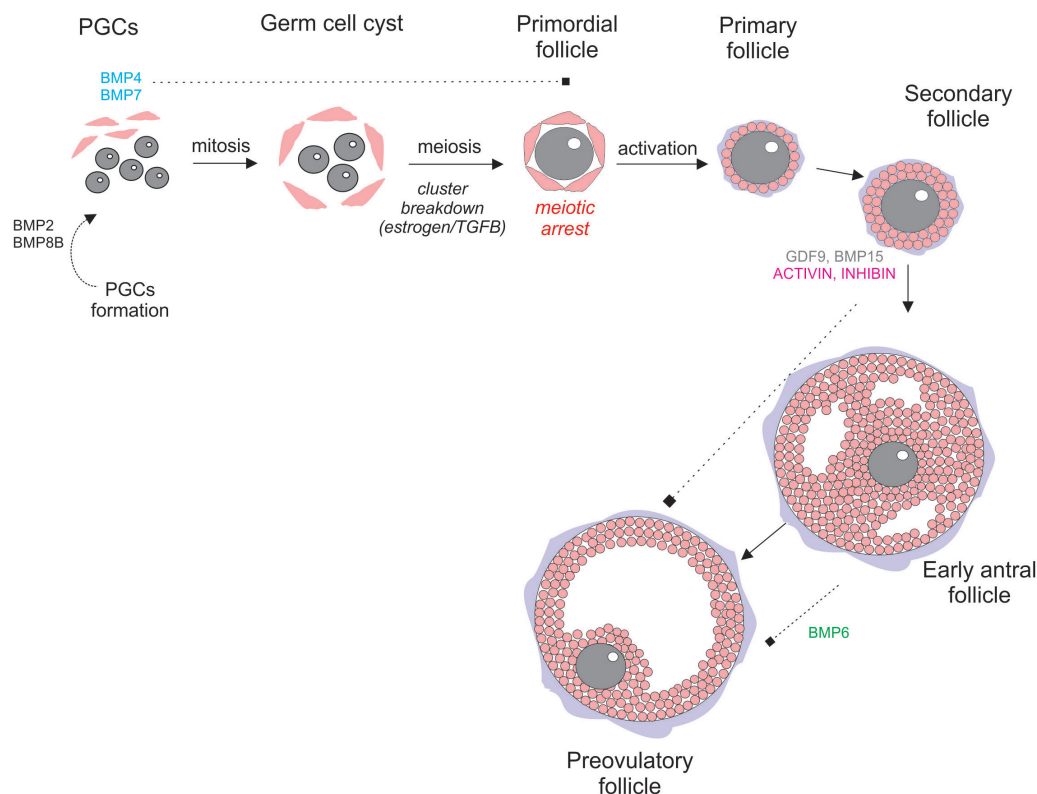


Figure 1. Factors involved in primordial germ cell (PGC) formation, oogenesis and folliculogenesis. Ovarian factors regulate oocyte and follicle development: produced by theca/stromal cells (blue), somatic/granulosa cells (pink), germ cells (gray) and in both germ cells and granulosa cells (green), at different stages of development. Proteins participating in PGC formation (from extra embryonic ectoderm) are indicated in black

2. The expression profile of genes of the TGFB superfamily during folliculogenesis

Several research groups have demonstrated that the TGFB superfamily genes and proteins are expressed in ovarian tissues (Ergin et al. 2008; Zhu et al. 2010; Hatzirodos et al. 2011; Nagashima et al. 2011). Recent studies have shown that the morphological and genetic transition from the primordial follicle stage through to ovulation and corpus luteum formation is associated with the activation of a growth factor signalling cascade that highly regulates the proliferation and differentiation of ovarian cells (Figure 1), (Nagashima et al. 2011; Joseph et al. 2012; Kawano et al. 2012). The most important growth factors include genes of the TGFB superfamily that are expressed in mammalian ovarian somatic cells and in oocytes in a stage-specific manner, and thereby, function as intraovarian regulators of folliculogenesis (Paradis et al. 2009; Nagashima et al. 2011; Corduk et al. 2012). The most important TGFB superfamily genes include bone morphogenic proteins 2, 4, 5, 6, 7, and 15 (BMP2, BMP4, BMP5, BMP6, BMP7, and BMP15) and growth differentiation factor 9 (GDF9), which are expressed throughout folliculogenesis and regulate key steps of follicle growth and development (Figure 2), (Knight and Glistler 2006). The role of TGFB superfamily gene expression in the regulation of mouse folliculogenesis has been thoroughly studied using mouse knockout models (Li et al. 2012; Pangas 2012). However, the possible functions of these proteins in follicular development and/or differentiation of ovarian somatic cells of domestic animals have yet to be highlighted. Shimizu et al. (2004) investigated the expression of GDF9, BMP4, BMP5 and BMP6 mRNA in neonatal pigs. They found that the molecular interaction between these growth factors significantly influenced the regulation of growth factor-derived folliculogenesis in the neonatal pig. They demonstrated also a species-specific expression of these growth factors in a follicle development-dependent manner. Consequently, these growth factors may play a distinct role during folliculogenesis. Along with the role of TGFB in the molecular regulation of follicle growth, multiple studies have highlighted the role of TGF- α (TGFA) during this process with regard to other proteins, e.g., laminin. There has not been substantial data supporting the association between the oestrous cycle in mammals and laminin versus

TGFA interaction during folliculogenesis (Irving-Rodgers et al. 2006; Tse and Ge 2010). Recently, Akkoyunlu et al. (2003) used the immunoperoxidase method to investigate the possible function of these factors during rat folliculogenesis. After analysing ovaries collected from adult virgin female rats, they found that TGFA was localised in the nuclei of oocytes, whereas the differential distribution of this protein was observed in granulosa and interstitial thecal cells. Laminin and fibronectin were localised in the vascular walls, in the outer layers of granulosa cells, suggesting that TGFA may play a role during follicular maturation. TGFA may also be involved in the formation of basement membranes of growing follicles. Drummond et al. (2002) studied the role of the TGFB/BMP/activin signalling pathway in the ovaries of postnatal rats. Using real-time PCR expression assays, they observed the presence of activin/BMP receptors (ActRIA, ActRIB, ActRIIA, and ActRIIB), beta glycan and Smad1-8 in the rat ovary. The expression of activin receptors and Smad superfamily genes were generally related to the formation of a secondary follicle. At the antral follicle stage, the activin receptors and Smad genes were expressed in a gene-dependent manner. Moreover, all of the identified activin-Smad superfamily gene expression was present in oocytes at all stages of follicle development, and both in granulosa and theca cells. These results confirm the supposition that the TGFB/activin/Smad signaling cascades are key regulators of follicle growth and development (Figure 2). A significant role of the TGFB superfamily genes in the regulation of mammalian folliculogenesis was previously investigated using a knockout mouse model for the GDF9 (GDF9^{-/-}) gene (Dong et al. 1996). These experiments demonstrated that folliculogenesis was arrested at the primary follicle stage in the absence of GDF9, leading to infertility. Laitinen et al. (1998), found a cDNA analogue of GDF9, designated as GDF9B, and this gene was also found to be involved in the regulation of folliculogenesis in mice. Furthermore, they showed that GDF9 and GDF9B were co-expressed and co-localized in the oocyte during follicle growth and development. Growth and development of follicles are regulated by the synthesis and secretion of several significant growth factors produced by oocyte/granulosa cells. These growth factors are secreted by the oocytes to the granulosa cells and thereby activate follicle-specific mechanisms via paracrine regulation. Recent

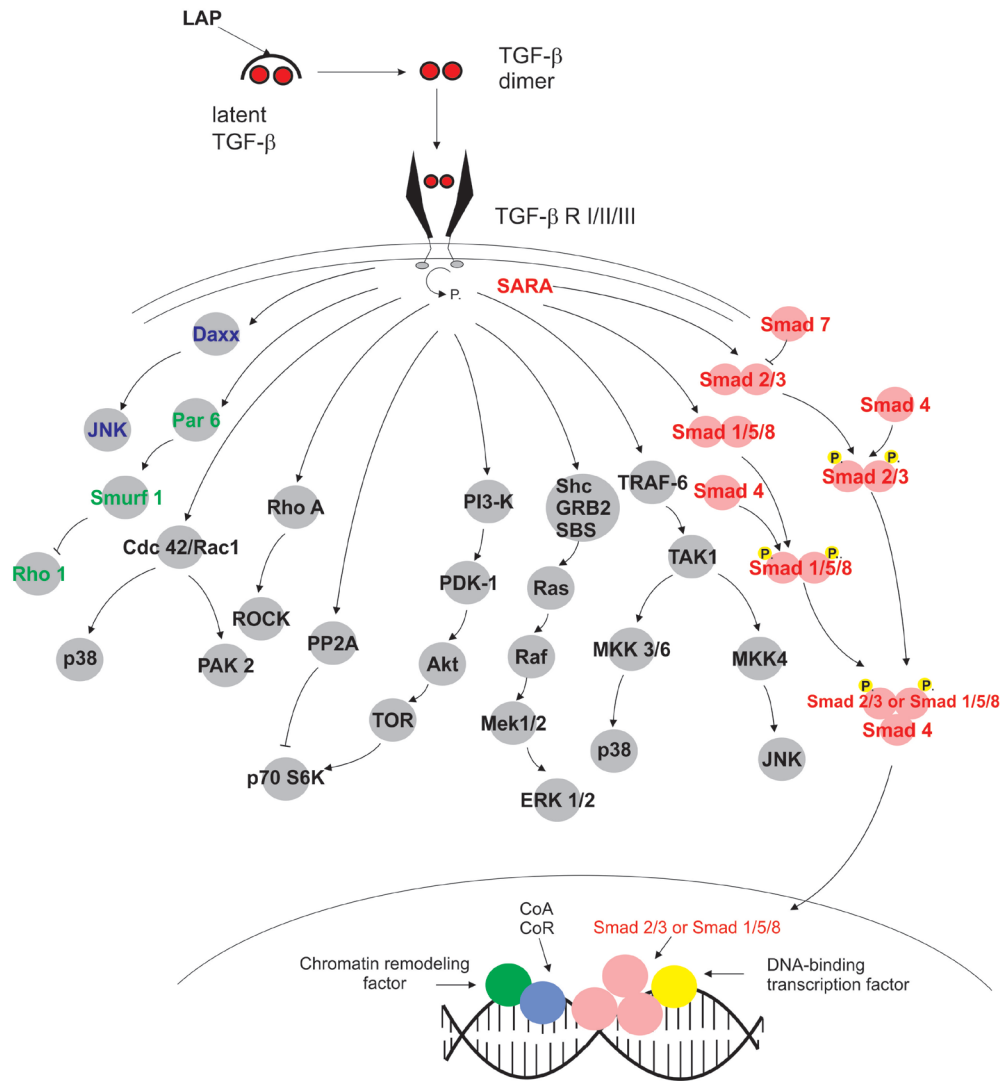


Figure 2. Representative figure of TGF- β signalling pathways. TGF- β cytokines are highly pleiotropic factors that regulate a wide range of cellular processes during development and adult tissue homeostasis (cell proliferation, apoptosis, epithelial-to-mesenchymal transition, angiogenesis). TGF- β can activate a number of Smad-independent signalling pathways in a cell type-specific and context-dependent manner, including Ras/MAPK, PI 3-K/Akt, p38, JNK, and RhoA/ROCK. Activation of these pathways may also contribute to the cellular responses induced by TGF- β . Based on leaflet product information (R&D Systems)

studies have demonstrated a role of oocyte-specific factors which belong to the TGFB superfamily, and which include BMP15 and GDF9. TGFB superfamily gene expression as well as immunohistochemical localisation of proteins was recently investigated in different animal models, including rats, pigs and sheep. Thus, Ergin et al. (2008), analysed the immunohistochemical expression of IGF-I, TGFB2, bFGF and epidermal growth factor receptor (EGFR) in rat ovaries, collected from newborn, one-month-old and adult females. An increased expression and distribution of all of these proteins was observed in oocytes and in ovaries

isolated from one-month-old rats. A moderate expression of only IGF-I was found in the theca cells and in the corpora lutea of adult females, and a similar expression pattern was observed in the granulosa cells of new-born ovaries. Furthermore, a moderate staining of TGFB2 protein was observed within the ovaries of adult rats, as well as of EGFR in the granulosa cells of one-month-old females. These results underline a role for growth factors in the regulation of ovarian development in rats. The expression of TGFB1 and TGFB2 in the ovaries of other mammals, including pigs, has been recently shown. However, little is known, e.g., about

the regulation of the expression of these growth factors expression during various stages of the oestrous cycle in pigs. Most of the recent studies highlighted the significance of TGFB1 and TGFB2. However, Steffl et al. (2008) investigated the expression of TGFB3 mRNA at different stages of the porcine oestrous cycle and follicle development. The expression pattern of TGFB3 was analysed using RT-PCR assays and immunohistochemistry. The expression of TGFB3 was observed throughout the oestrous cycle; however, there was an increase in expression at met- and dioestrous. Low levels of immunoreactivity were detected in follicular epithelial cells and in the oocytes of preantral follicles. The exclusive expression of the TGFB3 protein was observed in the theca internal cell layer of antral follicles, whereas this expression was significantly increased in large antral follicles. A higher distribution of TGFB3 protein in the theca cell layer, soon after ovulation, was also observed. Considering all of these results, it may be assumed that the expression pattern of TGFB3 modulates the development of theca cells of growing follicles and, therefore, actively regulates the differentiation of theca cells of pre- and post-ovulatory follicles in pigs. It was also shown in cows that ovarian follicle development requires the activation of several paracrine and endocrine factors that are synthesised by ovarian cells, and which regulate the growth and development of follicles. The role of bovine theca cells in this process was recently investigated (Spicer et al. 2008). In this study, the effect of GDF9 on theca cells isolated from large (8–22 mm) and small (3–6 mm) follicles was analysed and it was observed that the cultivation of theca cells isolated from small follicles with supplementation of LH, IGF1 and GDF9 leads to increased cell numbers and higher levels of DNA synthesis, whereas theca cells isolated from large follicles did not respond to incubation with GDF9. Furthermore, the cultivation of theca cells of small follicles resulted in down-regulated LHR and CYP11A1 mRNA expression, but the mRNA levels for IGF1R, STAR and CYP17A1 were not significantly altered. The expression of GDF9 mRNA was also analysed in granulosa cells and theca cells. While the GDF9 mRNA levels in granulosa cells isolated from small follicles were significantly higher compared to those from large follicles, no GDF9 mRNA expression was detected in theca cells. Thus, theca cells of small follicles may be more sensitive to stimulation by GDF9, and bovine

granulosa cells may synthesise GDF9, but only in small follicles. It has been proposed that GDF9 promotes theca cell proliferation as well as decreases steroidogenesis and may, therefore, also prevent theca cells from premature differentiation during folliculogenesis. It was also previously demonstrated that genetic alterations, e.g., mutations in the GDF9 gene lead to infertility in sheep due to ovarian dysfunction (Otsuka et al. 2011).

3. TGF gene expression in relation to angiogenesis and folliculogenesis

One important stage of follicle growth and development is angiogenesis, which requires the activation of several proteins and growth factors. Angiogenesis is a process that occurs during follicle growth and luteinisation. It is thought that FSH secreted by the pituitary gland regulates the expression of TGFB1 in oocytes-granulosa cells. Such a relationship between ovarian angiogenesis and the expression of TGFB1 stimulated by secretion of FSH was recently investigated (Kuo et al. 2011). Gonadotropin-primed granulosa cells of immature rats were incubated with FSH and/or TGFB. FSH and TGFB stimulated the angiogenic activity of granulosa cells as well as an up-regulation of angiogenic-specific gene expression (VEGF and PDGF-B). However, when specific inhibitors for VEGF (Ki8751) and/or for PDGF (AG1296) were additionally applied in the experiment, these inhibitors led to the suppression of microvessel growth in treated granulosa cell cultures. In summary, FSH and TGF up-regulate the expression of angiogenic-specific genes and both of these molecules stimulate the angiogenesis of ovarian-granulosa cells during follicle growth. Other studies were done by Sharma et al. (2010), where the effects of several growth factors, including IGF-I, TGFA, TGFB1 and bFGF used alone or in combination with FSH, on the development, survival, antrum formation and apoptosis of buffalo preantral follicles were investigated. TGFA and TGFB1 inhibited follicular survival and increased the incidence of oocyte apoptosis, whereas this effect was decreased by IGF-I + TGFA + TGFB1. Moreover, IGF-I alone significantly increased the survival and growth of follicles, and enhanced antrum formation. However, this stimulatory effect was strongest when FGF was used in the culture. It was also demonstrated that the concentrations of progesterone and oestradiol were higher in the presence of FGF and IGF-I when compared to TGFA and

TGFB1. Consequently, IGF-I and FGF enhanced the survival, development and antrum formation of buffalo follicles, whereas TGFA and TGFB1 increased the apoptosis of oocytes. Arunakumari et al. (2010) successfully derived *in vitro* embryos at the morula stage after the cultivation of ovine preantral follicles. The follicles were cultured in media supplemented with several growth factors, i.e., insulin-transferrin-selenite (ITS), IGF-I, TGFB, insulin and growth hormone (GH). A combination of ITS, IGF-I, insulin and GH stimulated ovine follicle growth and development, whereas, supplementation with TGFB inhibited the maturation of oocytes to the MII stage.

The TGFB superfamily genes act via pathways including the BMP/Smad signaling cascade. In a special type of sheep, called the Hu sheep, the effect of these signalling pathways on the high fecundity of this breed was studied (Xu et al. 2010). Ewes were analysed for FecB (A746G) and BMPRI gene mutations. RT-PCR expression analysis detected BMP associated genes (BMP2, BMP4, BMP6, BMP7 and BMP15), BMP receptors (BMPRIA, BMPRIB and BMPRII), Smad family genes (Smad1, Smad4 and Smad5), and other TGFB superfamily genes, such as TGFBRI and GDF9. All investigated animals were homozygous for mutations in BMPRIB (A746G), and all of the analysed TGFB superfamily genes were expressed in the Hu sheep ovary. It was also found that the expression of BMP4, BMPRIB, BMPRII, Smad4, GDF9 and TGF-betaRI mRNAs was increased in antral follicles of high-fecundity females compared to low-fecundity ewes, whereas BMP15 expression was decreased. Accordingly, the TGFB/BMP/Smad signalling cascade plays a significant role during Hu sheep ovarian folliculogenesis. However, there could be several other mutations in TGFB-related genes outside of BMPRIB that could influence proper folliculogenesis. In Woodland sheep, a reoccurring genetic mutation (FecX2(W)) was shown to increase the ovulation rate (Feary et al. 2007). The FecX2(W) mutation is characterised as a mutation present in the BMP15 gene and/or GDF9, both of which interact with BMP15 in the regulation of ovarian function, including ovulation. Furthermore, this mutation may regulate the expression pattern of genes contributing to the regulation of ovulation rate. Feary et al. (2007) analysed the sequence of the coding region of the BMP15 and GDF9 genes and performed RT-PCR expression analysis of *GDF9*, *BMP15*, *TGFBRI*, *BMPRIB*, and *BMPR2* mRNAs

during follicular development. No changes in the coding sequence of the BMP15 and GDF9 genes in females characterised by an increased ovulation rate were identified and the expression levels of mRNAs encoding GDF9 and BMPR2 did not differ. However, the expression of *BMP15* mRNA decreased in oocytes from FecX2(W) sheep in large preantral and antral follicles. However, the expression of *ALK5* was significantly increased in oocytes from ewes characterised by the Woodlands mutation. The mRNA levels of *BMPRIB* were lower in oocytes and in granulosa cells of FecX2(W) ewes. The number of antral follicles <1 mm in diameter in FecX2(W) ewes was also higher. Evidently, mutations in the BMP15 and/or BMPRIB genes and, subsequently, differential expression of *BMP15* and *BMPRIB* mRNAs may alter ovulation rate in sheep.

The role of FSH in the expression of TGFB superfamily genes has also been investigated. Chen et al. (2009a,b) investigated *in vitro* the effect of FSH treatment on the expression profile of *BMP15* and *GDF9* in ovine granulosa cells, as well as the effect of FSH and oestradiol (E_2) on the regulation of BMPRII, BMPRIB and ALK-5. An increased expression of *BMPRII*, *BMPRIB* and *ALK-5* was found in granulosa cells collected from large compared to smaller follicles. Moreover, the incubation of granulosa cells with different FSH concentrations inhibited the expression of *BMPRIB*, or the expression of *BMPRII* and *ALK-5* mRNAs was unchanged compared to control. Only high doses of FSH decreased the expression of *BMPRII* and *ALK-5* in granulosa cells. However, combined treatment with FSH and oestradiol increased the expression of all investigated genes, i.e., BMPRII, BMPRIB and ALK-5. This observation supported data from previous studies suggesting the existence of paracrine activation pathways between oocytes and granulosa cells, and the regulation of BMP15 receptors by FSH and E_2 secretion (Jayawardana et al. 2006; Chen et al. 2009a). Furthermore, it is evident that hormones of the pituitary and ovary have a significant role in the regulation of granulosa cell differentiation. Proper differentiation of granulosa cells also requires the activation of several other TGFB superfamily genes, such as Smad-related genes. It has been demonstrated that the ablation of Smad-related genes (mainly Smad2 and Smad3) leads to reduced female fecundity as a result of irregular ovarian processes and impaired follicular development, intrafollicular oocyte development and ovulation (Tomic et al. 2002). The role of the expression of Smad-related genes

during the growth and development of granulosa cells was recently investigated by (Li et al. 2008). They found that Smad2 and Smad3 are essential for normal follicle development and for oocyte maturation to achieve developmental competence.

The expression of mRNAs encoding Smads (-1, -2, -3, -5 and -9), Smad4 (co-Smad), Smad6 and Smad7 (inhibitory Smads) was observed in oocytes of preantral and antral follicles (Tian et al. 2010). The expression of Smad5, Smad6 and Smad7 was increased in growing compared to fully grown oocytes. In oocytes isolated from primordial, primary, secondary and antral follicles, pSMAD1/5/9, pSMAD2 and pSMAD3 were present. Immunostaining for pSMAD2/3 and pSMAD1/5/9 revealed the presence of these proteins in granulosa cells of primordial and secondary follicles. A significantly increased expression of pSMAD proteins was also detected in cumulus cells of antral follicles. Additionally, pSMADs were present in the oviductal epithelium.

4. TGFB superfamily gene expression during oogenesis

Although it is assumed that the TGFB gene expression signalling cascade is necessary for oocyte development during folliculo- and oogenesis, there is no substantial supporting evidence (Levacher et al. 1996; Bukowska et al. 2012). However, it is well known that normal mammalian oocyte maturation and development require somatic cumulus cell expansion (CCE) (Jain et al. 2012; Barberi et al. 2013; Caixeta et al. 2013; Gharibi et al. 2013; Mito et al. 2013). This process is regulated by, among others, paracrine signalling of oocyte-cumulus cell secreted growth factors (Su et al. 2009; Romaguera et al. 2010; Hussein et al. 2011). However, in three out of the four species studied to date, CCE did not require the presence of oocytes (Nagyova 2012; Watson et al. 2012; Gharibi et al. 2013). To study this process, Gilchrist and Ritter (2011) analysed the paracrine mechanisms of the Smad/MAPK signalling cascade comparing porcine and murine cumulus-oocyte-complexes (COCs). Treating COCs and oocyte-free complexes (OOXs) with FSH and TGFB superfamily antagonists, they found that the inhibition of TGFB superfamily genes, such as GDF9, TGFB, activin A, and activin B, and several BMP superfamily genes did not affect the CCE of porcine COCs. Additionally, they demonstrated that porcine oo-

cytes synthesise and secrete factors that activate Smad3 and Smad1/5/8 in granulosa cells, whereas murine oocytes only activate the Smad3 gene. The cultivation of porcine oocytes with the Smad2/3 phosphorylation inhibitor SB431542 partially affected the CCE, whereas the CCE of murine COCs was totally inhibited. This study also revealed that the inhibition of MAPK-related genes (ERK1/2 and p38) leads to decreased CCE in porcine oocytes. From this study it is evident that the Smad-related pathway is involved in cumulus expansion, but in a species-specific manner. Moreover, the activation of the Smad signalling cascade in porcine CC appears to be essential for the matrix formation of growing oocytes via the up-regulation of the MAP kinase signalling pathway.

Oogenesis is a complex process that is regulated by several intra- and extraovarian factors (Sanchez and Smitz 2012). In recent years, considerable attention has been focused on members of the TGFB superfamily as potential local regulators between the oocyte and the granulosa cell layer, facilitating appropriate oocyte development (Knight and Glistler 2006). Oocyte paracrine growth factors BMP-6, BMP15, GDF9 have been shown to be critical components of the intra-follicular communication pathways (Su et al. 2004).

The participation of TGFB proteins in the growth and maturation of oocytes begins at early stages of oogenesis during primordial germ cell (PGC) formation (Figure 1), (Ying et al. 2000; Ying and Zhao 2001). The precursors of PGCs are located in the proximal region of the epiblast adjacent to the extraembryonic ectoderm (Ginsburg et al. 1990) and can be identified as a cluster of alkaline phosphatase-positive cells located in the extraembryonic mesoderm posterior to the primitive streak (Ying et al. 2000). It has been demonstrated that BMP, BMP4, BMP8b (ectoderm-derived) and BMP2 (endoderm-derived) are required for PGC generation, proliferation and further colonisation (Lawson and Hage 1994). *In vitro* studies confirmed that BMP2 and BMP4 increase the number of murine PGCs in culture (Pesce et al. 2002), whereas Bmp7 mouse knockouts demonstrate a reduction in the number of germ cells around this period (Ross et al. 2007). Other studies provide species-dependent evidence for the role of TGF superfamily members. In human primordial germ cells, activin has been shown to increase the number of PGCs (Martins da Silva et al. 2004), whereas in mouse-derived PGC, activin acts as an inhibitor of proliferation (Richards et al. 1999).

PGCs progress step-wise and finally develop into primordial follicles with oocytes surrounded by pre-granulosa cells. Studies in rodents have revealed that steroids and members of the TGF superfamily, including GDF9 and BMP15, are involved in this process (Bristol-Gould et al. 2006; Sanchez and Smitz 2012). The development of primary follicles to the late preantral and early antral stages involves follicular growth, fluid-filled antrum formation and oocyte enlargement and an increase in volume and size (Bachvarova et al. 1985; Durinzi et al. 1995; Eppig and O'Brien 1996; Picton et al. 1998). Throughout this period, oocytes synthesise and accumulate RNAs and proteins that are vital for their appropriate growth and maturation (Picton et al. 1998).

It has been shown that TGF-superfamily members, including inhibin, activin and follistatin, which are expressed by companion granulosa cells, play an essential role in cytoplasmic and nuclear maturation of oocytes (Figure 2), (Eppig 2001). This was confirmed by the localisation of their receptors on the surface of oocytes in several species. *In vitro* studies have revealed that activin can accelerate meiotic maturation in primate and rodent oocytes in a follistatin-reversible manner (Sadatsuki et al. 1993; Alak et al. 1996, 1998). In cows it was demonstrated that activin A had no effect on the post-fertilisation cleavage stage of both denuded and cumulus-enclosed oocytes; however, their competence to form blastocysts was enhanced in a follistatin-dependent reversible manner (Silva and Knight 1998). These studies confirmed also a correlation between endogenous activin concentration in the cumulus-oocyte complex and oocyte developmental competence. However, some studies have also shown that inhibin, as well as its free α subunit, may have a negative influence on developmental competence and maturation of oocytes (WS et al. 1989; Silva et al. 1999).

From the summarised research data it becomes evident that members of the TGF superfamily are crucially involved in the processes of follicular and oocyte development. Although several molecular aspects have been already elucidated, much work remains to be done if we are to understand these vital processes in their entirety.

5. REFERENCES

- Akkoyunlu G, Demir R, Ustunel I (2003): Distribution patterns of TGF- α , laminin and fibronectin and their relationship with folliculogenesis in rat ovary. *Acta Histochemica* 105, 295–301.
- Alak BM, Smith GD, Woodruff TK, Stouffer RL, Wolf DP (1996): Enhancement of primate oocyte maturation and fertilization in vitro by inhibin A and activin A. *Fertility and Sterility* 66, 646–653.
- Alak BM, Coskun S, Friedman CI, Kennard EA, Kim MH, Seifer DB (1998): Activin A stimulates meiotic maturation of human oocytes and modulates granulosa cell steroidogenesis in vitro. *Fertility and Sterility* 70, 1126–1130.
- Arunakumari G, Shanmugasundaram N, Rao VH (2010): Development of morulae from the oocytes of cultured sheep preantral follicles. *Theriogenology* 74, 884–894.
- Bachvarova R, De L, V, Johnson A, Kaplan G, Paynton BV (1985): Changes in total RNA, polyadenylated RNA, and actin mRNA during meiotic maturation of mouse oocytes. *Developmental Biology* 108, 325–331.
- Barberi M, Di P, V, Latini S, Guglielmo MC, Cecconi S, Canipari R (2013): Expression and functional activity of PACAP and its receptors on cumulus cells: Effects on oocyte maturation. *Molecular and Cellular Endocrinology* 375, 79–88.
- Bristol-Gould SK, Kreeger PK, Selkirk CG, Kilen SM, Cook RW, Kipp JL, Shea LD, Mayo KE, Woodruff TK (2006): Postnatal regulation of germ cells by activin: the establishment of the initial follicle pool. *Developmental Biology* 298, 132–148.
- Bukowska D, Kempisty B, Piotrowska H, Zawierucha P, Brussow KP, Jaskowski JM, Nowicki M (2012): The in vitro culture supplements and selected aspects of canine oocytes maturation. *Polish Journal of Veterinary Sciences* 15, 199–205.
- Caixeta ES, Sutton-McDowall ML, Gilchrist RB, Thompson JG, Price CA, Machado MF, Lima PF, Buratini J (2013): Bone morphogenetic protein 15 and fibroblast growth factor 10 enhance cumulus expansion, glucose uptake, and expression of genes in the ovulatory cascade during in vitro maturation of bovine cumulus-oocyte complexes. *Reproduction* 146, 27–35.
- Chaves RN, de Matos MH, Buratini J Jr, de Figueiredo JR (2012): The fibroblast growth factor family: involvement in the regulation of folliculogenesis. *Reproduction, Fertility and Development* 24, 905–915.
- Chen AQ, Wang ZG, Xu ZR, Yu SD, Yang ZG (2009a): Analysis of gene expression in granulosa cells of ovine antral growing follicles using suppressive subtractive hybridization. *Animal Reproduction Science* 115, 39–48.
- Chen AQ, Yu SD, Wang ZG, Xu ZR, Yang ZG (2009b): Stage-specific expression of bone morphogenetic protein type I and type II receptor genes: Effects of folli-

- cle-stimulating hormone on ovine antral follicles. *Animal Reproduction Science* 111, 391–399.
- Corduk N, Abban G, Yildirim B, Sarioglu-Buke A (2012): The effect of vitamin D on expression of TGF beta1 in ovary. *Experimental and Clinical Endocrinology and Diabetes* 120, 490–493.
- Derynck R, Jarrett JA, Chen EY, Eaton DH, Bell JR, Assoian RK, Roberts AB, Sporn MB, Goeddel DV (1985): Human transforming growth factor-beta complementary DNA sequence and expression in normal and transformed cells. *Nature* 316, 701–705.
- Dong J, Albertini DF, Nishimori K, Kumar TR, Lu N, Matzuk MM (1996): Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature* 383, 531–535.
- Drummond AE, Le MT, Ethier JE, Dyson M, Findlay JK (2002): Expression and localization of activin receptors, Smads, and beta glycan to the postnatal rat ovary. *Endocrinology* 143, 1423–1433.
- Durinzi KL, Saniga EM, Lanzendorf SE (1995): The relationship between size and maturation in vitro in the unstimulated human oocyte. *Fertility and Sterility* 63, 404–406.
- Eppig JJ (2001): Oocyte control of ovarian follicular development and function in mammals. *Reproduction* 122, 829–838.
- Eppig JJ, O'Brien MJ (1996): Development in vitro of mouse oocytes from primordial follicles. *Biology of Reproduction* 54, 197–207.
- Ergin K, Gursoy E, Basimoglu KY, Basaloglu H, Seyrek K (2008): Immunohistochemical detection of insulin-like growth factor-I, transforming growth factor-beta2, basic fibroblast growth factor and epidermal growth factor-receptor expression in developing rat ovary. *Cytokine* 43, 209–214.
- Feary ES, Juengel JL, Smith P, French MC, O'Connell AR, Lawrence SB, Galloway SM, Davis GH, McNatty KP (2007): Patterns of expression of messenger RNAs encoding GDF9, BMP15, TGFBR1, BMPR1B, and BMPR2 during follicular development and characterization of ovarian follicular populations in ewes carrying the Woodlands FecX2W mutation. *Biology of Reproduction* 77, 990–998.
- Gharibi S, Hajian M, Ostadhosseini S, Hosseini SM, Forouzanfar M, Nasr-Esfahani MH (2013): Effect of phosphodiesterase type 3 inhibitor on nuclear maturation and in vitro development of ovine oocytes. *Theoriology* 80, 302–312.
- Gilchrist RB, Ritter LJ (2011): Differences in the participation of TGFB superfamily signalling pathways mediating porcine and murine cumulus cell expansion. *Reproduction* 142, 647–657.
- Ginsburg M, Snow MH, McLaren A (1990): Primordial germ cells in the mouse embryo during gastrulation. *Development* 110, 521–528.
- Hatzirodos N, Bayne RA, Irving-Rodgers HF, Hummertsch K, Sabatier L, Lee S, Bonner W, Gibson MA, Rainey WE, Carr BR, Mason HD, Reinhardt DP, Anderson RA, Rodgers RJ (2011): Linkage of regulators of TGF-beta activity in the fetal ovary to polycystic ovary syndrome. *Federation of American Societies for Experimental Biology Journal* 25, 2256–2265.
- Hussein TS, Sutton-McDowall ML, Gilchrist RB, Thompson JG (2011): Temporal effects of exogenous oocyte-secreted factors on bovine oocyte developmental competence during IVM. *Reproduction, Fertility and Development* 23, 576–584.
- Irving-Rodgers HF, Friden BE, Morris SE, Mason HD, Brannstrom M, Sekiguchi K, Sanzen N, Sorokin LM, Sado Y, Ninomiya Y, Rodgers RJ (2006): Extracellular matrix of the human cyclic corpus luteum. *Molecular Human Reproduction* 12, 525–534.
- Jain T, Jain A, Kumar P, Goswami SL, De S, Singh D, Datta TK (2012): Kinetics of GDF9 expression in buffalo oocytes during in vitro maturation and their associated development ability. *General and Comparative Endocrinology* 178, 477–484.
- Jayawardana BC, Shimizu T, Nishimoto H, Kaneko E, Tetsuka M, Miyamoto A (2006): Hormonal regulation of expression of growth differentiation factor-9 receptor type I and II genes in the bovine ovarian follicle. *Reproduction* 131, 545–553.
- Joseph C, Hunter MG, Sinclair KD, Robinson RS (2012): The expression, regulation and function of secreted protein, acidic, cysteine-rich in the follicle-luteal transition. *Reproduction* 144, 361–372.
- Jovanovic VP, Sauer CM, Shawber CJ, Gomez R, Wang X, Sauer MV, Kitajewski J, Zimmermann RC (2013): Intraovarian regulation of gonadotropin-dependent folliculogenesis depends on notch receptor signaling pathways not involving Delta-like ligand 4 (Dll4). *Reproductive Biology and Endocrinology* 11, 43.
- Kawano Y, Zeineh K, Furukawa Y, Utsunomiya Y, Okamoto M, Narahara H (2012): The effects of epidermal growth factor and transforming growth factor-alpha on secretion of interleukin-8 and growth-regulated oncogene-alpha in human granulosa-lutein cells. *Gynecologic and Obstetric Investigation* 73, 189–194.
- Kempisty B, Ziolkowska A, Piotrowska H, Ciesiolka S, Antosik P, Bukowska D, Zawierucha P, Wozna M, Jaskowski JM, Brussow KP, Nowicki M, Zabel M (2013): Short-term cultivation of porcine cumulus cells influences the cyclin-dependent kinase 4 (Cdk4) and connexin 43 (Cx43) protein expression-a real-time cell

- proliferation approach. *Journal of Reproduction and Development* 59, 339–345.
- Knight PG, Glistler C (2006): TGF-beta superfamily members and ovarian follicle development. *Reproduction* 132, 191–206.
- Kuo SW, Ke FC, Chang GD, Lee MT, Hwang JJ (2011): Potential role of follicle-stimulating hormone (FSH) and transforming growth factor (TGFbeta1) in the regulation of ovarian angiogenesis. *Journal of Cellular Physiology* 226, 1608–1619.
- Laitinen M, Vuojolainen K, Jaatinen R, Ketola I, Aaltonen J, Lehtonen E, Heikinheimo M, Ritvos O (1998): A novel growth differentiation factor-9 (GDF-9) related factor is co-expressed with GDF-9 in mouse oocytes during folliculogenesis. *Mechanisms of Development* 78, 135–140.
- Lawson KA, Hage WJ (1994): Clonal analysis of the origin of primordial germ cells in the mouse. *Ciba Foundation Symposium* 182, 68–84.
- Levacher C, Gautier C, Saez JM, Habert R (1996): Immunohistochemical localization of transforming growth factor beta 1 and beta 2 in the fetal and neonatal rat ovary. *Differentiation* 61, 45–51.
- Li Q, Pangas SA, Jorgez CJ, Graff JM, Weinstein M, Matzuk MM (2008): Redundant roles of SMAD2 and SMAD3 in ovarian granulosa cells in vivo. *Molecular and Cellular Biology* 28, 7001–7011.
- Li X, Tripurani SK, James R, Pangas SA (2012): Minimal fertility defects in mice deficient in oocyte-expressed Smad4. *Biology of Reproduction* 86, 1–6.
- Linke M, May A, Reifenberg K, Haaf T, Zechner U (2013): The impact of ovarian stimulation on the expression of candidate reprogramming genes in mouse preimplantation embryos. *Cytogenetic and Genome Research* 139, 71–79.
- Martins da Silva SJ, Bayne RA, Cambray N, Hartley PS, McNeilly AS, Anderson RA (2004): Expression of activin subunits and receptors in the developing human ovary: activin A promotes germ cell survival and proliferation before primordial follicle formation. *Developmental Biology* 266, 334–345.
- Mito T, Yoshioka K, Noguchi M, Yamashita S, Hoshi H (2013): Recombinant human follicle-stimulating hormone and transforming growth factor-alpha enhance in vitro maturation of porcine oocytes. *Molecular Reproduction and Development* 80, 549–560.
- Nagamatsu G, Kosaka T, Saito S, Takubo K, Akiyama H, Sudo T, Horimoto K, Oya M, Suda T (2012): Tracing the conversion process from primordial germ cells to pluripotent stem cells in mice. *Biology of Reproduction* 86, 182.
- Nagashima T, Kim J, Li Q, Lydon JP, DeMayo FJ, Lyons KM, Matzuk MM (2011): Connective tissue growth factor is required for normal follicle development and ovulation. *Molecular Endocrinology* 25, 1740–1759.
- Nagyova E (2012): Regulation of cumulus expansion and hyaluronan synthesis in porcine oocyte-cumulus complexes during in vitro maturation. *Endocrine Regulations* 46, 225–235.
- Otsuka F, McTavish KJ, Shimasaki S (2011): Integral role of GDF-9 and BMP-15 in ovarian function. *Molecular Reproduction and Development* 78, 9–21.
- Pangas SA (2012): Regulation of the ovarian reserve by members of the transforming growth factor beta family. *Molecular Reproduction and Development* 79, 666–679.
- Paradis F, Novak S, Murdoch GK, Dyck MK, Dixon WT, Foxcroft GR (2009): Temporal regulation of BMP2, BMP6, BMP15, GDF9, BMP1A, BMP1B, BMP2 and TGFBR1 mRNA expression in the oocyte, granulosa and theca cells of developing preovulatory follicles in the pig. *Reproduction* 138, 115–129.
- Pesce M, Klinger FG, De FM (2002): Derivation in culture of primordial germ cells from cells of the mouse epiblast: phenotypic induction and growth control by Bmp4 signalling. *Mechanisms of Development* 112, 15–24.
- Picton H, Briggs D, Gosden R (1998): The molecular basis of oocyte growth and development. *Molecular and Cellular Endocrinology* 145, 27–37.
- Richards AJ, Enders GC, Resnick JL (1999): Activin and TGFbeta limit murine primordial germ cell proliferation. *Developmental Biology* 207, 470–475.
- Romaguera R, Morato R, Jimenez-Macedo AR, Catala M, Roura M, Paramio MT, Palomo MJ, Mogas T, Izquierdo D (2010): Oocyte secreted factors improve embryo developmental competence of COCs from small follicles in prepubertal goats. *Theriogenology* 74, 1050–1059.
- Ross A, Munger S, Capel B (2007): Bmp7 regulates germ cell proliferation in mouse fetal gonads. *Sexual Development: genetics, molecular biology, evolution, endocrinology, embryology, and pathology of sex determination and differentiation* 1, 127–137.
- Sadatsuki M, Tsutsumi O, Yamada R, Muramatsu M, Taketani Y (1993): Local regulatory effects of activin A and follistatin on meiotic maturation of rat oocytes. *Biochemical and Biophysical Research Communications* 196, 388–395.
- Sanchez F, Smits J (2012): Molecular control of oogenesis. *Biochimica et Biophysica Acta* 1822, 1896–1912.
- Sharma GT, Dubey PK, Kumar GS (2010): Effects of IGF-1, TGF-alpha plus TGF-beta(1) and bFGF on in vitro survival, growth and apoptosis in FSH-stimulated buffalo (Bubalis bubalus) preantral follicles. *Growth Hormone and IGF Research* 20, 319–325.

- Shimizu T, Yokoo M, Miyake Y, Sasada H, Sato E (2004): Differential expression of bone morphogenetic protein 4–6 (BMP-4, -5, and -6) and growth differentiation factor-9 (GDF-9) during ovarian development in neonatal pigs. *Domestic Animal Endocrinology* 27, 397–405.
- Silva CC, Knight PG (1998): Modulatory actions of activin-A and follistatin on the developmental competence of in vitro-matured bovine oocytes. *Biology of Reproduction* 58, 558–565.
- Silva CC, Groome NP, Knight PG (1999): Demonstration of a suppressive effect of inhibin alpha-subunit on the developmental competence of in vitro matured bovine oocytes. *Journal of Reproduction and Fertility* 115, 381–388.
- Sobinoff AP, Sutherland JM, McLaughlin EA (2013): Intracellular signalling during female gametogenesis. *Molecular Human Reproduction* 19, 265–278.
- Songsasen N, Comizzoli P, Nagashima J, Fujihara M, Wildt DE (2012): The domestic dog and cat as models for understanding the regulation of ovarian follicle development in vitro. *Reproduction in Domestic Animals* 47 (Suppl 6), 13–18.
- Spicer LJ, Aad PY, Allen DT, Mazerbourg S, Payne AH, Hsueh AJ (2008): Growth differentiation factor 9 (GDF9) stimulates proliferation and inhibits steroidogenesis by bovine theca cells: influence of follicle size on responses to GDF9. *Biology of Reproduction* 78, 243–253.
- Steffl M, Schweiger M, Amselgruber WM (2008): Expression of transforming growth factor-beta3 (TGF-beta3) in the porcine ovary during the oestrus cycle. *Histology and Histopathology* 23, 665–671.
- Su YQ, Wu X, O'Brien MJ, Pendola FL, Denegre JN, Matzuk MM, Eppig JJ (2004): Synergistic roles of BMP15 and GDF9 in the development and function of the oocyte-cumulus cell complex in mice: genetic evidence for an oocyte-granulosa cell regulatory loop. *Developmental Biology* 276, 64–73.
- Su YQ, Sugiura K, Eppig JJ (2009): Mouse oocyte control of granulosa cell development and function: paracrine regulation of cumulus cell metabolism. *Seminars in Reproductive Medicine* 27, 32–42.
- Tian X, Halfhill AN, Diaz FJ (2010): Localization of phosphorylated SMAD proteins in granulosa cells, oocytes and oviduct of female mice. *Gene Expression Patterns* 10, 105–112.
- Tomic D, Brodie SG, Deng C, Hickey RJ, Babus JK, Malukas LH, Flaws JA (2002): Smad 3 may regulate follicular growth in the mouse ovary. *Biology of Reproduction* 66, 917–923.
- Tse AC, Ge W (2010): Spatial localization of EGF family ligands and receptors in the zebrafish ovarian follicle and their expression profiles during folliculogenesis. *General and Comparative Endocrinology* 167, 397–407.
- Watson LN, Mottershead DG, Dunning KR, Robker RL, Gilchrist RB, Russell DL (2012): Heparan sulfate proteoglycans regulate responses to oocyte paracrine signals in ovarian follicle morphogenesis. *Endocrinology* 153, 4544–4555.
- WS O, Robertson DM, de Kretser DM (1989): Inhibin as an oocyte meiotic inhibitor. *Molecular and Cellular Endocrinology* 62, 307–311.
- Xu Y, Li E, Han Y, Chen L, Xie Z (2010): Differential expression of mRNAs encoding BMP/Smad pathway molecules in antral follicles of high- and low-fecundity Hu sheep. *Animal Reproduction Science* 120, 47–55.
- Ying Y, Zhao GQ (2001): Cooperation of endoderm-derived BMP2 and extra embryonic ectoderm-derived BMP4 in primordial germ cell generation in the mouse. *Developmental Biology* 232, 484–492.
- Ying Y, Liu XM, Marble A, Lawson KA, Zhao GQ (2000): Requirement of Bmp8b for the generation of primordial germ cells in the mouse. *Molecular Endocrinology* 14, 1053–1063.
- Zhu Y, Nilsson M, Sundfeldt K (2010): Phenotypic plasticity of the ovarian surface epithelium: TGF-beta 1 induction of epithelial to mesenchymal transition (EMT) in vitro. *Endocrinology* 151, 5497–5505.

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Corresponding Author:

Bartosz Kempisty, Poznan University of Medical Sciences, Department of Histology and Embryology,
6 Swieczkiego St., 60-781 Poznan, Poland
Tel. + 48 61 8546515, Fax + 48 61 8546510, E-mail: etok@op.pl
