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The Fundamental Role of GDF9 In Mammalian Ovarian Function: A Computational Biology Analysis

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ABSTRACT

Endocrine hormones and other significant paracrine variables regulate ovarian follicle growth from the preantral to the antral stages. Growth differentiation factor 9 (GDF9), which is secreted by oocytes, is a paracrine factor that plays a vital role in follicular development. We performed a computational biology analysis to determine the role of GFD9 in mammalian ovarian function. We constructed a three-dimensional model of GDF9 using I-TASSER and human GDF9 protein sequences obtained from UniProt. We also analyzed GDF9 gene expression levels using GENEVESTIGATOR. Furthermore, we identified the proteins that interact with GDF9 using the Biological General Repository for Interaction Datasets and investigated their network formation using the STRING database. Finally, we performed a pathway analysis for GDF9 using the Kyoto Encyclopedia of Genes and Genomes databases and Wiki Pathways. Our results showed that GDF9 was composed of a protein, signal peptide, and mature protein and was highly expressed in oocytes. The interaction between GDF9 and other proteins was shown to activate various biological processes in the follicle, including the bone morphogenetic protein pathway, SMAD protein signaling, and the regulation of progesterone secretion. We concluded that GDF9 is important for female gonad development, ovulation cycle mechanisms, ovarian follicle growth, and female fertility.

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SR designed the study and wrote the manuscript. MR collected and analyzed the data. Widodo conducted the experiments and wrote the manuscript.

Key words
Bone morphogenetic protein pathway, Folliculogenesis, STRING database Fertility, SMAD protein signaling

INTRODUCTION

The ovary is the primary female reproductive organ, comprising follicles as its functional units. The ovarian follicles consist of somatic components (thecal and granulosa cells) and a germ cell (oocyte) (Edson *et al*., 2009). The granulosa cells are critical to reproductive function because of their role in estradiol and progesterone synthesis (Wen *et al*., 2010), ovulation (Dupuis *et al*., 2013), and the expression of luteinizing hormone receptors (Zhang *et al*., 2018). Thecal cells cannot produce estrogen. Instead, they release androstenedione, which granulosa cells convert to estrogen (Young and McNeilly, 2010).

The ovarian follicles occur in four stages during reproductive life: primordial, primary, preantral, and antral follicles (Hsueh *et al*., 2015), which is controlled by pituitary gonadotropins (Orisaka *et al*., 2009) and paracrine factors (Knight and Glister, 2006). Growth differentiation factor 9 (GDF9) is a paracrine factor released by oocytes during folliculogenesis that supports in the growth, development, and selection of follicles (Knight and Glister, 2006). GDF9 was shown to play a vital role in the process of cumulus expansion of porcine oocytes (Lin *et al*., 2014). In women, the high expression of GDF9 in granulosa cells has a positive correlation with oocyte maturation, successful fertilization, and the oocyte cleavage rate (Li *et al*., 2014), and GDF9 expression in oocytes was found to be reduced in patients with the polycystic ovarian syndrome (PCOS) (Wei *et al*., 2014). Furthermore, GDF9 deficiency in female mice was found to cause infertility because of an early block of folliculogenesis at the primary follicle stage (Elvin *et al*., 1999), and a GDF9 gene mutation was associated with increased fecundity and infertility in ewes (Otsuka *et al*., 2011). Thus, GDF9 is required for optimal follicular development and fertility. In this study, we performed a computational biology analysis to investigate the role of GFD9 in mammalian ovarian function.

MATERIALS AND METHODS

Determination of GDF9 molecule structure

The three-dimensional structure of GDF9 was

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constructed utilizing the I-TASSER server using protein sequences obtained from the UniProt database that were coded as O60383 (GDF9_HUMAN). I-TASSER software is widely used for determining the structure and function of proteins (Roy *et al*., 2010; Yang and Zhang, 2015). We displayed the structure of GDF9 using PyMOL software (Dey *et al*., 2021). The variation information was obtained from the UniProt database, containing information about proteins, their sequences, and variations and other information related to their functions and networks (The UniProt Consortium, 2017).

GDF9 expression

We used Genevestigator (https://genevestigator.com/gv/), a Web-based tool for examining gene expression of various species (Hruz *et al*., 2008), to analyze GDF9 expression levels.

Protein interactions and networks

Proteins interacting with GDF9 were identified using the Biological General Repository for Interaction Datasets (BioGRID) database (https://thebiogrid.org/), which contains >one million biological interactions that are curated from >55,000 publications covering 71 species (Oughtred *et al*., 2019; Chatr-Aryamontri *et al*., 2017) The proteins identified from the BioGRID database as interacting with GDF9 were examined to determine the networks formed using the STRING database (http://string-db.org/) (Szklarczyk *et al*., 2017), which is widely used to determine interactions between molecules and understand the function of protein interactions in cells.

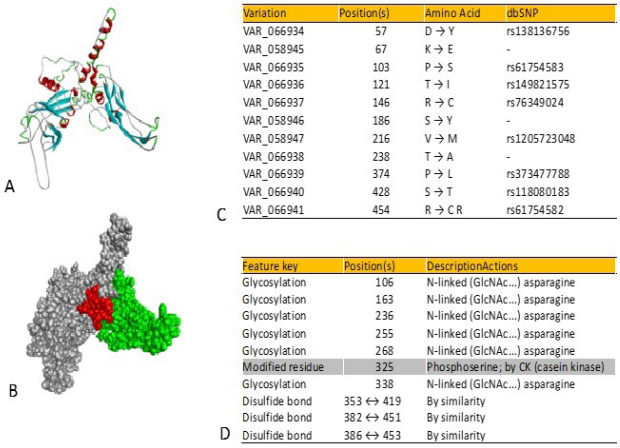
Pathway analysis

The proteins interacting with GDF9 were analyzed using STRING, and their role in cellular mechanisms and pathways was also investigated. The pathway analysis for GDF9 was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/) databases. KEGG has a variety of molecular pathway databases that can be employed to understand gene functions and a collection of gene sequences with up to date annotation of gene functions. The KEGG databases are updated on a daily basis and are open to the public (Kanehisa *et al*., 2017). Moreover, the role of GDF9 in the reproduction process was tracked down from the WikiPathways biological pathways database, which provides curated omics data and is a reliable and rich pathway database (Slenter *et al*., 2018).

RESULTS AND DISCUSSION

GDF9 protein model

The structure of the GDF9 protein was successfully predicted using I-TASSER software, based on O60383 UniProt sequences (GDF9_HUMAN). The GDF9 protein comprised three parts, viz., protein, signal peptide, and mature protein (Fig. 1). The mature GDF9 has a palm-like structure and is predominantly sheet-shaped. The structure of the modeling results resembled those obtained for MBP15 and GDF9 modelling using MODELLER software (https://salilab.org/modeller/) (Monestier *et al*., 2014); both of these proteins are essential for the development of follicle and pellucid zone structures, which are considered the cause of infertility in PCOS (Karagül *et al*., 2018). A homozygous mutation in GDF9 has been shown to cause premature ovarian failure 14, an ovarian disorder defined as the cessation of ovarian function under 40 years old (Laissue *et al*., 2006; Kovanci *et al*., 2007).



Variation	Position(s)	Amino Acid	dbSNP
VAR_066934	57	D → Y	rs138136756
VAR_058945	67	K → E	-
VAR_066935	103	P → S	rs61754583
VAR_066936	121	T → I	rs149821575
VAR_066937	146	R → C	rs76349024
VAR_058946	186	S → Y	-
VAR_058947	216	V → M	rs1205723048
VAR_066938	238	T → A	-
VAR_066939	374	P → L	rs373477788
VAR_066940	428	S → T	rs118080183
VAR_066941	454	R → C R	rs61754582

Feature key	Position(s)	DescriptionActions
Glycosylation	106	N-linked (GlcNAc...) asparagine
Glycosylation	163	N-linked (GlcNAc...) asparagine
Glycosylation	236	N-linked (GlcNAc...) asparagine
Glycosylation	255	N-linked (GlcNAc...) asparagine
Glycosylation	268	N-linked (GlcNAc...) asparagine
Modified residue	325	Phosphoserine; by CK (casein kinase)
Glycosylation	338	N-linked (GlcNAc...) asparagine
Disulfide bond	353 ↔ 419	By similarity
Disulfide bond	382 ↔ 451	By similarity
Disulfide bond	386 ↔ 453	By similarity

Fig. 1. The structure of GDF9 protein. GDF9 protein model (A) Red, helix; cyan, sheet; gray, coil. Components of the GDF9 protein (B) Red, signal peptide; gray, propeptide; green, mature GDF9. Natural variations of GDF9 (C). Protein processing by glycosylation, disulfide bonds, and post-translational modification by creatine kinase phosphorylation as shown in Golgi (D).

GDF9 gene expression

According to genevestigator analysis, GDF9 was shown to be strongly expressed in oocytes, but moderately expressed in lymphocytes and testicular cells (Fig. 2). GDF9 protein belongs to the transforming growth factor-beta (TGF-ß) family. Oocytes and granulosa cells both express it (Knight and Glister, 2006) as well as in many organs, including rabbit liver and kidney (Sun *et al*., 2017), rat testis (Nicholls *et al*., 2009), and sheep hypothalamus and pituitary gland (Tang *et al*., 2018).

GDF9 plays a role in folliculogenesis. Oocyte-expressed GDF9 interacts with BMR2 receptors found in granulosa cells to induce granulosa cell proliferation and differentiation during folliculogenesis (Russell and Robker,

2007). It also helps the cumulus cell act in glycolysis and cholesterol production, which is important for ovulation. Many steroid hormones, including progesterone, are constituted of cholesterol (Sugiura *et al*., 2005). GDF9 also plays a role in expanding cumulus cells by inducing the expression of the hyaluronan synthase 2 gene and the synthesis of hyaluronan and prostaglandin E2, which are essential for normal ovulation (Russell and Robker, 2007).

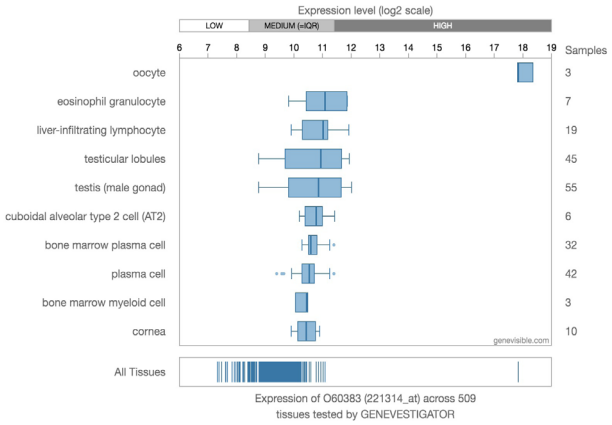
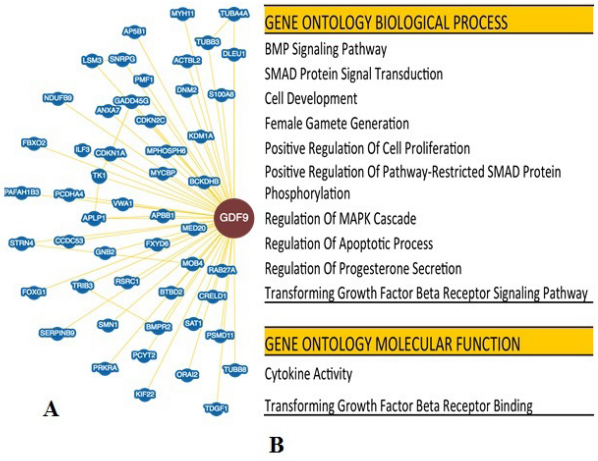


Fig. 2. GDF9 expression levels in different cells and organs, extracted from the Genevestigator database (Hruz *et al*., 2008).

Protein interaction and pathway analysis

The binding of proteins to GDF9 was investigated using the BioGRID database. This analysis was essential to map and resolve the functions of proteins that interact with GDF9. The results could be used in further pathway analysis to help elucidate the role of GDF9 in ovarian function. The results of the BioGRID analysis showed that GDF9 protein interacted with 51 proteins in various biological processes, including the bone morphogenetic protein (BMP) signaling pathway, SMAD protein signaling, and the regulation of progesterone secretion (Fig. 3). Furthermore, biological process analysis showed interactions between several proteins and GDF9 in the BMP signaling pathway (bone morphogenetic protein receptor [BMPR] type 2) (Vitt *et al*., 2002), the regulation of apoptotic processes (amyloid-beta precursor protein-binding family B member 1; dynamin 2; growth arrest and DNA-damage-inducible protein; proteasome 26S subunit, non-ATPase 11; S100 protein A; Tribbles homolog 3; and cyclin-dependent kinase inhibitor 1A) (Vinayagam *et al*., 2011), the TGF-ß receptor signaling pathway, and the regulation of progesterone secretion (c-Myc-binding protein) (Stelzl *et al*., 2005). GDF9, which is secreted by oocytes, is a specific ligand of the TGF-β group and promotes follicular growth and ovulation (Juengel *et al*., 2004). GDF9 also binds to BMPR2 in cumulus cells to activate the SMAD2/3 pathway (Gilchrist *et al*., 2008).



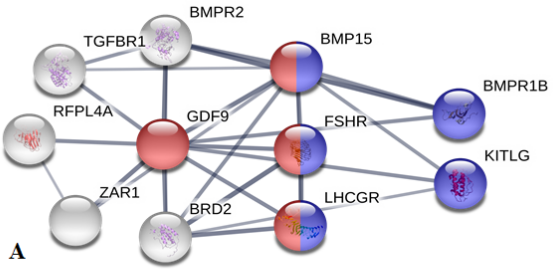
C

Protein	Evidence	Protein	Evidence
ACTBL2	Affinity Capture-MS	MYCBP	Two-hybrid
ANXA7	Two - hybrid	MYH11	Affinity Capture-MS
AP5B1	Affinity Capture-MS	NDUFB9	Two-hybrid
APBB1	Affinity Capture-MS	ORAI2	Two-hybrid
APLP1	Two – hybrid	PAFAH1B3	Two-hybrid
BCKDHB	Affinity Capture-MS	PCDHA4	Two-hybrid
BMPR2	Reconstituted Complex	PCYT2	Two-hybrid
BTBD2	Two – hybrid	PMF1	Two-hybrid
CCDC53	Two – hybrid	PRKRA	Two-hybrid
CDKN1A	Two – hybrid	PSMD11	Two-hybrid
CDKN2C	Two – hybrid	RAB27A	Two-hybrid
CRELD1	Two – hybrid	RSRC1	Two-hybrid
DLEU1	Two – hybrid	S100A8	Two-hybrid
DMN2	Two – hybrid	SAT1	Two-hybrid
FBXO2	Affinity Capture-MS	SERPINB9	Two-hybrid
FOXG1	Two – hybrid	SMN1	Two-hybrid
FXYD6	Two – hybrid	SNRPG	Two-hybrid
GADD45G	Two – hybrid	STRN4	Two-hybrid
GNB2	Affinity Capture-MS	TDGF1	Two-hybrid
ILF3	Two – hybrid	TK1	Two-hybrid
KDM1A	Two – hybrid	TRIB3	Two-hybrid
KIF22	Two – hybrid	TUBA4A	Affinity Capture-MS
LSM3	Two – hybrid	TUBB3	Affinity Capture-MS
MED20	Affinity Capture-MS	TUBB8	Affinity Capture-MS
M0B4	Two – hybrid	VWA1	Affinity Capture-MS
MPHOSPH6	Two – hybrid		

Fig. 3. GDF9-binding proteins. (A) GDF9 protein interaction results from the BioGRID database. (B) The role of GDF9 protein in gene ontology biological processes. (C) List of proteins that interact with GDF9, based on results from the BioGRID database.

The proteins in this network have two pathways associated with ovarian function signaling, ovarian steroidogenesis and cytokine-cytokine receptor interaction. Several proteins play a role in these pathways, including BMP15, follicle stimulating hormone receptor (FSHR), luteinizing hormone/ choriogonadotropin receptor, BMPR1B, and KIT ligand (Fig. 4). These pathways are involved in female gamete generation, oogenesis, and the ovulation cycle (Li *et al*., 2014). Based on the pathway analysis, the results show that GDF9 plays a role in ovarian steroidogenesis and cytokine-cytokine receptor interaction (Bornstein *et al*., 2004).

GDF9 is synthesized and secreted by oocytes to communicate with granulosa cells. GDF9 binds to BMPR and activates cascades via SMAD3 protein in the cytoplasm. SMAD3 protein is an activator capable of regulating several genes, such as Bax and Bcl-2, and is essential for regulating ovarian follicle growth and female fertility (Tomic *et al*., 2002, 2004). Research on Smad3−/− animal models has demonstrated growth retardation of follicles and increased atresia. Smad3 is assumed to interact with follicle-stimulating hormone signaling downstream of FSHR in the mouse ovary (Gilchrist *et al*., 2008) (Fig. 5).



A

Gene ontology biological processes

Gene ontology (GO) term	Pathway description	Count in the gene set	False discovery rate
GO:0008585	female gonad development	5	3.73E-06
GO:0022602	ovulation cycle process	5	3.73E-06
GO:0042698	ovulation cycle	5	3.73E-06
GO:0046545	development of primary female sexual	5	3.73E-06
GO:0046660	female sex differentiation	5	5.09E-06

B

KEGG Pathways

Pathway ID	Pathway description	Count in the gene set	False discovery rate
04913	Ovarian steroidogenesis	4	2.95E-06
04060	Cytokine-cytokine receptor interaction	3	0.04460

Fig. 4. GDF9 protein interaction network. (A) The network of GDF9 interaction based on the STRING database (B) Pathway description of GDF9 based on gene ontology and KEGG pathways.

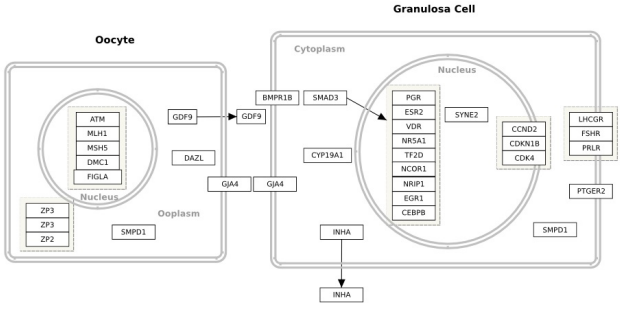


Fig. 5. GDF9 is produced by oocytes. It is involved in communication with granulosa cells and activates various genes via SMAD3 signaling. (Adapted from Ovarian Infertility Genes (Homo sapiens) in Wiki Pathways).

CONCLUSION

GDF9 was highly expressed in oocytes that interact with proteins involved in the biological processes of female gonad development, ovulation cycle processes, and female sexual development. Female gonad development, ovulation cycle processes, ovarian follicle growth, and female fertility are all affected by this protein. GDF9 regulates ovarian follicle formation and female fertility by activating SMAD3 through BMPR.

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Statement of conflict of interest

The authors have declared no conflict of interests.

REFERENCES

Bornstein, S.R., Rutkowski, H. and Vrezas, I., 2004. Cytokines and steroidogenesis. *Mol. Cell. Endocrinol.*, **215**: 135–141. https://doi.org/10.1016/j.mce.2003.11.022

Chatr-Aryamontri, A., Oughtred, R., Boucher, L., Rust, J., Chang, C., Kolas, N.K., O'Donnell, L., Oster, S., Theesfeld, C., Sellam, A., Stark, C., Breitkreutz, B.J., Dolinski, K. and Tyers, M., 2017. The BioGRID interaction database: 2017 update. *Nucl. Acids Res.*, **45**: D369–D379. https://doi.org/10.1093/nar/gkw1102

Dupuis, L., Schuermann, Y., Cohen, T., Siddappa, D., Kalaiselvanraja, A., Pansera, M., Bordignon, V. and Duggavathi, R., 2013. Role of leptin receptors in granulosa cells during ovulation. *Reproduction,* **147**: 221–229. https://doi.org/10.1530/REP-13-0356

Dey, S., Kaushik, G., Mahanta, S. and Chakraborty, A., 2021. Homology modeling of apoprotein Opsin and covalent docking of 11-cis retinal and 11-cis 3, 4-didehyroretinal to obtain structures of Rhodopsin and Porphyropsin from *Zebra danio*, *Danio rerio* (Hamilton, 1822*). Jordan J. Biol. Sci*., **14**: 727-732. https://doi.org/10.54319/jjbs/140413

Edson, M.A., Nagaraja, A.K. and Matzuk, M.M., 2009. The mammalian ovary from genesis to revelation. *Endocr. Rev.,* **30**: 624–712. https://doi.org/10.1210/er.2009-0012

Elvin, J.A., Yan, C., Wang, P., Nishimori, K. and Matzuk, M.M., 1999. Molecular characterization of the follicle defects in the growth differentiation factor

9-deficient ovary. *Mol. Endocrinol*., **13**: 1018-1034. https://doi.org/10.1210/mend.13.6.0309

Gilchrist, R.B., Lane, M. and Thompson, J.G., 2008. Oocyte-secreted factors: Regulators of cumulus cell function and oocyte quality. *Hum. Reprod. Update*, **14**: 159–177. https://doi.org/10.1093/humupd/dmm040

Hruz, T., Laule, O., Szabo, G., Wessendorp, F., Bleuler, S., Oertle, L, Widmayer, P., Gruissem, W. and Zimmermann, P., 2008. Genevestigator V3: A reference expression database for the meta-analysis of transcriptomes. *Adv. Bioinform*., **2008**: 1-5. https://doi.org/10.1155/2008/420747

Hsueh, A.J., Kawamura, K., Cheng, Y. and Fauser, B.C., 2015. Intraovarian control of early folliculogenesis. *Endocr. Rev*., **36**: 1–24. https://doi.org/10.1210/er.2014-1020

Juengel, J.L., Bodensteiner, K.J., Heath, D.A., Hudson, N.L., Moeller, C.L., Smith, P., Galloway, S.M., Davis, G.H., Sawyer, H.R. and McNatty, K.P., 2004. Physiology of GDF9 and BMP15 signaling molecules. *Anim. Reprod. Sci*., **82-83**: 447–460. https://doi.org/10.1016/j.anireprosci.2004.04.021

Kanehisa, M., Furumichi, M., Tanabe. M., Sato, Y. and Morishima, K., 2017. KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucl. Acids Res*., **45**: D353–D361. https://doi.org/10.1093/nar/gkw1092

Karagül, M.İ., Aktaş, S., Yılmaz, B.C., Yılmaz, M. and Temel, G.O., 2018. GDF9 and BMP15 expressions and fine structure changes during folliculogenesis in polycystic ovary syndrome. *Balkan med. J.,* **35**: 43–54. https://doi.org/10.4274/balkanmedj.2016.1110

Knight, P.G. and Glister, C., 2006. TGF-beta superfamily members and ovarian follicle development. *Reproduction*, **132**: 191–206. https://doi.org/10.1530/rep.1.01074

Kovanci, E., Rohozinski, J., Simpson, J.L., Heard, M.J., Bishop, C.E. and Carson, S.A., 2007. Growth differentiating factor-9 mutations may be associated with premature ovarian failure. *Fertil. Steril*., **87**: 143–146. https://doi.org/10.1016/j.fertnstert.2006.05.079

Laissue, P., Christin-Maitre, S., Touraine, P., Kuttenn, F., Ritvos, O., Aittomaki, K., Bourcigaux, N., Jacquesson, L., Bouchard, P., Frydman, R., Dewailly, D., Reyss, A.C., Jeffery, L., Bachelot, A., Massin, N., Fellous, M. and Veitia, R.A., 2006. Mutations and sequence variants in GDF9 and BMP15 in patients with premature ovarian failure. *Eur. J. Endocrinol*., **154**: 739–744. https://doi.org/10.1530/eje.1.02135

Li, Y., Li, R.Q., Ou, S.B., Zhang, N.F., Ren, L., Wei, L.N., Zhang, Q.X. and Yang, D.Z., 2014. Increased GDF9 and BMP15 mRNA levels in cumulus granulosa cells correlate with oocyte maturation, fertilization, and embryo quality in humans. *Reprod. Biol. Endocrinol*., **12**: 1-9. https://doi.org/10.1186/1477-7827-12-81

Lin, Z.L., Li, Y.H., Xu, Y.N., Wang, Q.L., Namgoong, S., Cui, X.S. and Kim, N.H., 2014. Effects of growth differentiation factor 9 and bone morphogenetic protein 15 on the in vitro maturation of porcine oocytes. *Reprod. Domest. Anim*., **49**: 219–227. https://doi.org/10.1111/rda.12254

Monestier, O., Servin, B., Auclair, S., Bourquard, T., Poupon, A., Pascal, G. and Fabre, S., 2014. Evolutionary origin of bone morphogenetic protein 15 and growth and differentiation factor 9 and differential selective pressure between mono- and polyovulating species. *Biol. Reprod.*, **91**: 83-89. https://doi.org/10.1095/biolreprod.114.119735

Nicholls, P.K., Harrison, C.A., Gilchrist, R.B., Farnworth, P.G. and Stanton, P.G., 2009. Growth differentiation factor 9 is a germ cell regulator of Sertoli cell function. *Endocrinology*, **150**: 2481–2490. https://doi.org/10.1210/en.2008-1048

Orisaka, M., Tajima, K., Tsang, B.K. and Kotsuji, F., 2009. Oocyte-granulosa-theca cell interactions during preantral follicular development. *J. Ovar. Res*., **2**: 9-17. https://doi.org/10.1186/1757-2215-2-9

Otsuka, F., McTavish, K.J., and Shimasaki, S., 2011. Integral role of GDF-9 and BMP-15 in ovarian function. *Mol. Reprod. Dev.,* **78**: 9–21. https://doi.org/10.1002/mrd.21265

Oughtred, R., Stark, C., Breitkreutz, B.J., Rust, J., Boucher, L., Chang, C., Kolas, N., O'Donnell, L., Leung, G., McAdam, R., Zhang, F., Dolma, S., Willems, A., Coulombe-Huntington, J., Chatr-Aryamontri, A., Dolinski, K. and Tyers, M., 2019. The BioGRID interaction database: 2019 update. *Nucl. Acids Res*., **47**: D529–D541. https://doi.org/10.1093/nar/gky1079

Roy, A., Kucukural, A. and Zhang, Y., 2010. I-TASSER: A unified platform for automated protein structure and function prediction. *Nat. Protoc*., **5**: 725–738. https://doi.org/10.1038/nprot.2010.5

Russell, D.L. and Robker, R.L., 2007. Molecular mechanisms of ovulation: co-ordination through the cumulus complex. *Hum. Reprod. Update*, **13**: 289–312. https://doi.org/10.1093/humupd/dml062

Slenter, D.N., Kutmon, M., Hanspers, K., Riutta, A., Windsor, J., Nunes, N., Mélius, J., Cirillo, E.,

Coort, S.L., Digles, D., Ehrhart, F., Giesbertz, P., Kalafati, M., Martens, M., Miller, R., Nishida, K., Rieswijk, L., Waagmeester, A., Eijssen, L., Evelo, C.T., Pico, A.R. and Willighagen, E.L., 2018. WikiPathways: A multifaceted pathway database bridging metabolomics to other omics research. *Nucl. Acids Res*., **46**: D661–D667. https://doi.org/10.1093/nar/gkx1064

Stelzl, U., Worm, U., Lalowski, M., Haenig, C., Brembeck, F.H., Goehler, H., Stroedicke, M., Zenkner, M., Schoenherr, A., Koeppen, S., Timm, J., Mintzlaff, S., Abraham, C., Bock, N., Kietzmann, S., Goedde, A., Toksöz, E., Droege, A., Krobitsch, S., Korn, B., Birchmeier, W., Lehrach, H. and Wanker, E.E., 2005. A human protein-protein interaction network: A resource for annotating the proteome. *Cell*, **122**: 957–968. https://doi.org/10.1016/j.cell.2005.08.029

Sugiura, K., Pendola, F.L. and Eppig, J.J., 2005. Oocyte control of metabolic cooperativity between oocytes and companion granulosa cells: Energy metabolism. *Dev. Biol.,* **279**: 20–30. https://doi.org/10.1016/j.ydbio.2004.11.027

Sun, C., Xie, S., Huang, T., Zhang, W., Wang, A., Wang, D., Li, M. and Sun, G., 2017. Molecular characterization and expression of the GDF9 gene in New Zealand white rabbits. *J. Genet.,* **96**: 313–318. https://doi.org/10.1007/s12041-017-0766-y

Szklarczyk, D., Morris, J.H., Cook, H., Kuhn, M., Wyder, S., Simonovic, M., Santos, A., Doncheva, N.T., Roth, A., Bork, P., Jensen, L.J. and von Mering, C., 2017. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. *Nucl. Acids Res*., **45**: D362–D368. https://doi.org/10.1093/nar/gkw937

Tang, J., Hu, W., Di, R., Liu, Q., Wang, X., Zhang, X., Zhang, J. and Chu, M., 2018. Expression analysis of the prolific candidate genes, BMPR1B, BMP15, and GDF9 in small tail han ewes with three fecundity (FecB Gene) genotypes. *Animals (Basel),* **8**: 166. https://doi.org/10.3390/ani8100166

The UniProt Consortium, 2017. UniProt: The universal protein knowledgebase. *Nucl. Acids Res*., **45**: D158–D169. https://doi.org/10.1093/nar/gkh131

Tomic, D., Brodie, S.G., Deng, C., Hickey, R.J., Babus, J.K., Malkas, L.H. and Flaws, J.A., 2002. Smad 3 may regulate follicular growth in the mouse ovary. *Biol. Reprod.,* **66**: 917–923. https://doi.org/10.1095/biolreprod66.4.917

Tomic, D., Miller, K.P., Kenny, H.A., Woodruff, T.K., Hoyer, P. and Flaws, J.A., 2004. Ovarian follicle development requires Smad3. *Mol. Endocrinol.,* **18**: 2224–2240. https://doi.org/10.1095/biolreprod66.4.917

Vinayagam, A., Stelzl, U., Foulle, R., Plassmann, S., Zenkner, M., Timm, J., Assmus, H.E., Andrade-Navarro, M.A. and Wanker, E.E., 2011. A directed protein interaction network for investigating intracellular signal transduction. *Sci. Signal.,* **4**: rs8. https://doi.org/10.1126/scisignal.2001699

Vitt, U.A., Mazerbourg, S., Klein, C. and Hsueh, A.J., 2002. Bone morphogenetic protein receptor type II is a receptor for growth differentiation factor-9. *Biol. Reprod*., **67**: 473–480. https://doi.org/10.1095/biolreprod67.2.473

Wei, L.-N., Huang, R., Li, L.-L., Fang, C., Li, Y. and Liang, X.-Y., 2014. Reduced and delayed expression of GDF9 and BMP15 in ovarian tissues from women with polycystic ovary syndrome. *J. Assist. Reprod. Genet.,* **31**: 1483–1490. https://doi.org/10.1007/s10815-014-0319-8

Wen, X., Li, D., Tozer, A.J., Docherty, S.M. and Iles, R.K., 2010. Estradiol, progesterone, testosterone profiles in human follicular fluid and cultured granulosa cells from luteinized pre-ovulatory follicles. *Reprod. Biol. Endocrinol*., **8**: 1-10. https://doi.org/10.1186/1477-7827-8-117

Yang, J. and Zhang, Y., 2015. I-TASSER server: New development for protein structure and function predictions. *Nucl. Acids Res*., **43**: W174–W181. https://doi.org/10.1093/nar/gkv342

Young, J.M. and McNeilly, A.S., 2010. Theca: The forgotten cell of the ovarian follicle. *Reproduction*, **140**: 489–504. https://doi.org/10.1530/REP-10-0094

Zhang, L., Wang, H., Yu, D., Chen, J., Xing, C., Li, J., Li, J. and Cai, Y., 2018. The effects of mouse ovarian granulosa cell function and related gene expression by suppressing BMP/ Smad signaling pathway. *Anim. Cells Syst.,* **22**: 317–323. https://doi.org/10.1080/19768354.2018.1497706