DOI: 10.21005/AAPZ2016.39.3.15

FOLIA POMERANAE UNIVERSITATIS TECHNOLOGIAE STETINENSIS
Folia Pomer. Univ. Technol. Stetin., Agric., Aliment., Pisc., Zootech. 2016, 328(39)3, 181–188

Review article

Wojciech MOŚCICKI

SELECTED FUNCTIONS OF GROWTH FACTORS FROM TGF-β SUPERFAMILY IN REPRODUCTION PROCESSES IN RUMINANTS

WYBRANE FUNKCJE CZYNNIKÓW WZROSTU Z NADRODZINY TGF-β W PROCESACH ROZRODCZYCH U PRZEŻUWACZY

Department of Biotechnology of Animal Reproduction and Environmental Hygiene
West Pomeranian University of Technology, Szczecin, Poland

Streszczenie. Transformujące czynniki wzrostu należą do najważniejszych i najszerzej oddziałujących substancji na funkcjonowanie układu rozrodczego samicy. Badania wielu ośrodków dotyczą szczególnie działania czynników wzrostu na warstwę korową jajnika, która składa się głównie z pęcherzyków jajnikowych i zawartych w nich oocytów. Pomimo wieloletnich badań nadal nie zostało wyjaśnionych wiele kwestii dotyczących samodzielnego wpływu poszczególnych czynników i efektów ich współdziałania. Ze względu na co raz szybszy rozwój prac hodowlanych, ale także ze względu na zapotrzebowanie na większą ilość informacji w kontekście fizjologii rozrodu hodowanych zwierząt w niniejszym artykule postanowiono przedstawić aktualny stan wiedzy na temat wpływu niektórych czynników wzrostu z nadrodziny TGF-β na układ rozrodczy krów, kóz oraz owiec.

Key words: BMP-15, GDF-9, BMP-6, cows, goats, sheep.
Słowa kluczowe: BMP-15, GDF-9, BMP-6, krowy, kozy, owce.

INTRODUCTION

The undoubted progress that has been made in recent years in the range of mammalian reproduction biology opens the new areas in the field of cognitive, as well as research sciences. One such issue is the accurate indication of the role of many families of peptide factors in the regulation of various reproductive processes in ruminants. Therefore, this paper presents the current state of knowledge on certain growth factors from TGF-β superfamily effect on reproduction physiology processes in the discussed animals.

GROWTH FACTORS EFFECT ON REPRODUCTION PROCESSES IN COWS

Transforming growth factors beta (TGF-β) are paracrine growth factors, which released by ovarian stroma or follicles, lead to the transformation of primary follicles to the basal form. In many species of mammals, factors such as bone morphogenetic protein-15 (BMP-15, also

Corresponding author – Adres do korespondencji: Wojciech Mościcki, Department of Biotechnology of Animal Reproduction and Environmental Hygiene, West Pomeranian University of Technology, Szczecin, Doktora Judyma 6, 71-466 Szczecin, Poland, e-mail: wojciech.moscicki@zut.edu.pl

known as GDF-9b), bone morphogenetic protein-6 (BMP-6) and growth and differentiation factor-9 (GDF-9) belonging to the discussed superfamily, may regulate their fertility during folliculogenesis. These include growth factors that secretory activity occurs closest to the oocyte (Knight and Glister 2006; Paradis et al. 2009; Rajput et al. 2013; Hoseini et al. 2014; Stankiewicz and Błaszczyk 2014; Sudiman et al. 2014).

Characteristic features of these growth factors include their individual or synergistic effect on the growth of granular cells as the result of an activity of follicle-stimulating hormone (FSH), or inhibition of atresia and apoptosis of granular cells. Moreover, the secretion of BMP-15 and GDF-9 organizes the phenomena of proliferation, apoptosis, metabolism, differentiation, as well as the expansion and regulation of cumulus oocyte complex necessary to achieve proper development of the ovum. Mutations of BMP-15 and GDF-9 related to the changes in nucleotide composition of DNA, i.e., deletion, may lead to unfavorable modification of reproductive function, as well as infertility in sheep and cattle. Such cases are also observed in human or mice (Hosoe et al. 2011; Karthirvel et al. 2013; Rajput et al. 2013).

One of interesting research was an experiment conducted by Hosoe et al. (2011) which compared the expression patterns of BMP-15 and GDF-9 in the ovarian cells of a calf and cow, in order to determine the relationship between these genes level, and low development of oocytes competence in calves. This experiment involved cattle tissues collected from 9–11 month-old calves and 4–6 year-old cows. The method of quantitative polymerase chain reaction (QPCR) and *in situ* hybridization were used in the study. It was noted in bovine oocytes that mRNA BMP-15 and GDF-9 in particular primary follicles was subject to an expression, which lasted until the 8-cell stage after fertilization, and also new differences in mRNA expression patterns were observed for ova and cumulus layer between calves and cows. In case of oocytes of calves and cows, no differences were found in the amount of intracellular mRNA BMP-15, however in the case of cumulus oophorus cells, mRNA content was higher in adult females. The data presented in the study may suggest that the amount of BMP-15 in the follicles is higher in the case of adult females. Lower development of calves oocyte competences can be explained by deficiency in bone morphogenetic protein-15 in cumulus cells.

Additionally, the authors of the experiment demonstrated that it was probably incorrect to assume that mRNA GDF-9 is not subject to an expression in cumulus cells and mural layer in cattle. The difference in expression may be related to follicle condition in the period of atresia or during the estrous cycle. It was found in the case of GDF-9, that the total number of genes subject to an expression was lower in cumulus cells of calves compared to the cells of cows, like in case of BMP-15. In this case, there is a probability of reduced expression of BMP-15 and GDF-9 in cumulus cells of calves which is associated with the modification of granular cells function. The study also provided the information that higher amount of GDF-9 genes subject to an expression is observed in oocytes of calves.

Having in mind the above experiment, it is worth to analyze the studies which demonstrated biological activity of these both growth factors with the use of various supplementation doses, as well as various combinations during *in vitro* maturation of the ovum in order to determine the effect of BMP-15 and GDF-9 on viable embryos production

after the stage of their transfer. The addition of GDF-9 and/or BMP-15 antagonist to cumulus-oocyte complex led to a significant reduction in blastocyst performance compared to untreated cumulus-oocyte complex (Hussein et al. 2006; Yeo et al. 2008). The exogenous supplementation of both discussed oocyte secretory factors has a significant effect on the development of oocyte competence, in particular after the dose given during the first 9 hours of *in vitro* maturation, when the native oocyte factors more effectively affect the maturation process. Reduced expression of GDF-9 and BMP-15 after 9 hours of bovine oocytes maturation may be associated with a decreased susceptibility of cumulus cells on BMP-15 and GDF-9 signaling after that time. The authors of the study suggest, however, that the positive effect of native oocyte secretory factors may be related to the presence of other oocyte factors that promote oocyte competence (Hussein et al. 2011).

The exact mechanism underlying the control of cumulus cells function by ovum derived from BMP-15 and GDF-9 is not completely understood. However, there is an increasing number of publications demonstrating that growth factors may affect cumulus cells and granular cells by binding to cumulus cells receptors (Sasseville et al. 2010; Pulkki et al. 2011). Activation of intracellular Smad signal is regulated by cumulus oophorus cells under the influence of FSH, which in turn leads to an accelerated growth and development of oocyte competence (Kaivo-oja et al. 2006). In the case of cattle, GDF-9 and BMP15 have a synergistic effect with other ligands in cattle like for example FSH. Such activity helps to promote the developmental competence of oocytes, alter metabolic functions of cumulus cells in the direction of nutrients transfer, as well as other factors in order to enhance the oocyte development (Sutton-McDowall et al. 2012; Rajput et al. 2013).

Interesting are the study on the effect of various forms of BMP-15 (early mature and mature), as well as the mature form of GDF-9, on the development of bovine embryos in the IVM method. It was demonstrated that only the early mature form of BMP-15 led to a significant increase in embryos development compared to the control group. Mature GDF-9 domain was ineffective, and mature form of BMP-15 only led to moderate, but not significant, increase in the efficiency of embryo development. Additionally, Sudiman et al. (2014) decided to use produced, premature form of human GDF-9 which demonstrated an effect on cumulus complex in cattle. However, the use of this protein in the culturing is problematic due to the high affinity to the plastic used in the concave cultures.

The mature domain of TGF-β superfamily proteins is the region binding a bioactive receptor. The pro-mature complex is formed while the mature domain is linked to the pro-region, and it may take the latent or active form, depending on the individual members of the superfamily. Pro-region plays important functions in proteins folding, disulfide bonds formation and biological activity regulation. It was presented in the study of Watson et al. (2012) that the pro-mature BMP-15 complex was very effective in an improvement of oocytes quality during IVM. BMP-15 pro-domains may probably interact with cumulus cells of extracellular matrix during maturation, and facilitate presentation of the mature domain to cumulus cells receptors. Richani et al. (2013) demonstrated significant interactions of somatic cells of the ovary with GDF-9 and BMP-15 pro-domains, which could explain in the future, why isolated mature regions of GDF-9 and BMP-15 have little influence on the proper development of oocytes.

Despite numerous studies demonstrating the positive effect of BMP-15 on embryonic development in cattle, there is no such certainty for GDF-9. Physiological role of bovine GDF-9 in the pituitary also remains to be determined. The same is true in case of BMP-15 expression, which biological significance in follicular somatic cells has not been fully understood. Hosoe et al. (2011) indicate that these factors probably affect the regulation of follicle selection for ovulation.

The growth factor affecting close to the oocyte is BMP-6, that is characterized by an inhibition of FSH and the IGF-induced progesterone production in granular cells in cattle and sheep. Moreover, it was found that it prevents cumulus cells apoptosis in cattle, that are associated with the granular cells of oocyte. BMP-6 also reduces mRNA FSHR expression, and also leads to an increased production of inhibin-A and activin-A (Zhu et al. 2013).

BMP-6 was used to examine the luteinized models of granular cells, and zona pellucida cells in cattle. Observation of BMP-6 and activin-A was related with an inhibition of forskolin--induced progesterone secretion (P^4) by luteinized granular cells, and zona pellucida cells suggesting a negative autocrine/paracrine activity. According to the negative effect on P^4 secretion by granular cells, BMP-6 also reduces forskolin-induced upregulation of mRNA CYP11A1 and HSD3B1 expression. It was observed in the study that luteinized granular cells exhibit weaker expression. An inhibition of forskolin-induced P^4 production was noted in case of luteinized zona pellucida cells. The authors of the study demonstrated that BMP-6 inhibits P^4 production (Kayani et al. 2009).

GROWTH FACTORS EFFECT ON REPRODUCTION PROCESSES IN GOATS

Araújo et al. (2010) examined an effect of BMP-6 on the development of primary follicles of goats in *in vitro* conditions. The experiment involved the samples of ovarian cortex, which were cultured in MEM media for a period of 7 days, and then the condition of follicles was examined using electron microscopy techniques. This study demonstrated a negative effect of BMP-6 on survival and ultrastructure of goats' primary follicles.

The study on periantral follicles in goats conducted by Leitao et al. (2014) demonstrated that they were subject to an increase despite FSH and GDF-9 absence. Goat secondary follicles were exposed to an activity of two above-mentioned factors, alone or together, for a period of six days, which caused an increase in follicles diameter compared to untreated secondary follicles, but not when they were cultured in MEM (minimal essential medium) separately. Most likely, a lack of GDF-9 and/or FSH effect in the early follicular development could have been related to insulin presence in the control medium. The authors, however, assumed that the follicles have a high demand for ascorbic acid in order to produce sufficient and basic amounts of the components of lamin proteins to maintain membrane expansion during its growth. It was demonstrated in the study that antrum formation in goat follicles was stimulated by FSH and GDF-9 after six days from the culturing. Recent findings also demonstrated that the medium with FSH does not stimulate mRNA expression for proteoglycans participating in antrum formation, i.e., for HAS-1, HAS-2, perlecan, versican, in developing follicles in cattle. As demonstrated by the scientists, the presence of GDF-9 in the culturing medium of bovine periantral follicles did not lead to an increase in the number of

follicles, in which antrum formation occurred. It was presented additionally, that GDF-9 addition to the culturing medium of goat secondary follicles after six days of culturing *in vitro* reduces the synthesis of mRNA for BMP-2 and BMP-15, but not for BMP-4, -6, -7, and in the case of FSH alone or in combination with GDF-9 it had no effect on the growth and expression of GDF-9, FSH-R and BMP in secondary follicles.

GROWTH FACTORS EFFECT ON REPRODUCTION PROCESSES IN SHEEP

Nicol et al. (2009) analyzed the mutation in Thoka breed sheep caused by GDF-9. The observed GDF-9 mutation led to the production of non-conservative amino acid changes at position 109 in the mature encoding region (S109R), which is probably responsible for proteins function. According to the researchers, this mutation affects the follicle development leading to infertile homozygosity, as well as reproductive heterozygosity. The ovaries of homozygous TT sheep (with a potential mutation) that were characterized by a very large number of primary follicles, numerous follicles with a single layer of granular cells, and a minimal number of follicles that developed to the primary stage, were used in the experiment. However, there was no formation of thecal cells of follicle zona pellucida in any case, and most of follicles after the primary stage was incorrect. Additionally, the authors indicate that there was no evidences of active cells proliferation or their increased death in primary follicles. The study involved an evaluation of phenotypic effects of the flock at a population level using genetic analysis combined with endocrine and anatomical analyzes, which led to the identification and characterization of new mutation in a particular protein GDF-9. Heterozygous sheep are characterized by an improved fertility, but the mechanisms in which a single wrong copy of GDF-9 leads to this effect has not been explained yet. In homozygous sheep, this mutation causes infertility due to the complete absence of follicles development despite apparently normal oocyte activation and expression of a range of genes, including these specific for oocyte involved in the formation of ZP. Identification of individual amino acids in this study provided new information in the range of structural analysis, modification of GDF-9 or sited of interaction with BMP-15 required to produce biologically active GDF-9.

It was demonstrated in the study by Campbell et al. (2009) that the introduction of BMP-6 to sheep ovary during the early follicular development not only affects the stimulation of ovarian hormones secretion, but also leads to a long-term period in which the follicles of small size produced estradiol, which resulted in premature LH release from pituitary and formation of small corpora lutea. In addition, an increase in the amount of inhibin A and androstenedione was noted. The authors of the study believe that the observed effect is a strong evidence confirming BMP-6 as an important local regulator of ovarian function, and additionally they indicate this growth factor as the future explanation of FecB mutation effects, which leads to dysregulation of follicles selection mechanism to further phases of their growth.

The current development of the reproductive biology of mammals is based mainly on determining the impact of the various elements involved in a wide range of processes affecting the reproductive system Presented in this article studies of research teams show

how difficult and extensive problem is to discover the effects of growth factors alone but also as a result of their cooperation. This leads scientists to intensify research and observation of TGF-β in different species of animals in order to expound the importance of growth factors in the course of reproductive processes occurring in the gonads of females both in physiological conditions as well as *in vitro*. It should also be noted that conducted experiments beyond undoubted increase awareness of the many issues for breeding, can translate into more in-depth analysis and have future impact on improving diagnostic methods in human medicine.

REFERENCES

Araújo V.R., Lima-Verde I.B., Name K.P.O., Báo S.N., Campello C.C., Silva J.R.V., Rodrigues A.P.R., Figueiredo J.R. 2010. Bone Morphogenetic Protein-6 (BMP-6) induces atresia in goat primordial follicles cultured in vitro. Pesquisa Veter. Brasil. 30(9), 770–781.

Campbell B.K., Kendall N.R., Baird D.T. 2009. Effect of direct ovarian infusion of bone morphogenetic protein 6 (BMP6) on ovarian function in sheep. Biol. Reprod. 81(5), 1016–1023.

Hoseini F.S., Mugahi S.M.H.N., Akbari-Asbagh F., Eftekhari-Yazdi P., Aflatoonian B., Aghaee--Bakhtiari S.H., Aflatoonian R., Salsabili N. 2014. A randomized controlled trial of gonadotropin-releasing hormone agonist versus gonadotropin-releasing hormone antagonist in Iranian infertile couples: Oocyte gene expression. DARU J. Pharmac. Sci. DOI: 10.1186/s40199-014-0067-4.

Hosoe M., Kaneyama K., Ushizawa K., Hayashi K., Takahashi T. 2011. Quantitative analysis of bone morphogenetic protein (BMP15) and growth differentiation factor 9 (GDF9) gene expression in calf and adult bovine ovaries. Reprod. Biol. Endocrinol. DOI: 10.1186/1477-7827-9-33.

Hussein T.S., Thompson J.G., Gilchrist R.B. 2006. Oocyte-secreted factors enhance oocyte developmental competence. Develop. Biol. 296(2), 514–521.

Hussein T.S., Sutton-McDowall M.L., Gilchrist R.B., Thompson J.G. 2011. Temporal effects of exogenous oocyte-secreted factors on bovine oocyte developmental competence during IVM. Repr. Fertil. Dev. 23(4), 576–584.

Kaivo-oja N., Jeffery L.A., Rivos O., Mottershead D.G. 2006. Smad signaling in the ovary. Repr. Biol. Endocrinol. DOI: 10.1186/1477-7827-4-21.

Karthirvel M., Soundian E., Kumanan V. 2013. Differential expression dynamics of Growth differentiation factor9 (GDF9) and Bone morphogenetic factor15 (BMP15) mRNA transcripts during in vitro maturation of buffalo (*Bubalus bubalis*) cumulus-oocyte complexes. SpringerPlus. DOI:10.1186/2193-1801-2-206.

Kayani A.R., Glister C., Knight P.G. 2009. Evidence for an inhibitory role of bone morphogenetic protein(s) in the follicular-luteal transition in cattle. Reproduction 137(1), 67–78.

Knight P.G., Glister C. 2006. TGF-β superfamily members and ovaria follicle development. Reproduction 132(2), 191–206.

Leitao C.C.F., Costa J.J.N., Brito I.R., Magalhaes-Padilha D.M., Almeida A.P., Figueiredo J.R., Hurk R., Silva J.R.V. 2014. Effects of GDF-9 and FSH on mRNA Expression for FSH-R, GDF-9 and BMPs in *in vitro* Cultured Goat Preantral Follicles. Braz. Arch. Biol. Technol. 57(2), 200–208.

Nicol L., Bishop S.C., Pong-Wong R., Bendixen C., Holm L.E., Rhind S.M., McNeilly A.S. 2009. Homozygosity for a single base-pair mutation in the oocyte-specific GDF9 gene results in sterility in Thoka sheep. Reproduction 138(6), 921–933.

Paradis F., Novak S., Murdoch G.K., Dyck M.K., Dixon W.T., Foxcroft G.R. 2009. Temporal regulation of BMP2, BMP6, BMP15, GDF9, BMPR1A, BMPR1B, BMPR2 and TGFBR1 mRNA expression in the oocyte granulosa and theca cells of developing preovulatory follicles in the pig. Reproduction 138(1), 115–129.

Pulkki M.M., Myllymaa S., Pasternack A., Lun S., Ludlow H., Al-Qahtani A., Korchynskyi O., Groome N., Juengel J.L., Kalkkinen N., Laitinen M., Ritvos O., Mottershead D.G. 2011. The bioactivity of human bone morphogenetic protein-15 is sensitive to C-terminal modification: Characterization of the purified untagged processed mature region. Mol. Cell. Endocrinol. 322(1–2), 106–115.

Rajput S., Lee K., Zhenhua G., Di L., Kolger J.K., Smith G.W. 2013. Embryotropic actions of follistatin: Paracrine and autocrine mediators of oocyte competence and embryo developmental progession. Reprod. Fertil. Dev. 26(1), 37–47.

Richani D., Ritter L.J., Thompson J.G., Gilchrist R.B. 2013 Mode of oocyte maturation affects EGF-like peptide function and oocyte competence. Mol. Hum. Reprod. 19(8), 500–509.

Sasseville M., Ritter L.J., Nguyen T.M., Liu F., Mottershead D.G., Russell D.L., Gilchrist R.B. 2010. Growth differentiation factor 9 signaling requires ERK 1/2 activity in mouse granulosa and cumulus cells. J. Cell Sci. 123, 3166–3176.

Stankiewicz T., Błaszczyk B. 2014. Concentrations of bone morphogenetic protein-15 (BMP-15) and growth differentiation factor-9 (GDF-9) in follicular cysts, mono- and polyoocyte follicles in gilts. Acta Veter. Beograd 64(1), 24–32.

Sudiman J., Sutton-McDowall M.L., Ritter L.J., White M.A., Mottershead D.G., Thompson J.G., Gilchrist R.B. 2014. Bone morphogenetic protein 15 in the pro-mature complex form enhances bovine oocyte developmental competence. PLOS ONE. DOI: 10.1371/journal.pone.0103563.

Sutton-McDowall M.L., Mottershead D.G., Gardner D.K., Gilchrist R.B., Thmopson J.G. 2012. Metabolic differences in bovine cumulus-oocyte complexes matured in vitro in the presence or absence of follicle-stimulation hormone and bone morphogenetic protein 15. Biol. Reprod. 84(4), 1–8.

Watson L.N., Mottershead D.G., Dunning K.R., Robker R.L., Gilchrist R.B., Russell D.L. 2012. Heparan sulfate proteoglycans regulate responses to oocyte paracrine signals in ovarian follicle morphogenesis. Endocrinology 153(9), 4544–4555.

Yeo Ch. X., Gilchrist R.B., Thompson J.G., Lane M. 2008. Exogenous growth differentiation factor 9 in oocyte maturation media enhances subsequent embryo development and fetal viability in mice. Human Reprod. 23(1), 67–73.

Zhu G., Cui Y., Qinglin-Wang, Yonggang-Kang, Yanzhi-Lv, Wang J., Song Y., Cao B. 2013. Bone morphogenetic proteins (BMP) 2, 4, 6 and 7 affect ovarian follicular .development through regulation of follicle-stimulating hormone receptor (FSHR) and luteinizing hormone receptor (LHR) epression in goat granulosa cells. J. Cell Biol. Genet. 3(1), 14–21.

Abstract. Transforming growth factors are considered as one of the most important substances, widely affecting the functioning of female reproductive system. Special attention of the research conducted in many centers is devoted to detailed description of growth factors effect on the ovarian cortex layer, which mainly consists of ovarian follicles and oocytes contained in them. Despite many years of research in this field, there are still many not explained issues concerning an influence of individual factors, as well as the effects of their interaction. Due to an increasingly faster development of breeding works, but also the need for more information in the context of reproductive physiology of livestock animals, it was decided to present in this article the current state of knowledge concerning the effects of certain growth factors from TGF-β superfamily on reproductive system of cows, goats and sheep.