

GDF-9 Affects the Development and Maturation of Oocytes

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Abstract: Within the intricate regulatory network of the female reproductive system, growth factors secreted by oocytes orchestrate follicular development through sophisticated paracrine signaling mechanisms. Among these factors, the transforming growth factor- β (TGF- β) superfamily serves as a central regulatory hub, playing a pivotal role in this process. Growth differentiation factor 9 (GDF-9), an essential member of the TGF- β superfamily, possesses multifaceted and indispensable biological functions. It not only finely regulates cellular proliferation and differentiation but also participates extensively in the entire course of oocyte growth and maturation, making it a crucial determinant for normal oocyte development. This review focuses on the key gene GDF-9, aiming to provide a comprehensive and in-depth overview of its fundamental characteristics and molecular structure, with particular emphasis on its dynamic expression patterns within ovarian tissues. Furthermore, we explore the complex regulatory mechanisms and specific functions of GDF-9 during the critical process of in vitro oocyte maturation (IVM), in order to offer a more systematic and insightful theoretical foundation and practical reference for research in female reproductive health.

Keywords: TGF- β ; GDF-9; Oocyte; In Vitro Maturation.

1. Introduction

According to the definition provided by the World Health Organization (WHO), infertility is a disease of the reproductive system, defined as the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. Data from the WHO indicate that approximately 48 million couples worldwide are affected by infertility, accounting for about 15% of couples of reproductive age [1]. The incidence of infertility has shown a gradually increasing trend in recent years. This phenomenon not only concerns the reproductive needs of individual families but has also attracted widespread attention at the public health level, making infertility an important issue in the field of global reproductive health that urgently requires further research and solutions. Common causes of infertility include ovulatory dysfunction in women, uterine and fallopian tube diseases, genetic factors, or male factor infertility [2]. Current treatment strategies for infertility include cyclic hormonal therapies or traditional Chinese medicine treatments, which regulate hormone levels and menstrual cycles, improve endometrial receptivity, and thereby facilitate embryo localization, adhesion, invasion, and eventual implantation. In addition to these approaches, tubal recanalization techniques play a crucial role in cases of infertility caused by fallopian tube factors. These techniques remove tubal obstruction and adhesions through physical or surgical methods, thereby restoring tubal patency and normal function. Furthermore, Assisted Reproductive Technology (ART) has become an important therapeutic option for many women with infertility. ART refers to all interventions involving the in vitro handling of human oocytes, sperm, or

embryos for the purpose of reproduction [3]. In Vitro Maturation (IVM) of human oocytes has emerged in recent years as a novel technique in the treatment of infertility, providing a unique therapeutic option for specific patient populations. This approach generally needs to be combined with in vitro fertilization technology to treat infertility [4]. IVM refers to the retrieval of oocytes from small- to medium-sized follicles in the ovary before the mean diameter of the largest follicle exceeds 13 mm. The retrieved oocytes are then cultured in a specific in vitro environment to reach maturation, after which in vitro fertilization is performed. The success of IVM largely depends on the regulation of cytoplasmic maturation, nuclear maturation, and the expression of genes associated with oocyte maturation [5]. Therefore, optimizing the culture conditions of IVM to improve oocyte maturation quality and fertilization capacity has become a key focus and challenge in current research in this field.

The development of female follicles into mature oocytes is a critical step in the realization of female reproductive function. This complex and highly coordinated process is precisely regulated by multiple factors, involving the endocrine system, the local ovarian microenvironment, and the coordinated actions of numerous protein molecules [6]. In recent years, increasing research evidence has provided strong support for the existence of stem cells. These stem cells possess the capacity for self-renewal and differentiation into various cell types and may play an important role in follicular growth and development, providing cellular sources and repair mechanisms for the continuous development of follicles [7]. During follicular growth and development, oocytes acquire the capacity to resume meiosis, achieve complete fertilization, and support early embryonic development [8]. This process depends on the coordinated

action of multiple regulatory factors. In addition to the well-known reproductive hormones such as estrogen, progesterone, Follicle-stimulating hormone (FSH), Anti-Müllerian hormone (AMH), and Luteinizing hormone (LH), as well as their corresponding receptors [9], several protein molecules are also involved in follicular development, oocyte maturation, and ovulation. These include Growth differentiation factor 9 (GDF-9), Bone morphogenetic protein 15 (BMP-15), Insulin-like growth factor 1 (IGF-1), and Octamer-binding transcription factor 4 (Oct4) [10].

Among these factors, Growth differentiation factor 9 (GDF-9) has attracted considerable attention because of its unique functions and critical role. Therefore, this paper focuses on the regulatory mechanisms and roles of GDF-9 in the in vitro maturation of oocytes. An in-depth investigation of GDF-9 will contribute to a comprehensive understanding of the molecular mechanisms underlying oocyte in vitro maturation, provide experimental insights for the regulation of ovarian function and the restoration of fertility, and offer valuable references for inducing ovarian stem cells to develop into mature oocytes.

2. Expression Distribution and Biological Significance of Growth differentiation factor 9 In Vivo

Growth differentiation factor 9 (GDF-9) is an oocyte-derived growth factor and a member of the Transforming growth factor- β superfamily (TGF- β) [11]. Its gene is located on the long arm of chromosome 5, and the synthesis of GDF-9 mRNA is highly specific to oocytes [12]. GDF-9 is considered one of the most important genes involved in oogenesis and is expressed throughout the stages of follicular development and embryogenesis (Figure 1). The expression of the GDF-9 gene increases during the formation of primary oocytes and decreases during follicular development [13]. It is expressed exclusively in oocytes from the primary unilaminar follicle stage to the pre-ovulatory stage [14], and it plays a crucial role in the development of Granulosa cells (GCs) and ovarian folliculogenesis [15].

During the early stages of folliculogenesis, GDF-9 plays a vital role through paracrine signaling. As an oocyte-secreted paracrine factor, GDF-9 not only regulates cumulus expansion but also maintains an optimal microenvironment for oocytes. It participates in regulating the expression of several key granulosa cell enzymes, including cyclooxygenase-2, hyaluronan synthase-2, progesterone, and urokinase-type plasminogen activator. Among these, the production of progesterone and prostaglandins is particularly important, as these substances stimulate cumulus expansion and thereby provide the necessary conditions for effective ovulation, fertilization, and the initiation of uterine implantation [16].

In studies of follicular development, Bone morphogenetic protein 15 (BMP-15) has attracted considerable attention in recent years as a key oocyte-derived growth factor that plays an irreplaceable role in oocyte quality, cumulus cell function, and the ovulatory process. Existing studies have shown that BMP-15 interacts with other factors and enhances oocyte developmental competence through the extension of gap junction coupling between oocytes and cumulus cells, thereby promoting normal follicular growth and maturation [17]. In recent years, the biological significance of the close interaction between BMP-15 and GDF-9 has increasingly

attracted the attention of researchers. Growing evidence suggests that GDF-9 demonstrates stronger predictive ability in reflecting ovarian reserve and follicular developmental dynamics.

In different species, including mice, rats, pigs, sheep, cattle, and deer, the mRNA expression levels of oocyte-derived GDF-9 and BMP-15 are highly correlated, indicating tight synergistic regulation between the genes encoding these two growth factors. However, the ratio of GDF-9 to BMP-15 mRNA expression exhibits significant species specificity. Oocytes from polyovulatory species typically display higher GDF-9/BMP-15 ratios. For example, the ratios in mice and rats are approximately 5.19 and 3.65, respectively. In contrast, species with lower ovulation rates, such as sheep, dairy cattle, and deer, show ratios of approximately 1.26, 0.24, and 0.10, respectively, demonstrating markedly lower GDF-9/BMP-15 ratios [18].

In serum, GDF-9 and BMP-15 levels show a positive correlation. Studies have indicated that, similar to BMP-15, GDF-9 is a stronger predictor of ovarian characteristics, including ovarian volume, antral follicle count, regular menstrual cycles, and Anti-Müllerian hormone (AMH) levels. Moreover, the association of GDF-9 with ovarian characteristics appears to be stronger than that of BMP-15. During primordial follicle activation and follicular development, GDF-9 enhances early follicular differentiation by downregulating the stimulatory function of α -inhibin production [19]. Evidence suggests that GDF-9 mRNA is expressed in primordial and early primary follicle populations, whereas BMP-15 mRNA expression begins at the early secondary follicle stage, with only occasional extremely low expression detected in late primary follicles. Therefore, during human folliculogenesis, GDF-9 expression precedes that of BMP-15 [20]. These findings indicate that GDF-9 may better reflect ovarian reserve than BMP-15 and may serve as a more critical potential biomarker of ovarian function and reserve [21].

Recent studies increasingly demonstrate that high levels of GDF-9 gene expression play an important role in maintaining reproductive capacity. Variations in the GDF-9 gene have been shown to affect the litter size of Mongolian sheep. Specific Single nucleotide polymorphisms (SNPs) in the GDF-9 gene, such as g.46547859C>T and c.1040T>C, can be used as markers for marker-assisted selection to increase the average litter size of Mongolian sheep [22]. Therefore, the significance of GDF-9 extends beyond evaluating oocyte quality. Its expression in vivo decreases with increasing age and has been regarded as a potential biomarker for screening ovarian aging [23].

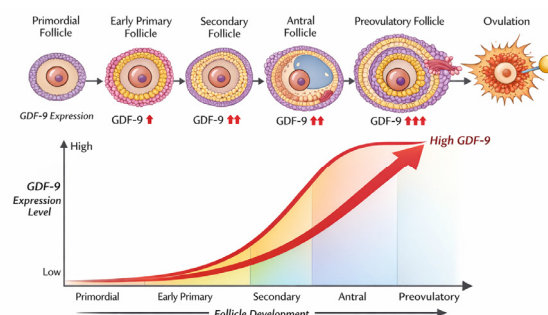


Figure 1. Temporal sequence of GDF-9 expression in oocytes during developmental stages

3. Regulation of the Expression and Functional Activity of Growth differentiation factor 9

Growth differentiation factor 9 (GDF-9) was initially identified as a bone-inducing cytokine that promotes bone and cartilage formation [24]. However, current research has primarily focused on its roles in the development of the female reproductive system. In addition to serving as a biomarker, the principal function of GDF-9 is to regulate folliculogenesis through paracrine and autocrine mechanisms [25]. The regulation of its expression and functional activity can be summarized as follows. GDF-9 regulates follicular development through both paracrine and autocrine pathways. It is mainly secreted by oocytes and acts on surrounding granulosa cells and cumulus cells, regulating their proliferation and differentiation and thereby maintaining normal follicular development. GDF-9 is also capable of regulating the resumption of meiosis in oocytes within preovulatory follicles. After the preovulatory surge of luteinizing hormone (LH), GDF-9 modifies cumulus cell function and activates the mitogen-activated protein kinase pathway, thereby contributing to meiotic resumption [26]. In addition, GDF-9 can directly promote nuclear maturation of oocytes [27]. Gene knockout studies have further demonstrated that follicular development in GDF-9 knockout mice is arrested at the primary follicle stage, indicating that GDF-9 is an essential local factor during the early stages of follicular development.

The expression and functional activity of GDF-9 have also been investigated in other physiological and pathological processes. (1) Prostate cancer: Studies have shown that GDF-9 is expressed in various normal and prostate cancer cell lines as well as in bone tissue. By altering its endogenous expression in human prostate cancer cells, such as PC-3 and DU-145, GDF-9 can promote cell growth by protecting these cells from caspase-3-mediated apoptosis [28]. (2) Gallbladder cancer: GDF-9 expression has also been detected in Gallbladder cancer, with relatively high expression observed in adenocarcinoma cases (50.9%). However, its expression is significantly reduced in poorly differentiated adenocarcinomas, cases with lymph node metastasis, and invasive tumors. Notably, the average survival time of patients with positive GDF-9 expression is significantly longer than that of GDF-9-negative patients [29]. (3) Keloids: The expression of GDF-9 has also attracted attention in Keloid tissues. GDF-9 promotes the proliferation and migration of keloid fibroblasts by regulating the activity of Smad2/3, a mechanism that may be associated with the invasive hyperplasia observed in keloids [30].

4. Effects of Growth Differentiation Factor 9 on the In Vitro Maturation of Oocytes

(1) Effects of Growth differentiation factor 9 on Cumulus cells (CCs)

Cumulus cells (CCs) are somatic cells surrounding the oocyte and play crucial roles in mammalian oocyte growth, meiotic maturation, ovulation, and fertilization. They originate from undifferentiated granulosa cells (GCs) during the transition from primordial follicles to preantral follicles [31]. Recent studies have shown that the developmental and maturation processes of CCs can be influenced by regulating

the expression of Growth differentiation factor 9 (GDF-9). GDF-9 can exert direct effects on CCs and can also act synergistically with other factors to regulate their function. Treatment of cultured CCs with exogenous GDF-9 has been shown to increase blastocyst survival rates, suggesting that GDF-9 - treated oocytes may potentially enhance reproductive success [32]. A modified version of GDF-9, known as super-GDF-9, has been developed in which the receptor-binding region contains unique residues derived from Bone morphogenetic protein 15 (Arg301, Gly304, His307, and Met369). This modification enables efficient activation of the SMAD2/3 signaling pathway. The biological potency of super-GDF-9 is approximately 1000 times greater than that of wild-type human GDF-9. Super-GDF-9 can effectively promote the in vitro expansion of mouse CCs and improve oocyte quality [33]. Furthermore, during standard oocyte in vitro maturation, super-GDF-9 enhances oocyte developmental competence [34], significantly increasing the blastocyst formation rate. In addition, circular RNAs (circRNAs) can upregulate GDF-9 mRNA expression, thereby regulating the functional state of CCs and promoting follicular development and maturation [35]. Oocytes may also enhance the proliferation of CCs and inhibit their apoptosis by increasing the secretion of GDF-9, which upregulates the expression of Bone morphogenetic protein receptor type 2 (BMPR2) and subsequently downregulates the endogenous expression of miR-375 in surrounding CCs [36].

(2) Effects of Growth differentiation factor 9 on Granulosa cells (GCs) in Follicular cells

Granulosa cells (GCs) are the earliest differentiated follicular cells. From the initiation of primordial follicle formation, their differentiation and growth are closely associated with follicular development [37]. Within ovarian tissue, GCs represent the largest cellular population of the follicle. They surround the oocyte and play a pivotal role in determining follicular development and fate. GCs not only protect the oocyte and facilitate bidirectional communication but also possess steroidogenic and endocrine functions [38]. GCs actively participate in all stages of follicular maturation, from primary and secondary oocytes to fully developed follicles. Oocyte-derived Growth differentiation factor 9 (GDF-9) contributes to the formation of the follicular antrum, while the synthesis of GDF-9 is, in turn, regulated by GCs [39]. In patients with poor outcomes following in vitro fertilization and embryo transfer (IVF-ET), GDF-9 expression in both follicular fluid and GCs is significantly reduced [40]. Similarly, in patients with diminished ovarian reserve, GDF-9 expression in GCs is markedly decreased [41]. These findings highlight the reciprocal influence of oocyte-secreted GDF-9 and GCs during follicle development, where crosstalk between the two cell types is essential for their growth and functional competence [42]. Functionally, GDF-9 in follicular development inhibits GC apoptosis and promotes their proliferation. It enhances the responsiveness of GCs to Follicle-stimulating hormone (FSH) and Insulin-like growth factor 1 (IGF-I), increasing estradiol (E2) production and supporting glycolytic activity in oocytes [43]. Downregulation of GDF-9 induces GC apoptosis, reduces proliferation, causes follicular atresia, and inhibits follicular growth [44]. GCs also produce Anti-Müllerian hormone (AMH), and GDF-9 can recruit the coactivator p300 to the AMH promoter via the PI3K/Akt- β type I and II receptors, notably via Activin A receptor type 1B (ACVR1B, ALK5) and Bone Morphogenetic Protein type II receptor (BMPRII),

leading to phosphorylation of Smad2/3. Activated Smad2/3 forms a complex with Smad4 and translocates into the nucleus, where it regulates transcription of downstream target genes. Through this canonical Smad-dependent pathway, GDF-9 not only promotes mitotic activity in GCs but also prevents premature luteinization, ensuring normal follicular maturation and developmental progression [47].

(3) Effects of Growth differentiation factor 9 on the Cumulus-oocyte complex (COCs)

The conventional and widely accepted definition of oocyte in vitro maturation (IVM) [48] refers to the process in which immature cumulus - oocyte complexes (COCs) collected from antral follicles mature from the germinal vesicle (GV) stage to metaphase I (MI), and subsequently, following the recovery of follicles not previously exposed to preovulatory triggers, reach metaphase II (MII) [49]. During this process, cumulus cells remain closely associated with the oocyte, forming the COCs. Numerous signaling interactions within COCs sustain oocyte growth [50]. The extracellular matrix of COCs plays a key role in ovulation and fertilization and serves as a major predictor of oocyte quality [51]. Studies have shown that treatment of COCs with progesterone, combined with coculture with fallopian tube cells, significantly improves both COC development and subsequent embryonic development. Progesterone-treated COCs exhibit upregulated expression of EGFR-related genes, and GDF-9 expression in oocytes is also increased [52]. Earlier research reported that GDF-9 promotes COC development and survival [53]. Furthermore, treatment with L-cysteine during oocyte IVM significantly increases both the number of COCs and the degree of expansion of individual COCs. L-cysteine exerts these effects by activating the oocyte maturation - related gene GDF-9, thereby effectively enhancing both cytoplasmic and nuclear maturation of in vitro oocytes [54]. Recent studies have demonstrated that GDF-9 binds to Bone morphogenetic protein type II receptor/ALK5, triggering phosphorylation of Smad2/3 (p-Smad2/3). Activation of this pathway subsequently upregulates the mRNA and protein expression of estrogen receptor β (ER β) and G protein - coupled estrogen receptor (GPER) in goat COCs, ultimately enhancing COC proliferation [55]. During oocyte IVM, the cumulus - oocyte complex maintains oocyte growth through close intercellular signaling. As a key regulatory factor, GDF-9 promotes COC expansion and improves oocyte maturation quality via multiple signaling pathways.

5. The application of traditional embroidery art in modern fashion design

(1) To strengthen the combination of traditional hand embroidery skills and modern industrialization

In the development of contemporary embroidery art, traditional embroidery is made by hand, so the overall production quality has been greatly guaranteed. However, because of labor, the cost is higher and the efficiency is slower. Facing the contemporary fashion design needs, mass production cannot be carried out. Therefore, it is necessary to meet the needs of consumers, properly carry out industrialization treatment, and make the development of the whole embroidery art serve the marketization, thus providing help for the promotion of artistic influence. For relevant

personnel, let hand embroidery and modern industry be effectively combined. By using intelligent modern manufacturing technology instead of traditional manual methods, embroidery art can develop sustainably. New technologies and processes can improve the quality of embroidery production. Moreover, through the new technology, under the requirements of high standards, it can not only preserve the original aesthetic feeling, but also satisfy people's love for free art. Therefore, when designing clothing, it is necessary to consider whether the machinery and equipment can be produced, print and dye the pattern in the clothing, and form a hollow design, which

6. Key Factors Regulating the Expression of Growth differentiation factor 9

Growth differentiation factor 9 (GDF-9) is a key regulatory factor in germ cell development, and its expression and functional activity are finely controlled by a multilayered regulatory network. Evidence from current studies indicates that the regulation of GDF-9 primarily involves activation of signaling pathways, synergistic interactions with other oocyte-derived growth factors, and transcriptional, post-transcriptional, and epigenetic mechanisms.

At the level of signal transduction, GDF-9 predominantly binds to Activin A receptor type 1B (ALK5) and Bone morphogenetic protein type II receptor (BMPRII) to activate the canonical Smad2/3 signaling pathway. Phosphorylated Smad2/3 forms a complex with Smad4 and translocates into the nucleus, where it regulates the transcription of target genes associated with granulosa cell (GC) and cumulus cell proliferation, differentiation, and apoptosis inhibition. This mechanism maintains normal follicular development and prevents premature luteinization. In specific physiological contexts, GDF-9 can also synergistically activate noncanonical pathways, such as PI3K/Akt, further enhancing its regulatory effects on follicular survival and function, and participates in the regulation of oocyte meiotic resumption during folliculogenesis [56].

Regarding interactions with other growth factors, GDF-9 exhibits a significant synergistic effect with Bone morphogenetic protein 15 (BMP-15), another member of the TGF- β superfamily, during follicular development. Both factors bind to serine/threonine kinase receptors on the GC surface, co-activating the Smad2/3 signaling axis with highly coordinated temporal expression and receptor utilization. This synergy enhances the biological efficacy of the relevant signaling pathways, collectively promoting GC and cumulus cell proliferation, acquisition of oocyte developmental competence, and maintenance of follicular microenvironmental homeostasis. These observations suggest that GDF-9 and BMP-15 have complementary, rather than completely overlapping, functions during folliculogenesis [57].

At the transcriptional and post-transcriptional level, GDF-9 expression is directly regulated by oocyte-specific transcription factors, such as NOBOX and USF1, which precisely modulate its transcriptional activity via the GDF-9 promoter. Additionally, regulatory networks involving circular RNAs (circRNAs) and microRNAs, RNA methylation modifications (e.g., NSUN5-mediated m5C), and histone acetylation act through effects on GDF-9 mRNA stability, translational efficiency, and chromatin accessibility,

finely tuning its expression and functional activity [58]. Beyond endogenous regulation, exogenous pharmacological interventions can also influence GDF-9 expression and biological effects. Studies have shown that sitagliptin and metformin significantly upregulate GDF-9 protein levels in follicles of patients with polycystic ovary syndrome, thereby improving fertilization rates and embryo quality. These findings suggest that GDF-9 may serve as a critical molecular target for pharmacological modulation of ovarian function and reproductive outcomes [59] (Figure 2).

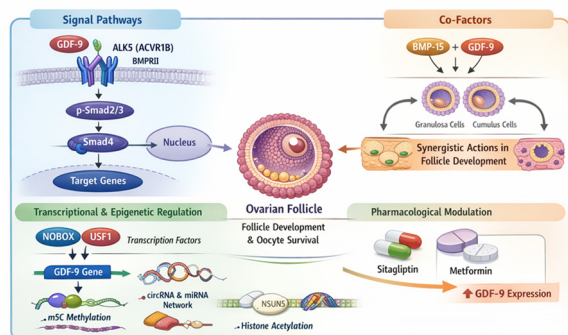


Figure 2. Major factors regulating GDF-9 expression

7. Conclusion

As an oocyte-specific growth factor, Growth differentiation factor 9 (GDF-9) holds landmark academic significance in the field of reproductive biology. Firstly, the synergistic regulatory network between GDF-9 and Bone morphogenetic protein 15 (BMP-15) has elucidated the molecular mechanisms underlying oocyte-granulosa cell communication, providing a critical theoretical framework for understanding the paracrine regulation of follicular development. Secondly, as a biomarker of oocyte quality, studies correlating GDF-9 expression levels with embryonic developmental potential offer a scientific basis for oocyte selection in clinical assisted reproductive technologies, such as in vitro fertilization and embryo transfer (IVF-ET). Moreover, the development of engineered variants, such as super-GDF-9, and the discovery that pharmacological agents, including sitagliptin and metformin, can improve follicular function by modulating GDF-9 expression, have not only expanded the interventional approaches in reproductive endocrinology but also opened new avenues for investigating the interplay between metabolic disorders and reproductive function. Collectively, these advances deepen our understanding of mammalian reproductive regulation and provide important theoretical support and technical groundwork for addressing human fertility disorders and enhancing the success rates of assisted reproductive technologies.

Research on Growth differentiation factor 9 (GDF-9) is currently at a critical transitional stage, shifting from basic mechanistic exploration toward clinical translational applications. In the future, integrated multi-omics analyses, cross-species model validation, and clinical cohort studies are expected to comprehensively elucidate the central role of GDF-9 in reproductive health. These efforts will advance assisted reproductive technologies toward more personalized and precise approaches, ultimately contributing innovative strategies and solutions to address challenges in human reproductive health.

References

- [1] MARIAN S, JACEK S J A M S. Proven and unproven methods for diagnosis and treatment of infertility [J]. 2020, 65(1).
- [2] CARSON S A, KALLEN A N. Diagnosis and Management of Infertility: A Review [J]. *Jama*, 2021, 326(1): 65-76.
- [3] ESTEVES S C, HUMAIDAN P, ROQUE M, et al. Female infertility and assisted reproductive technology [J]. *Panminerva medica*, 2019, 61(1): 1-2.
- [4] CHA K Y, KOO J J, KO J J, et al. Pregnancy after in vitro fertilization of human follicular oocytes collected from nonstimulated cycles, their culture in vitro and their transfer in a donor oocyte program [J]. *Fertility and sterility*, 1991, 55(1): 109-113.
- [5] STRĄCZYŃSKA P, PAPIS K, MORAWIEC E, et al. Signaling mechanisms and their regulation during in vivo or in vitro maturation of mammalian oocytes [J]. *Reproductive biology and endocrinology: RB&E*, 2022, 20(1): 37.
- [6] YANG Q, ZHU L, JIN L. Human Follicle in vitro Culture Including Activation, Growth, and Maturation: A Review of Research Progress [J]. *Frontiers in endocrinology*, 2020, 11: 548.
- [7] LI J, FAN H, LIU W, et al. Mesenchymal stem cells promote ovarian reconstruction in mice [J]. *Stem cell research & therapy*, 2024, 15(1): 115.
- [8] ANDERSON R A, TELFER E E. Being a good egg in the 21st century [J]. *British medical bulletin*, 2018, 127(1): 83-89.
- [9] LIU M N, ZHANG K, XU T M. The role of BMP15 and GDF9 in the pathogenesis of primary ovarian insufficiency [J]. *Human fertility (Cambridge, England)*, 2021, 24(5): 325-332.
- [10] GARCIA P, ASPEE K, RAMIREZ G, et al. Influence of growth differentiation factor 9 and bone morphogenetic protein 15 on in vitro maturation of canine oocytes [J]. *Reproduction in domestic animals = Zuchthygiene*, 2019, 54(2): 373-380.
- [11] FUSHII M, YAMADA R, MIYANO T. In vitro growth of bovine oocytes in oocyte-cumulus cell complexes and the effect of follicle stimulating hormone on the growth of oocytes [J]. *The Journal of reproduction and development*, 2021, 67(1): 5-13.
- [12] DONG J, ALBERTINI D F, NISHIMORI K, et al. Growth differentiation factor-9 is required during early ovarian folliculogenesis [J]. *Nature*, 1996, 383(6600): 531-535.
- [13] YAN C, WANG P, DEMAYO J, et al. Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function [J]. *Molecular endocrinology (Baltimore, Md)*, 2001, 15(6): 854-866.
- [14] LEE Y M, KIM T H, LEE J H, et al. Overexpression of Oct4 in porcine ovarian stem/stromal cells enhances differentiation of oocyte-like cells in vitro and ovarian follicular formation in vivo [J]. *Journal of ovarian research*, 2016, 9: 24.
- [15] JAVVAJI P K, DHALI A, FRANCIS J R, et al. IGF-1 treatment during in vitro maturation improves developmental potential of ovine oocytes through the regulation of PI3K/Akt and apoptosis signaling [J]. *Animal biotechnology*, 2021, 32(6): 798-805.
- [16] MAZERBOURG S, HSUEH A J. Growth differentiation factor-9 signaling in the ovary [J]. *Molecular and cellular endocrinology*, 2003, 202(1-2): 31-36.
- [17] INCERTI B, DONG J, BORSANI G, et al. Structure of the mouse growth/differentiation factor 9 gene [J]. *Biochimica et biophysica acta*, 1994, 1222(1): 125-128.
- [18] ZHU P, LI H, HUANG G, et al. Molecular Cloning, Identification, and Expression Patterns of Myostatin Gene in Water Buffalo (*Bubalus Bubalis*) [J]. *Animal biotechnology*, 2018, 29(1): 26-33.

- [19] ELVIN J A, CLARK A T, WANG P, et al. Paracrine actions of growth differentiation factor-9 in the mammalian ovary [J]. *Molecular endocrinology* (Baltimore, Md), 1999, 13(6): 1035-1048.
- [20] MCGRATH S A, ESQUELA A F, LEE S J. Oocyte-specific expression of growth/differentiation factor-9 [J]. *Molecular endocrinology* (Baltimore, Md), 1995, 9(1): 131-136.
- [21] VERMA K P, THOMPSON B, WOLFE J, et al. A homozygous truncating variant in GDF9 in siblings with primary ovarian insufficiency [J]. *Journal of assisted reproduction and genetics*, 2021, 38(6): 1539-1543.
- [22] ELVIN J A, YAN C, MATZUK M M. Oocyte-expressed TGF-beta superfamily members in female fertility [J]. *Molecular and cellular endocrinology*, 2000, 159(1-2): 1-5.
- [23] DE LOS REYES M, PALOMINO J, ARAUJO A, et al. Cyclooxygenase 2 messenger RNA levels in canine follicular cells: interrelationship with GDF-9, BMP-15, and progesterone [J]. *Domestic animal endocrinology*, 2021, 74: 106529.
- [24] PARAMIO M. Exploring the Potential of Prepubertal Oocytes: A Small Ruminant Model [J]. *Reproduction, fertility, and development*, 2025.
- [25] BELLI M, SHIMASAKI S. Molecular Aspects and Clinical Relevance of GDF9 and BMP15 in Ovarian Function [J]. *Vitamins and hormones*, 2018, 107: 317-348.
- [26] CRAWFORD J L, MCNATTY K P. The ratio of growth differentiation factor 9: bone morphogenetic protein 15 mRNA expression is tightly co-regulated and differs between species over a wide range of ovulation rates [J]. *Molecular and cellular endocrinology*, 2012, 348(1): 339-343.
- [27] CAMPOS L B, SILVA A M, PRAXEDES É C G, et al. Effect of growth differentiation factor 9 (GDF-9) on in vitro development of collared peccary preantral follicles in ovarian tissues [J]. *Animal reproduction science*, 2021, 226: 106717.
- [28] GRØNDAHL M L, BORUP R, VIKESÅ J, et al. The dormant and the fully competent oocyte: comparing the transcriptome of human oocytes from primordial follicles and in metaphase II [J]. *Molecular human reproduction*, 2013, 19(9): 600-617.
- [29] KRISTENSEN S G, ANDERSEN K, CLEMENT C A, et al. Expression of TGF-beta superfamily growth factors, their receptors, the associated SMADs and antagonists in five isolated size-matched populations of pre-antral follicles from normal human ovaries [J]. *Molecular human reproduction*, 2014, 20(4): 293-308.
- [30] ZHANG Y, YAN Z, QIN Q, et al. Transcriptome Landscape of Human Folliculogenesis Reveals Oocyte and Granulosa Cell Interactions [J]. *Molecular cell*, 2018, 72(6): 1021-1034.e1024.
- [31] RIEPSAMEN A H, DONOGHOE M W, INDRAN I R, et al. Serum GDF9 and BMP15 as potential markers of ovarian function in women with and without polycystic ovary syndrome [J]. *Clinical endocrinology*, 2023, 98(4): 567-577.
- [32] TONG B, WANG J, CHENG Z, et al. Novel Variants in GDF9 Gene Affect Promoter Activity and Litter Size in Mongolia Sheep [J]. *Genes*, 2020, 11(4).
- [33] PARK M J, AHN J W, KIM K H, et al. Prediction of ovarian aging using ovarian expression of BMP15, GDF9, and C-KIT [J]. *Experimental biology and medicine* (Maywood, NJ), 2020, 245(8): 711-719.
- [34] WANG E A, ROSEN V, CORDES P, et al. Purification and characterization of other distinct bone-inducing factors [J]. *Proceedings of the National Academy of Sciences of the United States of America*, 1988, 85(24): 9484-9488.
- [35] CHANG H M, QIAO J, LEUNG P C. Oocyte-somatic cell interactions in the human ovary-novel role of bone morphogenetic proteins and growth differentiation factors [J]. *Human reproduction update*, 2016, 23(1): 1-18.
- [36] DE CASTRO F C, CRUZ M H, LEAL C L. Role of Growth Differentiation Factor 9 and Bone Morphogenetic Protein 15 in Ovarian Function and Their Importance in Mammalian Female Fertility - A Review [J]. *Asian-Australasian journal of animal sciences*, 2016, 29(8): 1065-1074.
- [37] NORRIS R P, FREUDZON M, MEHLMANN L M, et al. Luteinizing hormone causes MAP kinase-dependent phosphorylation and closure of connexin 43 gap junctions in mouse ovarian follicles: one of two paths to meiotic resumption [J]. *Development* (Cambridge, England), 2008, 135(19): 3229-3238.
- [38] KONA S S R, KUMAR A, PUNYAKUMARI B, et al. Influence of TCM 199B, α -MEM, Waymouth MB 752/1 culture media, VEGF, Estradiol-17 β , GDF-9 and FGF on in vitro development of preantral follicles in sheep [J]. *Veterinary and animal science*, 2021, 13: 100189.
- [39] BOKOBZA S M, YE L, KYNASTON H G, et al. GDF-9 promotes the growth of prostate cancer cells by protecting them from apoptosis [J]. *Journal of cellular physiology*, 2010, 225(2): 529-536.
- [40] SHU G S, YANG L P, YANG Z L, et al. Expression of CDC6 and GDF-9 and their clinicopathological significances in benign and malignant lesions of the gallbladder [J]. *Cancer biomarkers : section A of Disease markers*, 2012, 11(2-3): 107-114.
- [41] JIANG Z, YU Q, XIA L, et al. Growth Differentiation Factor-9 Promotes Fibroblast Proliferation and Migration in Keloids through the Smad2/3 Pathway [J]. *Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology*, 2016, 40(1-2): 207-218.
- [42] TURATHUM B, GAO E M, CHIAN R C. The Function of Cumulus Cells in Oocyte Growth and Maturation and in Subsequent Ovulation and Fertilization [J]. *Cells*, 2021, 10(9).
- [43] FU Y, JIANG H, LIU J B, et al. Genome-wide analysis of circular RNAs in bovine cumulus cells treated with BMP15 and GDF9 [J]. *Scientific reports*, 2018, 8(1): 7944.
- [44] LIU C, YUAN B, CHEN H, et al. Effects of MiR-375-BMPR2 as a Key Factor Downstream of BMP15/GDF9 on the Smad1/5/8 and Smad2/3 Signaling Pathways [J]. *Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology*, 2018, 46(1): 213-225.
- [45] CHATROUDI M H, KHALILI M A, ASHOURZADEH S, et al. Growth differentiation factor 9 and cumulus cell supplementation in in vitro maturation culture media enhances the viability of human blastocysts [J]. *Clinical and experimental reproductive medicine*, 2019, 46(4): 166-172.
- [46] STOCKER W A, WALTON K L, RICHANI D, et al. A variant of human growth differentiation factor-9 that improves oocyte developmental competence [J]. *The Journal of biological chemistry*, 2020, 295(23): 7981-7991.
- [47] AKIN N, RICHANI D, LIAO X, et al. Effect of cumulin and super-GDF9 in standard and biphasic mouse IVF [J]. *Journal of assisted reproduction and genetics*, 2022, 39(1): 127-140.
- [48] WEI Q, LI J, HE H, et al. miR-23b-3p inhibits chicken granulosa cell proliferation and steroid hormone synthesis via targeting GDF9 [J]. *Theriogenology*, 2022, 177: 84-93.
- [49] TAN W, DAI F, YANG D, et al. MiR-93-5p promotes granulosa cell apoptosis and ferroptosis by the NF-kB signaling pathway in polycystic ovary syndrome [J]. *Frontiers in immunology*, 2022, 13: 967151.
- [50] SHAO T, KE H, LIU R, et al. Autophagy regulates differentiation of ovarian granulosa cells through degradation of WT1 [J]. *Autophagy*, 2022, 18(8): 1864-1878.
- [51] ALAM M H, MIYANO T. Interaction between growing oocytes and granulosa cells in vitro [J]. *Reproductive medicine and biology*, 2020, 19(1): 13-23.
- [52] ALAM M H, LEE J, MIYANO T. GDF9 and BMP15 induce development of antrum-like structures by bovine granulosa

- cells without oocytes [J]. *The Journal of reproduction and development*, 2018, 64(5): 423-431.
- [53] HUANG T H, CHEN F R, ZHANG Y N, et al. Decreased GDF9 and BMP15 in follicle fluid and granulosa cells and outcomes of IVF-ET among young patients with low prognosis [J]. *Journal of assisted reproduction and genetics*, 2023, 40(3): 567-576.
 - [54] OMRIZADEH M, MOKHTARI P, EFTEKHARI-YAZDIP, et al. Altered Expression of GDF9 and BMP15 Genes in Granulosa Cells of Diminished Ovarian Reserve Patients: A Case-Control Study [J]. *Cell journal*, 2022, 24(9): 540-545.
 - [55] LI Y, LI R Q, OU S B, et al. Increased GDF9 and BMP15 mRNA levels in cumulus granulosa cells correlate with oocyte maturation, fertilization, and embryo quality in humans [J]. *Reproductive biology and endocrinology: RB&E*, 2014, 12: 81.
 - [56] HOBEIKA E, ARMOUTI M, KALA H, et al. Oocyte-Secreted Factors Synergize With FSH to Promote Aromatase Expression in Primary Human Cumulus Cells [J]. *The Journal of clinical endocrinology and metabolism*, 2019, 104(5): 1667-1676.
 - [57] SUGIURA K, SU Y Q, DIAZ F J, et al. Oocyte-derived BMP15 and FGFs cooperate to promote glycolysis in cumulus cells [J]. *Development (Cambridge, England)*, 2007, 134(14): 2593-2603.
 - [58] E F, ZHANG H, YIN W, et al. CPEB3 deficiency in mice affect ovarian follicle development and causes premature ovarian insufficiency [J]. *Cell death & disease*, 2021, 13(1): 21.
 - [59] ROY S, GANDRA D, SEGER C, et al. Oocyte-Derived Factors (GDF9 and BMP15) and FSH Regulate AMH Expression Via Modulation of H3K27AC in Granulosa Cells [J]. *Endocrinology*, 2018, 159(9): 3433-3445.
 - [60] MONTE A P O, BEZERRA M É S, MENEZES V G, et al. Involvement of Phosphorylated Akt and FOXO3a in the Effects of Growth and Differentiation Factor-9 (GDF-9) on Inhibition of Follicular Apoptosis and Induction of Granulosa Cell Proliferation After In Vitro Culture of Sheep Ovarian Tissue [J]. *Reproductive sciences (Thousand Oaks, Calif)*, 2021, 28(8): 2174-2185.
 - [61] ZHANG S, LIU H, ZHANG T, et al. Repair of female reproductive function by GDF-9-overexpressing extracellular vesicles via ACVR1B/SMAD2 regulation in ovarian granulosa [J]. *Science advances*, 2025, 11(50): eadw9006.
 - [62] EDWARDS R G. Maturation in vitro of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes [J]. *Nature*, 1965, 208(5008): 349-351.
 - [63] In vitro maturation: a committee opinion [J]. *Fertility and sterility*, 2021, 115(2): 298-304.
 - [64] XIE J, XU X, LIU S. Intercellular communication in the cumulus-oocyte complex during folliculogenesis: A review [J]. *Frontiers in cell and developmental biology*, 2023, 11: 1087612.
 - [65] PATIL K, SHINDE G, HINDUJA I, et al. Compromised Cumulus-Oocyte Complex Matrix Organization and Expansion in Women with PCOS [J]. *Reproductive sciences (Thousand Oaks, Calif)*, 2022, 29(3): 836-848.
 - [66] LEE S H, OH H J, KIM M J, et al. Interaction of the EGFR signaling pathway with porcine cumulus oocyte complexes and oviduct cells in a coculture system [J]. *Journal of cellular physiology*, 2019, 234(4): 4030-4043.
 - [67] GUO J, SHI L, GONG X, et al. Oocyte-dependent activation of MTOR in cumulus cells controls the development and survival of cumulus-oocyte complexes [J]. *Journal of cell science*, 2016, 129(16): 3091-3103.
 - [68] ELGEBALY M M, HAZAA A B M, AMER H A, et al. L-Cysteine improves bovine oocyte developmental competence in vitro via activation of oocyte-derived growth factors BMP-15 and GDF-9 [J]. *Reproduction in domestic animals = Zuchthygiene*, 2022, 57(7): 734-742.
 - [69] TANG Y, LU S, WEI J, et al. Growth differentiation factor 9 regulates the expression of estrogen receptors via Smad2/3 signaling in goat cumulus cells [J]. *Theriogenology*, 2024, 219: 65-74.
 - [70] LIN Z L, LI Y H, XU Y N, et al. Effects of growth differentiation factor 9 and bone morphogenetic protein 15 on the in vitro maturation of porcine oocytes [J]. *Reproduction in domestic animals = Zuchthygiene*, 2014, 49(2): 219-227.
 - [71] FOUNTAS S, PETINAKI E, BOLARIS S, et al. The Roles of GDF-9, BMP-15, BMP-4 and EMMPRIN in Folliculogenesis and In Vitro Fertilization [J]. *Journal of clinical medicine*, 2024, 13(13).
 - [72] BAYNE R A, KINNELL H L, COUTTS S M, et al. GDF9 is transiently expressed in oocytes before follicle formation in the human fetal ovary and is regulated by a novel NOBOX transcript [J]. *PloS one*, 2015, 10(3): e0119819.
 - [73] DATTA T K, RAJPUT S K, WEE G, et al. Requirement of the transcription factor USF1 in bovine oocyte and early embryonic development [J]. *Reproduction (Cambridge, England)*, 2015, 149(2): 203-212.
 - [74] DING C, LU J, LI J, et al. RNA-methyltransferase Nsun5 controls the maternal-to-zygotic transition by regulating maternal mRNA stability [J]. *Clinical and translational medicine*, 2022, 12(12): e1137.
 - [75] GAO L, WANG S, XU J, et al. SET improved oocyte maturation by serine/threonine protein phosphatase 2A and inhibited oocyte apoptosis in mouse oocytes [J]. *Reproductive biology*, 2022, 22(3): 100668.
 - [76] DANESHJOU D, MEHRANJANI M S, ZADEHMODARRES S, et al. Sitagliptin/metformin improves the fertilization rate and embryo quality in polycystic ovary syndrome patients through increasing the expression of GDF9 and BMP15: A new alternative to metformin (a randomized trial) [J]. *Journal of reproductive immunology*, 2022, 150: 103499.
 - [77] Cheng Qiyun, Sun Caixin, Zhang Xiaoxing, et al. Short-Term load forecasting model and method for power system based on complementation of neural network and fuzzy logic [J]. *Transactions of China Electrotechnical Society*, 2004, 19(10): 53-58.
 - [78] Fangfang. Research on power load forecasting based on Improved BP neural network [D]. Harbin Institute of Technology, 2011.
 - [79] Amjady N. Short-term hourly load forecasting using time series modeling with peak load estimation capability [J]. *IEEE Transactions on Power Systems*, 2001, 16(4): 798-805.
 - [80] Ma Kunlong. Short term distributed load forecasting method based on big data [D]. Changsha: Hunan University, 2014.
 - [81] SHI Biao, LI Yu Xia, YU Xhua, YAN Wang. Short-term load forecasting based on modified particle swarm optimizer and fuzzy neural network model [J]. *Systems Engineering-Theory and Practice*, 2010, 30(1): 158-160.