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# Gene knockouts that affect female fertility: novel targets for contraception

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## TABLE OF CONTENTS

*1. Abstract*
*2. Introduction*
*3. Discussion*
*3.1. Gene knockouts affecting germ cell development, folliculogenesis, and endocrine milieu*
*3.2. Gene knockouts affecting ovulation, fertilization, and post-fertilization embryonic development*
*3.3. Gene knockouts affecting reproductive structures and/or other mechanism*
*4. Conclusions*
*5. Acknowledgement*
*6. References*

## 1. ABSTRACT

The knockout gene technology is an excellent tool to investigate gene function. In 2005, we reviewed 83 gene knockouts that were reported to affect female fertility. This article is an update of the previous review published in early 2005. It describes genes discovered (2004-2010) since the last review that affect female fertility. Using the database search in the Pubmed and Google Scholar search engines, 67 new genes were discovered using knockout technology that have been reported to affect female fertility. These genes were grouped into three main categories based upon the aspect of female reproductive biology that is affected by their knockout. Some of these genes may provide novel targets for developing better methods for contraception and specific diagnosis and treatment of female infertility.

## 2. INTRODUCTION

The human population continues to explode at an alarming rate. The current population is estimated to be over 6.8 billion, which is causing significant health issues. In addition to the population explosion, unintended pregnancies also are posing health hazards worldwide. In the United States, half of all pregnancies are unintended resulting in over 1.2 million elective abortions annually (1,2). Half of these women were using some form of contraceptive. It calls for better and acceptable contraceptive methods (3).

Investigating the genes that affect female fertility may help to provide novel methods for contraception and for diagnosis and treatment of female infertility. We published an earlier review in 2005 on genes that affect

female fertility (4). This article is an update of the genes that were discovered since the last review. The knockout genes presented in this article are from reports spanning from January 1, 2004 through January 19, 2010. The keywords "female infertility/sterility/knockout/knockdown/gene deletion" were used in the Pubmed and Google Scholar search engines. The Pubmed search produced 600 articles; some were repeats. When searching the same keywords in the Google Scholar database, 51,700 articles were found. Again, this number includes many repeats of several articles. Using these two search engines, 67 genes were found to be novel and relevant to female fertility. The genes were then divided into three categories based on which aspect of female reproductive biology was affected by their knockout.

## 3. DISCUSSION

### 3.1. Gene knockouts affecting germ cell development, folliculogenesis, and endocrine milieu

βB2-crystallin (*Crybb2*) gene encodes one of the primary proteins of the ocular lens. It is involved in cataracts, a condition in which lens transparency cannot be maintained and the lens turns opaque. A recent study found that βB2-crystallin is expressed in both female and male mouse reproductive organs, and mice exhibiting the "Philly mutation" (homozygous for a 12 nucleotide inframe deletion mutation) are subfertile (5). Females display less frequent and smaller litters and a 70% reduction in ovarian weight (5). Consequently, *Crybb2*$^{Phil}$ mice on the C57B1/6NHsd genetic background show normal fertility (5). A complete null mutation is needed to further examine its role in reproductive biology (5). Scaffold Attachment Factor B1 (SAFB1) belongs to a family of multifunctional proteins with highly conserved domains. SAFB1 contains an RNA recognition motif and nuclear localization signal. It can bind to RNA processing proteins and RNA polymerase II (6). SAFB1 corepresses estrogen receptor α, which can affect various aspects of reproduction (6). Both *SAFB1*$^{-/-}$ and *SAFB*$^{-/+}$ mice show varying degrees of embryonic and neonatal lethality. If the mice survive, only homozygous mice display growth defects (6). The homozygous knockout exhibits retarded growth, low serum levels of insulin growth factor 1 (IGF1), and infertility/subfertility in both sexes. Females are subfertile, attributable to loss of embryos after fertilization (6). In the *SAFB1*$^{-/-}$ knockout, embryos are found isolated from the oviduct, proposing an oviduct transport defect (6). Other phenotype attributes observed were fewer secondary follicles and atrophy of the ovaries, which suggests a defect in circulating hormone levels (6). Fibroblast growth factor 23 (Fgf-23) has been identified as a factor in numerous isolated renal phosphate wasting disorders, most notably autosomal dominant hypophosphataemic rickets (7). Its biological function is the regulation of phosphate homeostasis and vitamin D metabolism (7). Fgf$^{-/-}$/*h*FGF-23-Tg double mutants were generated to ensure complete knockdown of all endogenous Fgf-23. Premature aging occurs that affects several organ systems of the knockout mice, and lifespan is also reduced (7). Additional phenotype attributes include kyphosis, osteopenia, pulmonary emphysema, tissue calcifications, widespread tissue atrophy, and hypogonadism (7). Hypogonadism, along with overall premature aging of the body, leads to infertility (7).

Aquaporin-4 (AQP-4) is a member of a family of 13 isoforms of integral-membrane proteins (9). Aquaporin-4 is the main water-channel protein of the brain, where it is highly expressed in periventricular areas and in the paraventricular hypothalamic nucleus (9). These areas of the brain are tightly correlated with neuroendocrine function of gonadotropin-releasing hormone, which suggests a possible role in reproductive physiology (9). AQP4-deficienct mice were generated using targeted gene disruption. The homozygous knockout produces fewer and smaller litters. Although deletion does not alter estrus cycling, the knockout has smaller ovaries and fewer oocytes. Fewer antral follicles and corpora lutea are present (9). Additionally, the uteri become less hypertrophic and endometrial thickness is also reduced (9). Leptin receptor (*LEPR*-neuronal) is expressed in several tissues throughout the body. The research that linked *LEPR* to female infertility focused on neuron-specific leptin receptor. Leptin is often called the "satiety hormone" because it can reduce appetite and speed up the expenditure of food through communication with the central nervous system (10). Leptin also is needed to achieve the luteinizing hormone (LH) surge required for ovulation (10). However, the influence of neuronal leptin on luteinizing hormone (LH) surge induction has been questioned; opposing research findings in the past have suggested leptin receptors are not needed in the brain to maintain fertility (10). New research supports neuronal leptin receptor's function in fertility, as neuron-specific *LEPR* female knockout mice show acyclicity, have lower fresh weight of reproductive organs, and are unable to produce a LH surge (10).

GPR54, a member of the G-protein coupled receptor family or more commonly referred to as the "kisspeptin receptor," regulates the onset of puberty and human fertility (12). Its high expression in the pituitary, placenta, and pancreas supports its role as an endocrine axis regulator. More recently, a knockout of *GPR54* indicated its loss blocks LH and gonadotropin-releasing hormone (GnRH) surges (12). Without the release of these hormones, ovulation cannot occur. The kisspeptin-GPR54-GnRH-neuron axis must be functional for routine onset puberty (11). Kisspeptin neurons may have estrogen receptor α (ERα), prolactin receptor (PR), and/or c-FOS present, which engage in positive feedback with the axis to stimulate a LH surge (11). Brain and Muscle ARNT-like protein 1 (*BMAL1*) serves as one of the primary circadian clock genes. Recently, these genes have been gaining significance as important regulators of reproductive function. Both sexes of *BMAL1* knockout mice are infertile because of defective steroidogenesis (13). *SCA2* produces a protein designated as ataxin-2 (Spinocerebellar ataxia type 2). This gene and its protein structure are well-known, but the cellular function remains elusive. Ataxin-2 has been shown to be associated with neurodegenerative diseases. Ataxin-2 is primarily found in the brain, but it has been found in several other tissues (14). Knockout

mice were generated by an exon 1 knockout of *SCA2*, which encodes for about 16.6% of the protein ataxin-2 (14). *SCA2*$^{-/-}$ mice are obese and display insulin resistance, which have been linked to infertility. The *SCA2*$^{-/-}$ female produces smaller litter sizes along with less frequent birthing (14).

The neuropeptide Pituitary adenylate cyclase-activating polypeptide (PACAP) is the most highly conserved member of the glucagon superfamily and can be found in high concentrations in the ovary, hypothalamus, pituitary, and several other areas of the brain. It acts on adenylate cyclase to raise cAMP level in cells. PACAP is expressed in preovulatory follicles, and administration of LH and follicle stimulating hormone (FSH) increases expression of PACAP in granulosa cells (15). Increase in PACAP can cause accumulation of progesterone in granulosa cells which elicits luteinization (15). The physiological effects of PACAP suggest that it may have a role in regulation of ovulation.

Folliculogenesis, fertilization, and first cleavage of *PACAP*$^{-/-}$ mice are normal, but implantation is significantly reduced (15). PACAP may act on various parts of the reproductive system to affect implantation, such as in the ovary affecting progesterone secretion, in the uterus affecting blood flow and decidualization, and in the anterior pituitary regulating prolactin release (15). Nuclear protein transcription regulator 1 **(**NUPR1) is involved in the transient expression of the β subunit of LH. Expression of NUPR1 is higher at later stages of gonadotroph development, which are the specialized cells of the anterior pituitary that produce LH and FSH. LH and FSH are both composed of a common α subunit and unique β subunit. NUPR1 is thought to be involved in stage-specific gonadotroph transcription needed during cellular specification for *Lhb* gene expression (17). Although *NUPR1* knockouts are fertile, they exhibit delayed sexual maturation and absence of corpora lutea (17).

Androgen receptor (AR) has a distinct role in male reproductive physiology, but its role in female reproductive physiology is not clear. Androgen receptor, which is mainly found in the hypothalamus, pituitary, and male and female gonads, is a member of the nuclear receptor superfamily which modulates neuroendocrine function and acts as transcription factors. In a recent study, the whole body knockout of *AR* was generated by an in-frame *Ar* exon 3 deletion (18). Transplants between knockout mice and wild-type mice were also performed; AR knockouts (*AR*KOs) received a wild-type ovary, and wild-type mice received an *AR*KO ovary (18). *AR*$^{-/-}$ mice with wild-type transplants are infertile, although they display a slightly longer but normal estrus cycle (18). *AR*$^{+/+}$ mice with knockout transplants display normal estrus cycles, along with the control mice (18). Defects seem to be due to a neuroendocrine defect, as estradiol and FSH are increased and LH is more vigorously suppressed by estradiol (18). Both transplant groups have less frequent and smaller litters. *AR*$^{-/-}$ females display abnormal uterine histology, with longer horn length and decreased size (14). Transplant groups also have reduced uterine diameters (18). Androgen abnormalities, similar to the phenotypes of ARKO mice, are linked to polycystic ovarian syndrome.

Glucokinase (GK) is an enzyme highly involved in carbohydrate metabolism through its key function of the phosphorylation glucose. This is a critical step in the pathway of deriving adenosine triphosphate (ATP) from carbohydrates. It is primarily expressed in the hypothalamus, other parts of the brain, pancreas, liver, and less so in several other tissues. Accessory roles of glucokinase have recently been studied using knockout mice, and its deletion causes reproductive impairment resulting in reduced litter size and pups with lower birth weight (19). This supports the hypothesis that hypothalamic glucose metabolism is involved in the secretion of gonadotropins (19). The heterozygous knockout of *glucokinase* also exhibits increased food intake, maturity-onset diabetes, increased plasma levels of corticosterone, leptin deficiency, and hypothalamic gene expression similar to that of diabetes (19). Luteinizing hormone subunit β (Lhβ) is a subunit of LH. The β subunit is the specific receptor for this hormone, which differentiates it from other pituitary and placental glycoprotein hormones such as FSH, thyroid-stimulating hormone (TSH), and chorionic gonadotropin (CG). All of the aforementioned hormones share the same α subunit. LH receptors are expressed in Leydig cells in the male, and ovarian granulosa and theca cells in the female. To observe the effects of Lhβ loss, *Lhβ* null mice were generated (20). Female mice exhibit thin uterine horns and hypergonadism (20). Estrus cycles are impaired and normal antral, preovulatory follicles, and corpa lutea are absent (20). The endometrium is thinned, and estradiol and progesterone serum levels are reduced (20).

Response of silent information regulator 2α (Sir2α) is a homolog of the yeast *sir2* gene. The yeast sir2 protein helps maintain telomeric and mating genes in an inactive form. To examine its function in mammals, knockouts were generated. Two allele knockouts are much smaller than wild-type embryos and most consequently die early in postnatal life (21). Chances of survival of one allele knockouts are much higher, but both sexes are sterile (21). Surviving *sir2α* null pups are also smaller at birth, and experience development and growth hindrance (21). Other phenotypic effects include abnormal eye development, neutrophil infiltration of lungs (suggesting infection), pulmonary edema, right ventricular atrophy, and pancreatic atrophy of exocrine epithelium (21). Small ovaries, absent corpora lutea, thin-walled uteri, and hormonal inadequacy are the primary reproductive physiological defects (21). *Sir2α* null mice also seem to be arrested in diestrus (21). Nhlh2, Nascient helix loop helix 2 is a transcription factor which binds as a dimer to E-box motifs in the promoter region of genes, along with other members of the family basic helix-loop-helix domain. Nhlh2 is expressed in the nervous system of developing embryos and in the adult hypothalamus and pituitary (22). Male knockouts of *Nhlh2* are completely infertile while females display an interesting phenotype (22). Females have stringy uteri and late vaginal opening, but some are able to become pregnant and deliver pups (22). However,

null females experience an overall decrease in lifetime pregnancies and a decrease in hormone-induced lordosis behavior and ovulation (22).

Alpha-fetoprotein (AFP), a member of the albumin family of protein, is a serum glycoprotein which is highly expressed in embryos but its expression drastically diminishes after birth. Expression of AFP in adult life is associated with liver pathologies and tumor development. Although there is much debate over its biological function, it is known that AFP can specifically bind estrogen. Human AFP displays antiestrogenic activity (23). It is proposed that this is to sequester estrogen to protect the developing female brain, which is even further supported by its presence in neurons (23). *AFP* double knockouts are sterile. Knockouts display decreased expression of genes in the gonadotropins-releasing hormone pathway and decreased gonadotropin-releasing hormone 1 (Gnrh1) in the hypothalamus (23). This leads to anovulation (23). Interestingly, the sterility can be alleviated with the administration of an aromatase inhibitor (23). ERK1 and ERK2 (extracellular signal-regulated kinases) are coexpressed in all mammals and govern proliferation, differentiation, and oocyte maturation. ERK1/2 are commonly referred to as MAPK3/1, and are present in the phosphorylation cascade to regulate the cell cycle. The crucial LH spike to induce ovulation activates both ERK1 and ERK2. *ERK1* knockout mice thrive and reproduce normally, while *ERK2* knockout mice are embryonic lethal (24). Mice with tissue specific knockouts of *ERK1* and *ERK2* in granulosa and cumulus cells do not ovulate, which causes complete infertility (24). Progesterone is in serum at normal concentrations, but estradiol level is elevated (24).

The appropriately named multidomain protein, Dicer, is an RNAse III endonuclease which produces the enzymatic products of miRNAs and siRNAS. MicroRNAs affect many processes, such as cellular differentiation and proliferation, embryonic development, and apoptosis, through binding to 3'-untranslated regions and coding regions of mRNA to regulate expression (25). Small interfering RNAs (siRNAS) can knockout gene expression and can also mimic the function of miRNAs if they associate with an RNA-inducing silencing complex (RISC) (25). Reported phenotypes of various *Dicer* knockout mice include meiotic defects, luteal deficiency, reduction of equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG), increased number of atretic follicles, improper follicle recruitment, shortened oviduct tubules length, loss of oviduct coils, abnormal histology of the reproductive tract, and impaired embryonic development (25).

Mice have two polyubiquitin B genes (*Ubb*), along with two *Ub*-ribosomal fusion genes. These four functional genes encode products which carry out the programmed destruction of cell cycle regulators by the ubiquitin-proteasome system (27). Sufficient Ub concentrations must be maintained to ensure cell cycle progression with high unidirectional fidelity (27). Homozygous knockouts specific for the *Ubb* gene are infertile (27). $Ubb^{-/-}$ females have smaller ovaries and lack the appropriate number of mature follicles and corpora lutea (27). Even after superovulation with exogenous hormones, the females rarely ovulate normally and if they do, the oocyte is abnormal (27). It seems that *Ubb* is needed during meiotic prophase and oocyte development for fertility (27). Foxl2 (Forkhead box protein L2) is a member of the forkhead transcription factor family. It is highly evolutionarily conserved in females throughout many distant species due to its vital role in regulating ovarian development. In mice, it is expressed in the embryonic eyelids, pituitary, and follicles of the ovary (28). *Foxl2* deletion impairs granulosa cell transition from squamous to cuboidal, which impedes follicle growth (28). Oocyte atresia leads to premature ovarian failure (POF) (28). Foxl2 is also involved in female sex differentiation. Due to its exclusiveness in females, much debate has ensued regarding its classically accepted role as merely an "early ovarian" gene transcription factor (29).

An oocyte-specific *Mgat1* (Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase 1) knockout was created to assess the role of complex or hybrid N-glycans in embryogenesis. *Mgat1* gene produces the protein N-acteylglucosaminyltransferase I, which is needed to initiate complex and hybrid N-glycan production (30). In deficient oocytes, there is complete absence on N-glycans in the zona pellucida,which appears thinner. Mutant females have a lower birth rate, although a full-term pregnancy is possible. Some mutant embryos display remissive development. It seems *Mgat1* is crucial for some aspects of embryogenesis but is not required for fertilization, blastogenesis, or implantation (30). Guanylyl cyclase B receptor (GC-Br) is the receptor for guanylyl cyclase B, the member of the guanylyl cyclase family that has not been intensively studied due to lack of specific inhibitors. The ligand identified for GC-B receptor is C-type natriuretic peptide which is locally expressed in several tissues. To investigate its biological role, the *CG-B receptor* gene was disrupted. The resulting phenotype indicated that it is needed for proper skeletal growth and female reproductive organ maturation (31). All female mice are sterile and show multiple reproductive organ malformations (31). The uterus manifested stringy, abnormal uterine horns and a thin myometrium and endometrium (31). Ovary size is decreased and corpora lutea are absent (31). Follicle development ceases past the secondary follicle stage (31). Effects on other tissues include GC-B2 overexpression in growth-plate chondrocytes and dwarfism, which become more prominent as the mice age (31).

Fatty Acid Desaturase 2 (FADS2) is crucial for the proper synthesis of polyunsaturated fatty acids and eicosanoids. A knockout of FADS2 arrests folliculogenesis, resulting from failure of zona pellicuda formation. The granulosa cells never proliferate to become stratified (as seen in normal follicular maturation) and gap junctions between granulosa cells are diminished (32). FADS2 initiates the cascade of polyunsaturated fatty acid (PUFA) synthesis, which is an important aspect of membrane formation and provide precursors for

eicosanoids. Due to its unspecific enzymatic role, it is found throughout the body. *FADS2* research is gaining attention because it has recently been linked to attention deficient hyperactivity disorder (ADHD), metabolic syndrome, and breast tumors in humans (33, 34). 1,3-phosphoinositide-dependent protein kinase-1 (PDK1) functions as a master kinase, ubiquitously expressed throughout body tissues. It co-binds and activates Akt (protein kinase B) with aid from phosphatidylinositol 3, 4, 5,-trisphosphate ($PIP_3$) (35). Complete depletion of PDK1 elicits death, and its depletion in crucial organs causes serious disease such as heart failure, diabetes, liver failure, and impaired T-cell formation (35). PDK1 depletion in the ovary decreases the lifespan of primordial follicles leading to POF.

Retinoblastoma (Rb) protein binds to and inhibits the E2F transcription factors to prevent the cell cycle from transitioning from G1 to S phase. Cyclin D and E can phosphorylate Rb bound to E2F causing Rb to release E2F, then allows transcription of several genes for cell cycle progression. Although significant for its cell cycle role, Rb protein is also involved in cell differentiation. The investigation of effects of Rb was impossible, as complete deletion of Rb causes embryonic death (36). In the granulosa-cell specific knockout, females show increased infertility due to accelerated follicular recruitment, follicular atresia and apoptosis (36). Knockouts also birth less frequent litters with a decrease in litter size. Altered hormone and/or growth factor input along with abnormal gene expression is the primary cause of the POF (36). Phosphotidylcholine is an important part of biological membranes, and its synthesis is partially regulated by CTP:Phosphocholine cytidylyltransferase (CCT), which includes the isoform of interest CCTβ2. Loss of the more crucial isoform α causes lethality in early embryogenesis while deletion of the β2 isoform harbors significant infertility (37). The homozygous knockout displays a disorganized ovarian structure and failure of normal follicle growth (37). Additional phenotypes include fewer junctions between granulosa cells, decreased penetration of granulosa microvilli through the zona pellucida, distorted Golgi apparatus, and decreased rough endoplasmic reticulum with an increase in free ribosomes (37).

Mouse Y-box protein 2 (MSY2) is a transcriptional coactivator that is part of the highly conserved family of Y-box proteins which regulate mRNA expression. MSY2 is associated with long-term mRNA storage and stabilization. MSY2 accumulates in oocytes prior to fertilization, but is degraded by the late two-cell stage. MSY2 affects oocyte growth, and there is an increase in degree of infertility is seen with an increase in depletion of MSY2 (60-75% protein reduction causes subfertility; 95% reduction causes infertility) (38). Upon reaching sexual maturation, fewer follicles are present in the mutant mice along with granulosa cell disorganization (38). Various other phenotypic attributes are exhibited such as ovarian cysts, lack of cumulus cells, and significant oocyte death (38). The Long Pentraxin 3 (PTX8) is a member of the pentraxin superfamily of proteins that are produced in the liver in response to inflammatory signals, such as interleukin-6 (IL-6) and/or toll-like receptor (TLR). PTX3 is a highly conserved protein found in several tissues, more specifically mononuclear phagocytes, dendritic cells, fibroblasts, and endothelial cells (39). Knockouts of the *PTX3* gene are more prone to infection and are sterile (39). PTX3 causes a disruption in the assembly of the cumulus oophorus hyaluronan-rich extracellular matrix that results in infertility (39).

Lim Homeobox 8 (Lhx8) expresses transcription factor Lim Homeobox Protein 8 in primordial through antral follicles and oocytes. Its function in folliculogenesis is poorly defined, inspiring further assessment of its function through an ovarian-specific *Lhx8* knockout (40). Female newborns show signs of accelerated follicle degeneration and oocyte loss (40). Gene expression of oocyte-specific genes of *Gdf9, Pou5f1, Nobox, Kit,* and *Kitl* is drastically disrupted. (40). *Lhx8p* also aids in regulation of the Nobox pathway (40). Female mice that are homozygous null for the *Lfng* (lunatic fringe) gene are usually infertile. There has been debate over whether *Lfng* is truly a target gene for infertility, as a recent study reported that all female knockout mice were interfile, only to publish later that some were in fact fertile. *Lfng* seems to be associated with infertility, although it may not be associated with complete sterility (37). *Lfng* deletion causes follicle and meiotic maturation defects (41, 42). Lfng, which is found in granulosa cells, is also a crucial mediator of the Notch signaling pathway which has been proven to affect fertility (42). Coilin is the marker protein of the "coiled" Cajal Body which is involved in small ribonucleic protein maturation within the nucleus (43). It is found in all tissues of mice, more significantly in the brain and testis (43). Mice with a mutation in the gene produce less frequent and sizable litters, possibly due to abnormal uterine conditions (43). A definite link between this protein and decreased fertility in unclear.

### 3.2. Gene knockouts affecting ovulation, fertilization, and post-fertilization embryonic development

Although it has been long-known that fertility is adversely affected in neuron-and pituitary specific (*Esr1)* knockout mice, the effect of *Esr1* deletion in the ovary was not examined. The disruption of *Esr1* gene, specifically in theca cells, affects the estrous cycle resulting in complete infertility by age 6 months (45). SULT1E1, encoding estrogen sulfotransferase, is a gene that was first thought to be testis-specific but is now known to be expressed in the placenta, uterus, and ovary (46). Its function is to catalyze the sulfate conjugation of various hormones and biological substrates including estrone (46). Female *SULT1E1* knockout mice have been previously reported to become infertile due to placental defect, but current research suggests that infertility may also arise from impaired ovulation (46). Hurp, hepatoma up-regulated protein (Hurp) is a cell cycle gene which binds to microtubules at its N-terminal region to facilitate attachment of microtubules to the kinetochore of chromosomes (47). Hurp is expressed at high concentration in testis, thymus, and spleen, and at low concentration in ovary, small intestine, and colon (47). Its localization coincides with its

role in cell proliferation. Defects of *hurp* can cause chromosomal misalignment during the metaphase plate, causing failure of mitosis. Recent research supports the role of hurp as both a cell-cycle regulator and also uterine phenotype modulator. *Hurp*$^{-/-}$ female mice display normal reproductive histology and function, except for defects of implantation. *Hurp* $^{-/-}$ female mice fail to implant blastocysts as result of defective decidualization of the uterus (47).

Retina and anterior neural fold homeobox (RAX) protein has the ability to activate the interferon-inducible dsRNA-dependent protein kinase (PKR) and a component of RNA-induced silencing complex (RISC). Various stressors can promote phosphorylation of RAX, which activates PKR and inhibition of eIF2α-dependent protein biosynthesis (48). *PACT* is the human ortholog of *RAX,* and they have 96% similarity (48). Whole body homozygous knockouts of *RAX* are lethal at the pre-implantation (48). Integrin alpha-9 (ITGA-9) which is present in several tissues, is primarily involved in cell:cell and cell:matrix adhesion. Both mouse and human eggs express ITGA-9 and a knockout of this gene decreases surface proteins on the egg (49). Egg integrins seem to be involved in gamete interactions. When ITGA-9 is inhibited 50% or more, there is a significant decrease in sperm-egg binding, and subsequent fusion and fertilization (49). ADAM (disintegrin and metalloprotease on sperm) acts as a ligand to various integrins, as many different integrins are thought to be associated with gamete membrane fusion.

Steroidogenic Factor 1 (SF-1) is a nuclear receptor involved in fetal gonadal development, and a more recently found to be involved in postnatal ovarian function. Mice with gene deletions of *SF-1* exhibit underdeveloped adrenals and gonads, retarded pituitary gonadotropin expression, and irregularities of the ventromedial hypothalamic nucleus (50). The deletion of *SF-1* from rodent granulosa cells, which is naturally found both in human and rodent ovaries, causes detrimental effects in multiple aspects of female reproductive system development and maintenance. Granulosa cell-specific knockouts show highly irregular or absent estrus cycles, underdeveloped ovaries, impaired response to gonadotropins, depletion of follicle reserves, and abnormal histology of the uterus (51). Cytochrome P450 of the family 11, subfamily B, polypeptide 1 (Cyp11b1) is involved in congenital hyperplasia, but it was found that it can cause female infertility. *Cyp11b1* and the related *Cyp11b2* have over 95% similarity and encode glucocorticoid and mineralcorticoid enzymes, the 11β-hydroxylase and aldosterone synthase (52). Deletion of these genes provides a novel mechanism to study congenital hyperplasia, which is landmarked by significant decrease in glucocorticoid synthesis from reduced or abolished 11β-hydroxylase (52). Homozygous females display normal cycling patterns and ovulation although they are infertile (52). Females lack corpora lutea and lobular amorphous cells are present in the ovary, which are possibly non-secreting luteinized granulosa cells (52).

Liver receptor homolog 1 (Lhr1) is a member of the NR5A family which participates in bile metabolism, steroidogenesis, and cell proliferation, and more recently found essential for ovulation (53). Granulosa cell-specific knockout mice of *Lhr1* do not produce pups. They display abnormal estrus patterns and do not display corpora lutea (53). The anovulatory phenotype is not due to lack of gonadotropins, but rather due to abnormal progression of pre-ovulatory events. Unlike wide-type controls, the *Lhr1*-deficient females show no changes in the theca interna post human chorionic gonadotropin (hCG) administration. Additionally, there is an absence of vascularity of the granulosa cells, decrease in mRNA of crucial ovulatory proteases, and underdevelopment of cumulus oophorus (53). TR4 (testicular orphan nuclear receptor 4) is a ligand-activated transcription factor needed for normal spermatogenesis in male mice, and recently found important for optimal female fertility and estrus cycling. It is suspected that it belongs to the estrogen receptor/thyroid hormone receptor subfamily (55).*TR4*$^{-/-}$ females show decrease in litter size and birthing frequency, prolonged and erratic diestrus and estrus cycles, smaller ovaries, decrease in oocyte production, and defective folliculogenesis (54). Other phenotypic attributes of *TR4* knockout mice include increase in granulosa cell apoptosis and reduction in luteinizing hormone receptor expression (54). A decrease in preovulatory follicles, which causes a low ovulation rate, is the primary cause of suboptimal fertility (54).

Follistatin (FST), a protein found either as circulating or tissue-bound isoform, can control the action of activins (which can also affect fertility). Full deletion of the gene causes lethal lung, skin, and musculoskeletal defects. To overcome the lethal effects of a whole body knockout of the gene, a novel method was used in which engineered human *FST* gene was created to express each isoform of FST through endogenous promoters. Using this targeted disruption method, the FST isoforms (circulating FST315 and bound FST228) were introduced (56). Mice expressing FST228 die after birth, and mice expressing FST315 are able to escape lethality although they have various abnormalities (56). Infertility was attributed to shorter uterine horns, malformed vagina, defunct Mullerian duct development, follicle depletion leading to POF, absence of corpora lutea, and possible mating-induced inflammation in the uterus (56). Effect on other tissues include decreased liver and spleen weight at birth, retarded body growth, and abnormal coloration and tail development (56). Cyclin-Dependent Kinase Inhibitor 1B (CDKN1B) or more commonly referred to as *p27* or *p27*$^{kip1}$, is a negative regulator of cell cycle, and is a tumor suppressor gene. Previous findings have shown that p27 is a critical protein involved in ovulation and luteinization in mice. The current study focused on p27 and its role in the PI3K (phosphatidylinositol 3 kinase) pathway and its association with Foxo3a, an infertility-related protein. Knockouts of *p27* display an elevated follicle number possibly due to hypermitotitic activity, accelerated primordial follicle development, and premature follicle recruitment and depletion (57). These findings support the paradigm that

Foxo3a and p27 work independently of one another to suppress early follicle activation (57).

An ovarian-specific knockout of the *Inhbb/Inhba* gene helped clarify the role of activins in female fertility. Activins are now known for their role in FSH biosynthesis and regulation. Double knockouts of *βA/βB* subunits are infertile, while knockouts of *βA* are only subfertile (58). Phenotypic attributes include increased corpora lutea and distorted expression of the c genes in granulosa cells in the double knockout mutants (58). Mice show a decrease in litter size and birthing frequency. Effect on fertility increases with gradient deletion of β-subunit and reaches infertility when a total absence of activins is achieved (58). A disintegrin and metalloproteinase with thrombospondin type 1 motifs-1 (ADAMTS-1) is a protease which cleaves large aggregating molecules of protoeglycans, including versican and aggrecan. It is produced in granulosa cells and cleaves versican in the oocyte cumulus complex. More recently, *ADAMTS-1* has been found to be involved in follicle structural remodeling. Mice with a disruption of the *ADAMTS-1* gene are subfertile (59). Observed dysfunctional phenotypes include improper follicle development (especially when transitioning into the antral stage), disorganized granulosa cell layer, absent theca cell layer, and other folliclular dysgenesis (59).

2′,5′ -oligoadenylate synthetase 1D (OAS1D) is an ovary specific 2′,5′-oligoadenylate synthetase-like protein. It has 59% similarity to OAS1A, which is the protein activated by interferon. Upon interferon activation, OAS1A is induced to bind to dsRNA and convert ATP into a series of 2′,5′-linked oligoadenylates. Conversely, OAS1D can bind to dsRNA but lacks the linking ability. OAS1D actually interferes with the action of OAS1A. *OAS1D*$^{-/-}$ female mice experience impaired follicle development, abnormal ovulation, and one-cell stage arrest of fertilized oocytes (60). Mutant mice are not able to suppress the cellular death induced by OAS1A because OAS1D is not suppressed (60). PAD6, Peptidylarginine deaminase 6, is the newest member of the PAD family which is composed of $Ca^{2+}$-dependent enzymes which modify arginine residues by citrullination. Citrullination is crucial during development and cell differentiation because it modifies protein stability and degradation. PAD6 expression is localized to the mammalian oocyte, sperm cell, early embryo, and is involved in the development of cytoskeletal sheets (61). Oocyte-specific knockout mice were generated to further assess the role of PAD6 in folliculogenesis and embryogenesis. *PAD*-deficient females have no apparent histological abnormalities and cycle normally; however, they are infertile (61). Oocytes progress only to the two-cell stage, implying that the infertility is due to inhibition of embryonic development rather than fertilization (61). The absence of citrullination appears to disperse the cytoskeletal sheets in oocytes causing infertility (61).

Cluster of Differentiation 81 (CD81) is a tetraspanin that is associated with CD9, and the complex also involves a variety of other molecules to form proteolipidic complexes (62). Like most members of the transmembrane 4 superfamily, CD81 is a surface protein involved in development and growth. The role of CD9 as a sperm fusion protein has been reported. Due to the 45% similarity between CD9 and CD81, it is not surprising that CD81 may also be involved in sperm-oocyte fusion and its disruption can affect fertility (62). Although the infertility effect of CD9 loss is more severe, double knockout mice of CD81 display 40% reduction in female fertility (62). The Phosphatidylinositol glycan class-A (*Pig-a*) gene encodes a subunit of N-acetyl glucosaminyl transferase involved in glycosylphosphatidylinositol-anchored protein (GPI-AP) biosynthesis. GIP proteins are found on the egg surface and have been thought to function in gamete fusion. Oocyte-specific knockout mice were generated, as full knockouts are embryonic lethal. Females produce normal numbers of oocytes and display normal mating behavior; however, they do not become pregnant (63). Infertility seems due to failure of sperm binding (63). Additionally, there is a lack of fusion-induced block of polyspermy (63).

Argonaute2 (Ago2) helps direct the function of microRNAs and is a component of the RISC. It binds miRNAs to alter post-transcriptional gene silencing. Argonaute 2 can "slice" targeted mRNA, a specific function different from the other members of the silencing argonaute family (64). Whole body knockout mice of *Ago2* are embryonic lethal, so an oocyte-specific knockout was created (64). MicroRNA expression decreases over 80% and spindle formation is disrupted in mutant oocytes (64). TAp73, a transdomain transcription factor (protein) 73, is encoded by *Trp73* which is a member of the *Trp53* gene family. *Trp73* also encodes ΔNp73, and together these proteins have anti-apoptotic and pro-apoptotic functions. The proteins of the *Trp53* family regulate cell development, death, proliferation, renewal, and tumorigenesis (65). TAp73 displays a pro-apoptotic bias, and *TAp73*$^{-/-}$ mice of both sexes are infertile (65). Although *TAp73*-deficient female mice display normal cyclicity, no oocyte is found in the fallopian tubes after hCG (human choriogonadotropin) injection (65). Deficient mice also produce fewer gametes, and the oocytes are more frequently trapped within luteinizing granulosa cells (65). Abnormal spindle generation and arrest in early cleavage mimic maternal cell aging (65). Many fertility defects found in *TAp73*$^{-/-}$ mice are congruent with defects found in aged mice, and it has been shown that TAp73 diminishes with age (65).

Belonging to the Shugoshin/Mei-S322 family, Shugoshin-2 (SGOL2) maintains the viability of multiprotein cohesion complexes involved in regulation of sister chromatid cohesion. It is found in various species, from yeast to human. *SGOL2* deletion does not disrupt embryogenesis or somatic cell development (66). Histological examination of the ovary shows no phenotypic abnormalities (66). However, *SGOL2*-deficient mice are infertile due to early loss of centromeric cohesion at meiosis, leading to abnormal chromatid number in gametes (66). A member of the tristetraprolin family, Zpf36l2 is zinc-finger protein that binds to the 3'untranslated regions of RNA which is rich in AU sequences. It can destabilize both tumor necrosis factor (TNF) and granulocyte

macrophage – colony stimulating factor (GM-CSF) (67). Female mice with first exon deletion of *Zpf36l2* are completely infertile (67). The females display normal estrus cycling and fertilization, although development of the embryo is impeded at the 2-cell stage (67). Knockout mice generated through homologous recombination, in which majority of the tandem zinc finger domain is deleted, die within several weeks of birth from intestinal hemorrhage and pallor (67). Thus, Zpf36l2 is critical for RNA stability during hematopoiesis.

Lipoma Preferred Partner (LPP) is involved in rearrangement in lipomas and soft tissue tumors. It is primarily found in focal adhesions, where it can bind to vasodilator-stimulated phosphoprotein and alpha-actinin. It then migrates to the nucleus to co-activate polyomavirus enhancer activator 3 (PEA3). PEA3 is associated with breast cancer and has a role pertaining to ejaculation in males. LPP also interacts with the tumor suppressor scribbled homolog, Scrib (68). The *LPP* knockout shows partial lethality (68). $LLP^{-/-}$ females are less fertile as a result of aberrant pregnancies and physiological abnormalities during gestation (68). The corpus luteum expresses cytochrome P450 subfamily XIX (CYP19) and synthesized estradiol, but the specific luteal 17β-hydroxysteroid dehydrogenase involved in this process was not known. This novel enzyme, PRAP/17βHSD-7 (prolactin-receptor associated protein/17β-hydroxysteroid dehydrogenase 7), converts estrone to estradiol and is involved in postsqualene cholesterol synthesis converting zymosterone to zymosterol (69). It has been found in all species investigated thus far including humans. Although pregnant $HSD17B7^{+/-}$ mice display normal development, gross anatomy, and cyclicity, fetuses die *in utero* (69). Embryonic death is attributed to a smaller fetus body size, underdevelopment of the brain, heart and nervous system defects, and increased embryo resorption (69).

Pleomorphic adenoma gene 1 (*Plag1*) is a proto-oncogene transcription factor that has a crucial role in the development of multiple human tumors. Overexpression of *Plag1* as result of promoter swapping leads to oncogenic activation (70). Although its C2H2 zinc-fingers and serine-rich C-terminus structure are well defined, its role in development is not clear. To examine this, both alleles of *Plag1* gene were silenced in embryonic stem cells using homologous recombination. $Plag^{-/-}$ mice are 30% smaller at birth than their wild-type littermates, with proportionately smaller organs and have more difficulty gaining weight (70). Null female mice that mated with wild-type males reproduce normally with a slight reduction in litter size (70). However, when null female and null male mice are mated, conception is infrequent and litter size is greatly reduced (70). Females display normal reproductive organ histology, while males exhibit atrophy in the seminal vesicles and hyperplastic epithelium rich in secretory vesicles (70).

### 3.3. Gene knockouts affecting reproductive structures and/or other mechanisms

THO Complex Subunit 1 (Thoc1) is involved in various aspects of transcription and early ribonucleoprotein (RNP) biogenesis. Its functions include recruitment of RNA processing and export factors, association with RNA POL II, interaction with retinoblastoma protein 1 (Rb1) tumor suppressor gene product, and occupation of promoter proximal regions of genes (71). Complete deletion of *Thoc1* causes early embryonic death; therefore, recent studies are being conducted on mice with a hypomorphic allele of *Thoc1 (*71). $Thoc1^{H/H}$ females display reduced fertility and litter size. The mechanism by which the infertility is caused needs to be further investigated (71). Hyperhomocysteinemia in mice can be caused by a deficiency of the enzyme cystathionine β-synthase (cbs), which is expressed in the liver and decidual tissue (72). A knockout model was generated to further investigate hyperhomocysteinemia and infertility. *Cbs* knockout causes infertility in females, but not in males. Female mutants show increased progesterone levels, decreased litter size, reduced follicle development, increased lipid accumulation in corpora lutea, and shortened and irregular estrus cycling (72). Placental and uterine abnormalities are recorded on day 18 of pregnancy. Further investigation reported that infertility is primarily due to uterine failure (72).

Phospholipase C-β1 (PLC-β1) has a role in a plethora of biological processes. Twelve isoforms of the phospholipase family have been identified, which are involved in transmembrane signal transduction pathways (73). PLC-β1 catalyzes the hydrolytic breakdown of phosphatidylinositol 4,5-bisphosphate ($PIP_2$) into two products: 1,2-diacylglycerol (DAG) and inositol 1,4,5-trisphosphate ($IP_3$) (73). These products activate protein kinase C (PKC) which controls the release of intracellular calcium ion (73). A recent study focused on the phenotypic results of a transgenic line which carries a recessive insertional mutation from integration of the goat βLG transgene in the intron 30 of murine PLC-β1 (73). When mRNA levels for PLC-β1 are significantly reduced, the homozygous mutants have smaller body size at birth which continues throughout life (73). Knockouts also display reduced vitality (73). Females are infertile, which is possibly due to defective oocyte maturation and activation (73). Transforming growth factor β (*TGFβ*) protein family is involved in cellular proliferation and differentiation, and in many aspects of fertility. A member of this family, bone morphogenetic protein (BMP) can induce bone and cartilage generation. TGFβ receptor signals through SMAD 2 and 3 ("AR-SMADS"), while BMP receptors signal through SMAD 1, 5, and 8 ("BR-SMADS") (74). "SMAD" is an acronym derived from the two homologs of the gene, mothers again decapentaplegic (MAD) and the *Caenorhabditis elegans* protein (SMA). After phosphorylation, BR-SMADs can bind to SMAD 4 to regulate gene transcription. A recent study found that the simultaneous knockdown of SMAD 1, 5, and 8 causes infertility and metastatic granulosa cell tumors in female, but not in male mice (74). Double knockdown of *SMAD 1 and 5* causes infertility as well as tumor development (74).

Adrenomedullin (Adm) acts as a vasodilator through its G-protein receptor, calcitonin receptor-like receptor, which must also have receptor activity-modifying

**Table 1.** Gene knockouts that affect female fertility

| **I. gene knockouts affecting germ cell development, folliculogenesis, and endocrine milieu** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **No. Gene** | **Protein** | **Knockout** | **Infertility Target** | **Phenotype** | **Effect on other tissues** | **Phenotype** | **Effect on other tissues** | **Ref.** |
| | **Name** | **Function** | **Localization** | | | | | |
| *1. Crybb2* | **βB2-cry**stallin | Major protein of ocular lens; involved in maintaining transparency | Ocular lens, retina, brain, testis | "philly mutation" in outbred Swiss Webster colony | Ovary | 70% decrease in ovarian mass due to lack of developing follicles; subfertility could be strain-specific | In males, defective testis function; cataracts | 5 |
| *2. SAFB1* | **S**caffold **A**ttachment **F**actor **B1** | Multifunctional protein which binds to RNA processing proteins and RNA polymerase II | Multiple tissues | Homozygous and heterozygous knockout | Oviduct & endocrine milieu | Alteration of hormones, possible oviduct transport defect, and partial neonatal lethality | Retarded growth and low level of insulin-like growth factor 1 (IGF1) | 6 |
| *3. Fgf-23* | **F**ibroblast **g**rowth **f**actor **23** | Vitamin D metabolism and phosphate homeostasis | Brain and thymus | $Fgf^{-/-}$/*h*FGF-23-Tg double mutant | Ovary | Hypogonadism associated with premature ageing-like features | Kyphosis, osteopenia, pulmonary emphysema, tissue calcifications, atrophy of numerous tissues | 7, 8 |
| *4. AQP4* | **Aq**ua**p**orin-**4** | Expressed in the brain acting as predominant water-channel protein in mammals | Kidney, brain, uterus, testis | Homozygous whole body knockout | Ovary & endocrine milieu | Deceased number of oocytes, small ovaries, decreased antral follicles and corpora lutea, less hypertrophic uteri, and decreased endometrial thickness | Needs to be investigated | 9 |
| *5. LEPR (neuron specific)* | **Lep**tin **R**eceptor | Gonadatropin release | Brain neurons | Neuron-specific knockout | Ovary & endocrine milieu | Blocks luteinizing hormone (LH) surge | Doubling of body weight compared to wild type | 10 |
| *6. GPR54* | **G**-**p**rotein coupled **r**eceptor **54** (Kisspeptin receptor) | Crucial for onset of puberty and human fertility | Highly expressed in pituitary, pancreas, and placenta | Whole body knockout | GnRH neuron | Absence of luteinizing hormone (LH) and gonadotropin-releasing hormone (GnRH) surge | Hypogonadotropic hypogonadism and precocious puberty affecting many organs | 11, 12 |
| *7. BMAL1* | **B**rain and **m**uscle **a**ryl hydrocarbon receptor nuclear translocator (ARNT)-**l**ike **1** | Central circadian clock gene | Lipophilic tissues such as brain and adipose | Whole body knockout | Female reproductive tract & endocrine milieu | Decreased steroidogenesis | Whole body knockout displays lipid metabolism, obesity, and metabolic syndrome | 13 |
| *8. SCA2* | **S**pino**c**erebellar **a**taxia type **2** (Ataxin-2) | Polyglutamine expansion causes neurodegeneration; cellular function unknown | Brain and various other tissues | Homozygous whole body knockout | Female reproductive tract & endocrine milieu | Decrease frequency of liters and decreased liter sizes | Involved in neurodegenerative diseases, obesity, insulin resistance, hepatosteatosis, dyslipidemia | 14 |
| *9. PACAP* | **P**ituitary **a**denylate **c**yclase-**a**ctivating **p**olypeptide | Stimulates adenylate cyclase to increase cAMP levels in cells | Brain (hypothalamus and pituitary), peripheral nervous system, uterus | Homozygous whole body knockout | Uterus & endocrine milieu | Decreased implantation due to low levels of progesterone and prolactin | Inhibits prolactin release in the anterior pituitary | 15 |
| *10. NUPR1* | **Nu**clear **pr**otein, transcription regulator **1** | Upregulated in response to stress; many functions which are unclear, implicated in numerous signaling pathways | Developing pituitary, pancreas, various tissues | Homozygous whole body knockout | Ovary & endocrine milieu | Delayed luteinizing hormone subunit β (LHβ) expression leading to delayed ovarian maturation | Cancers of the breast, pancreas, thyroid, and pituitary; Sertoli-cell-only syndrome like phenotype in males | 16, 17 |
| *11. AR* | **A**ndrogen **r**eceptor | Receptor for androgen found | Hypothalamus- pituitary- | Homozygous whole body | Ovary, uterus, & | Heterogeneous ability to cycle, | Needs to be investigated | 18 |

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| | | on X chromosome involved in male and female reproductive physiology; ability to influence stimulation in both reproductive organs and neuroendocrine pathways | gonadal axis | knockout with transplants: (1) AR+ mice with AR- ovary transplant (2) AR- mice with AR+ ovary transplant | endocrine milieu | deceased litter size and frequency, ovarian weight, and corpus luteum; abnormal uterine horns, reduced implantation sites, $AR^{-/-}$ has increased baseline follicle stimulating hormone (FSH) | | |
| *12. GK* | **G**luco**k**inase | Acts as glucose sensor and aids in phosphorylation of glucose | Pancreas, hypothalamus, liver, brain | Whole body heterozygous knockout | Endocrine milieu | Reduced liter size, lower birth weight in cross-over of wild-type and knockout | Elevated food consumption, adult onset diabetes, hyperphagia, hypothalamic gene expression similar to that under hypoglycemia | 19 |
| *13. LHβ* | **L**uteinizing **h**ormone **β** subunit | Specific receptor subunit for luteinizing hormone | Leydig cells in male, ovarian granulosa and theca cells in female | Homozygous whole body knockout | Endocrine milieu | Hypogonal, decreased uterine horn size and endometrium, low serum levels of estradiol and progesterone, absence of normal antral and preovulatory follicles along with corpora lutea | In males, hypergonadism, reduced testis size and glands; decreased serum testosterone | 20 |
| *14. SIR2α* | Response of **s**ilent **i**nformation **r**egulator **2α** | Homolog of the yeast sir2 gene | High expression during embryogenesis | Whole body knockout | Ovary & endocrine milieu | Sterility due to hormonal inadequacy; small ovaries, thin-walled uterus, and absence of corpora lutea | Two null alleles cause neonatal lethality and low birth weight/death; if survive experience pulmonary infection, atrophy of pancreas, and eye malformation | 21 |
| *15. Nhlh2* | **N**ascient **h**elix **l**oop **h**elix **2** | Member of the basic helix-loop-helix family of transcription factors that bind to E-box motifs in promoter regions of genes | Central and peripheral nervous system in embryos; hypothalamus and pituitary in adults | Whole body knockout | Ovary, uterus, & endocrine milieu | Impairs neuronal regulation of fertility, stringy uteri, delay in vaginal opening, decreases lifetime pregnancies, reduces ovulation, reduces hormone-induced lordosis behavior | Needs to be investigated | 22 |
| *16. AFP* | **A**lpha-**f**eto**p**rotein | Serum glycoprotein belonging to the albumin family that is expressed highly in embryos; has anti-estrogenic activity | Mainly embryos, can also be expressed in tumors and infected livers | Homozygous whole body knockout | Endocrine milieu | Anovulation due to deficiency in hypothalamic-pituitary-gonal axis | Needs to be investigated | 13 |
| *17. ERK1/2* | **E**xtracellular signal-**r**egulated **k**inases **1** and **2** | Kinases involved in cascade to regulating the cell cycle, activated by luteinizing hormone (LH) | All mammalian tissues | Granulosa and cumulus cell-specific knockout | Ovary and endocrine milieu | Failure to ovulate, increased serum estradiol, blocks LH surge | Needs to be investigated | 24 |
| *18. Dicer1* | Dicer dsRNA **dicing** protein | Two RNAse III domains, DS-RNA-binding domain, role in biogenesis of | Expressed in several tissues | Multiple tissue-specific (oviduct/ovary/ | Ovarian granulosa and luteal cells, oocyte, | *Oviduct knockout*: fluid filled sacs, loss of coil and smooth muscle | Numerous tissues shown to be affected (cancer/disease) mainly due to lack of miRNA and | 25, 26 |

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| | | miRNA and siRNA | | oocyte/uterus) knockouts | oviduct, possibly uterus | *Ovary knockout:* luteal insufficiency, decreased weight and ovulation rate<br>*Oocyte knockout:* disorganized spingle formation, misaligned chromosomes<br>*Uterus knockout:* decreased weight and size – fertility unknown | siRNA expression | |
| *19. Ubb* | Poly**ub**iquitin **B** | Ensures normal cell cycle progression; its covalent conjugation is crucial step in process of programmed destruction of cell cycle regulators | Several tissues | Homozygous whole body knockout | Ovary | Hypogonadism and failure to progress through meiosis I | Hypogonadism and meiotic defects | 27 |
| *20. FOXL2* | **F**orkhead B**ox** Protein **L2** | Forkhead family transcription factor - may play role in ovarian development and function | Embryonic eyelids, pituitary cells, and follicle cells of the ovary in wide range of phylogenetically distant species | Ovary-specific homozygous knockout (with or without mutation in *Wnt4* or *Kit/c-Kit*) | Granulosa cells | Granulosa cell impairment; impedes secondary follicles, oocyte atresia, causing premature ovarian failure (POF) | Murine eyelid/craniofacial malformation; human blepharophimosis-ptosis-epicanthus inversus syndrome and eyelid malformations | 28, 29 |
| *21. Mgat1* | Alpha-1,3-**m**annosyl-**g**lycoprotein 2-beta-N-**a**cetylglucosaminyl**t**ransferase **1** | Encodes enzyme needed for N-glycan synthesis | Several tissues | Oocyte-specific knockout | Embryo | Retarded development of eggs, fewer eggs, thinner zona pellucida (ZP) lacking N-glycans | Multiple defects depending on tissue where deficient | 30 |
| *22. GC-Br* | **G**uanylyl **c**yclase-**B** **r**eceptor | Member of the guanylyl cyclase family of receptors, has a counterregulatory role to many growth factors (GFs) | Several tissues | Homozygous whole body knockout | Uterus & ovary | Stringy, malformed uterine horns with thinned myometrium and endometrium; absence of corpora lutea and failure of follicle development past secondary stage | Dwarfism, overexpression of GC-B2 in growth-plate chondrocytes | 31 |
| *23. FADS2* | **F**atty **A**cid **D**e**s**aturase **2** | Enzymatic action to regulate unsaturation of fatty acids | Present in several tissues | Whole body knockout | Ovary | Failure of development of zona pellucida, stratified granulosa cells, and gap junctions in granulosa cells | Dermal and intestinal ulceration, in humans - possible prevention of metabolic syndrome, possible role in attention deficient hyperactivity disorder (ADHD), low expression in breast tumors | 32, 33, 34 |
| *24. Pdk1* | 3-**P**hosphoinositide-**d**ependent protein **k**inase-**1** | Master kinase that activates kinases of the AGC family and activates Akt | Present in numerous tissues | Oocyte-specific gene deletion | Ovary | Primordial follicles depleted at puberty, premature ovarian failure (POF) | Whole body knockout causes lethal effects | 35 |
| *25. Rb* | **R**etino**b**lastoma | Binds to E2F transcription | Replicating cells | Granulosa cell- | Ovary | Premature ovarian failure, high levels | Whole body knockout is | 36 |

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| | | factors to facilitate regulation of the cell cycle | | conditional knockout | | of follicle-stimulating hormone receptor (FSHr), FSH, KIT ligand/stem cell factor (KITL), and anti-mullerian hormone (AMH), retinoblastoma protein (RB) and E2F disregulation in preantral follicles | correlated with various cancers | |
| *26. CCTβ2* | **C**TP:phosphocholine **c**ytidylyl**t**ransferase **β2** | Regulates synthesis of phosphatidylcholine | Throughout body, primarily in gonads and brain | Homozygous whole body knockout | Ovary | Disorganization of ovary, impaired follicle development | Reduced steroidogenesis leading to increased FSH and LH levels | 37 |
| *27. MSY2* | **M**ou**s**e **Y** box protein **2** | Member of highly conserved Y-box proteins that serve as transcriptional coactivators involved in translation | Germ cells | Whole body knockout | Ovary | Absence of corpora lutea and decreased number of growing follicles | Disruption of spermatogenesis in male mice | 38 |
| *28. PTX2* | The long **p**en**t**ra**x**in **3** | Member of the pentraxin superfamily, proteins produced in the liver in response to inflammatory elements | Several tissues; mononuclear phagocytes, dendritic cells, fibroblasts, endothelial cells | Whole body knockout | Oocyte | Failure of oocyte to construct proper cumulus oophorus hyaluronon-rich extracellular matrix | Decreased resistance to pathogens and disease | 39 |
| *29. Lhx8* | **L**IM-**h**omeobo**x** **8** | Transcription factor involved in folliculogenesis | Primordial follicle through oocyte | Ovary-specific knockout | Oocyte | Altered oocyte-specific gene expression, rapid depletion of oocytes | Needs to be investigated | 40 |
| *30. Lfng* | **L**unatic **fr**i**ng**e | Methyltransferase that mediates Notch pathway | Granulosa cells | Whole body knockout | Ovary | Impaired folliculogenesis and meiotic defects | Needs to be investigated | 41, 42 |
| *31. Coilin* | Coilin-Cajal Body marker protein found in **coiled** Cajal Bodies | Small RNP biogenesis and Cajal body homeostasis | All tissues, particularly high concentration in brain and testis | Whole body knockout | Ovary & possibly uterus | Produce fewer oocytes, decreased frequency and litter size | Possible culprit in human neuromuscular disease and spinal muscular atrophy through SMN protein | 43, 44 |
| **II. gene knockouts affecting ovulation, fertilization, and post-fertilization embryonic development** | | | | | | | | |
| *32. Esr 1* | **Es**trogen **R**eceptor α | Preferentially bindsto estrone and 17-beta-estradoil | Endometrium, breast cancer cells, ovarian stroma cells, hypothalamus, other estrogen receptive tissues | Theca-cell specific knockout | Ovary (germinal epithelium and theca-interstitial cells) and uterus | Erratic patterns of estrous, fewer oocytes, hemorrhagic cysts, disrupted thecal development, follicle arrest, lack of corpora lutea, underdeveloped uterus, hyperemic ovaries | Needs to be investigated | 45 |
| *33. SULT1E1* | **Sul**fo-**t**ransferase | Catalyzes conjugation of sulfate to various hormones, including estrone | Testis, placenta, ovary, pregnant uterus | Whole body knockout | Ovary & uterus | Disrupted uterine environment and impeded ovulation, low COX-2 expression, reduced cumulus expansion | Hyperplasia and hypertrophy of testis and low sperm motility | 46 |
| *34. Hurp* | **H**epatoma **u**p-**r**egulated **p**rotein | Factor for mitosis, expressed at high levels during $G_2$/M phase, cell cycle associated gene | High expression in testis, spleen, thymus; Low expression in colon, ovary, | Whole body knockout | Uterus (endometrial stroma) | Implantation defect due to impaired decidualization | Chromosomal misalignment | 47 |

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| | | | and small intestine | | | | | |
| *35. Rax* | **R**etina and **a**nterior neural fold homeobo**x** | Activates interferon-inducible dsRNA-dependent protein kinase and component of RISC | Several tissues | Whole body knockout | Embryo | Lethal at pre-implantation stage | Translation inhibition | 48 |
| *36. ITGA9* | **I**n**t**e**g**rin **a**lpha-**9** | Cell:cell and cell:matrix binding integral membrane glycoprotein | Present in several tissues | Not a knockout, but examined by using specific inhibitor | Ovum | Reduction in sperm-binding | Needs to be investigated | 49 |
| *37. SF-1* | **S**teroidogenic **F**actor **1** | Regulates expression of genes via hypothalamic-pituitary-gondal axis | Reproductive tissues | Granulosa cell-specific knockout | Theca & granulosa cells | Impaired follicle development, irregular estrus cycles, impaired gonadotropin-induced superovulation | Whole body knockout displays underdeveloped adrenals, abnormalities of ventromedial hypothalamic nucleus | 50, 51 |
| *38. Cyp11b1* | **Cy**tochrome **P**450, family **11**, subfamily **B**, polypeptide **1** | Encodes 11β-hydroxylase and aldosterone synthase to produce glucocorticoids and mineralcorticoids | Mainly adrenal glands | Whole body knockout | Ovary | Absence of corpora lutea, disorganized steroidogenic tissue | Congenital adrenal hyperplasia | 52 |
| *39. Lrh1* | **L**iver **r**eceptor **h**omolog **1** | Bile acid metabolism, steroidogenesis, cell proliferation | Granulosa and luteal cells in ovary, other several tissues | Granulosa-cell specific knockout | Ovary | Lack of ovulation due to failed cumulus expansion, luteinization, and rupture | Needs to be investigated | 53 |
| *40. TR4* | **T**esticular orphan nuclear **r**eceptor **4** | Ligand-activated transcription factor needed for proper male spermatogenesis and normal female folliculogenesis | Central nervous system and several other organs | Homozygous whole body knockout | Ovary | Decrease in preovulatory follicles leading to decrease in ovulation rate | Cerebellum development | 54, 55 |
| *41. FST* | **F**olli**st**atin | Two isoforms created by alternative splicing; one binds heparin sulphate to direct activin toward lysosomal degradation, the other form does not bind heparin sulphate | Several tissues | Whole body knockout (Human FST isoforms created to prevent neonatal lethality) | Ovary | Subfertility due to POF | Whole body knockout is lethal; FST -/- pups show respiratory difficultly, decrease mass of diaphragm and intercostal muscles; decrease in body size; skeletal defects; other abnormalities of skin and teeth | 56 |
| *42. CDKN1B* | **C**yclin-**d**ependent **k**inase i**n**hibitor **1B** | Tumor suppressor/cell cycle suppressor, activates caspase cascade in follicles by inhibiting Cdk2/Cdc2-cyclin A/B1 | Nuclei of oocytes | Ovary-specific knockout | Ovary | Overactivated follicular pool causing POF | Needs to be investigated | 57 |
| *43. Inhbb/Inhba* | **Inh**ibin **b**eta-**B**/**Inh**ibin **b**eta-**A** ***(Activins)*** | Positive regulator of pituitary FSH synthesis and | Multiple tissues during embryonic and postnatal | Activin beta-A/beta-B conditional knockouts | Ovary | Decreased liter size and frequency, increased number of corpora lutea, | Lethality in whole body knockout | 58 |

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| | | release | development | | | upregulation of granulosa cell gene expression | | |
| *44. ADAMTS-1* | **A** **d**isintegrin **a**nd **m**etalloproteinase with **t**hrombo**s**pondin motifs **1** | Extracellular matrix metalloprotease which degrades large proteoglycans such as versican and aggrecan | Several tissues | Homozygous whole body knockout | Ovary | Severely reduced ovulation, impaired tissue remodeling for folliculogenesis, follicular atresia, dysplasic ovarian vasculature | Needs to be investigated | 59 |
| *45. OAS1D* | 2',5'-**o**ligo**a**denylate **s**ynthase **1D** | Activated by interferon to bind to dsRNA, which activates RNAse L to degrade viral and host RNA | Oocyte and early embryo | Whole body knockout | Ovary & Egg | Abnormal follicle development and ovulation, arrest of zygote at one-cell stage | Needs to be investigated | 60 |
| *46. PAD6* | **P**eptidyl**a**rginine **d**emininase **6** | Newest member of the PAD family, a group of calcium-dependent enzymes involved in citrullination which affects protein stability | Mammilian oocyte, sperm cell, early embryo, associated with cytoskeletal sheet | Oocyte-specific knockout | Oocyte | Failure of zygote to progress past two-cell stage due to lack of citrullination | No abnormal phenotypes exhibited | 61 |
| *47. CD81* | **C**luster of **D**ifferentiation **81** | Member of the tetraspanin family of surface proteins which regulate signal transduction to influence growth, development, and motility | Hemopoietic, endothelial, and epithelial cells | Homozygous whole body knockout | Oocyte | 40% decrease in fertility due to less sperm binding to egg | Needs to be investigated | 62 |
| *48. Pig-a* | **P**hosphatidyl**i**no sitol **g**lycan class-**A** | Encodes enzyme involved in biosynthesis of egg surface glycosylphosphatidylinositol-anchored proteins | Several tissues | Oocyte-specific knockout | Oocyte | Failure of gamete binding and fusion | Needs to be investigated | 63 |
| *49. Ago2* | **Argo**naute **2** | Main component of miRNA-induced silencing complex (RISC) | Several tissues | Oocyte-specific knockout | Oocyte | Abnormal spindle formation and 80% decrease in miRNA expression | Needs to be investigated | 64 |
| *50. TAp73 (exon of Trp73)* | **Tra**nsactive domain **p**rotein **73** | Transcription factor with proapoptotic activities | Various tissues | Whole body knockout | Ovary & Zygote | Fewer gametes ovulated, less primordial follicles, oocytes trapped in luteinizing granulosa cells, spindle abnormalities | Neuronal defects; spontaneous tumors | 65 |
| *51. SGOL2* | **S**hu**go**shin-**l**ike **2** | Protects multiprotein cohesion complexes crucial for sister chromatid cohesion; role in regulation of chromosomal separation | Cells undergoing meiosis or mitosis | Whole body knockout | Gametes (oocyte and sperm) | Premature loss of centromeric cohesion during meiosis; gametes with abnormal number of chromatids | In males, causes infertility | 66 |
| *52. Zpf3612* | CCCH (cysteine-cysteine-cysteine-histidine) tandem **z**inc | Physiological significance unknown; can destabilize AU-rich element containing | Several tissues | Whole body knockout | Embryo | Embryo ceases progression beyond two-stage cell | Systemic inflammatory syndrome, skin lesions, autoimmunity, arthritis, myeloid | 67 |

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| | **f**inger RNA-binding **p**rotein | transcripts | | | | | hyperplasia | |
| *53. LPP* | **L**ipoma **P**referred **P**artner | Structural support for cell adhesion and also cell migration | Several tissues | Whole body knockout | Embryo | Aberrant pregnancies and decreased litter size | Role in mammary oncogenesis, turnover of migrating smooth muscle cells, and possible tumor suppression through interaction with Scrib | 68 |
| 54. PRAP/17βHSD-7 | **P**rolactin **r**eceptor **a**ssociated **p**rotein/**17β**-**H**ydroxy**s**teroid **d**ehydrogenase **7** | 17-βHydroxysteroid dehydrogenase that converts estrone to estradiol; cholesterol biosynthesis | Corpus luteum | Whole body knockout | Fetus | Brain malformation and heart defect *in utero* leading to fetal death | Needs to be investigated | 69 |
| *55. Plag1* | **P**leomorphic **A**denoma **G**ene **1** | Proto-oncogene transcription factor involved in human tumorigenesis | Differentially expressed in pituitary and reproductive organs | Embryonic stem cell-specific homozygous knockout | Needs to be investigated | Females mated with wild-type males produce smaller litters; females mated with null males frequently fail to conceive and also have smaller litters | Low body weight at birth | 70 |
| **III. gene knockouts affecting reproductive structures and/or other mechanisms** | | | | | | | | |
| *56. Thoc1* | **THO** complex subunit **1** | Recruits RNA processing and exports factors to developing mRNA; associates with RNA POLII | Several tissues | Whole body knockout (hypomorphic allele) | Female reproductive tract | Limits pregnancy compared with wild-type by unknown mechanism; if pregnancy occurs the litter is significantly smaller | Inhibits expression of specific genes; smaller body size | 71 |
| *57. Cbs* | **C**ystathionine **β**-**s**ynthase | Enzyme responsible for trans-sulfuration of homocysteine | Liver and decidual tissue | Homozygous whole body knockouts | Ovary & endocrine milieu | Increases progesterone during pseudo-pregnancy; shorted and irregular estrus cycle, placental and uterine abnormalities at day 18 of pregnancy | Needs to be investigated | 72 |
| *58. PLC-β1* | **P**hospho**l**ipase **C-β1** | Catalyzes reaction involved in transmembrane signal transduction pathways to regulate intracellular calcium ions | All tissues | Transgene-induced insertional mutation at the PLC-β1 locus | Female reproductive tract; possibly oocyte | Reduced fertility by unclear mechanism; possibly oocyte maturation and activation | Small body size and reduced vitality | 73 |
| *59. Smad1/5* | **M**others **a**gainst **D**PP homolog **1/5** | Alter the activity of TGFβ family ligands | All tissues | Floxed and null allele knockouts | Not known | Cause of infertility not stated | Metastatic tumor growth in granulosa cells | 74 |
| *60. Adm* | **Ad**reno**m**edullin | G-protein coupled receptor protein that acts as vasodilator | Endothelial cells and smooth muscle cells | Whole body heterozygous knockout | Uterus | Decreased uterine receptivity; uterine overcrowding; placental abnormalities; reduced pinopodes | Homozygous bull mutants are embryonic lethal | 75 |
| *61. Rspo1* | **R**-**spo**ndin**1** | Soluble protein similar to Wnt proteins; involved in | Multiple tissues | Homozygous whole body knockouts | Female reproductive tract | Pseuohermaphroditism, depletion of female oocytes, various forms of | Increased intestinal epithelial healing; protects against adverse effects of | 76 |

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| | | transcriptional activity mediated by the β-catenein/T cell factor signaling pathway | | | | ovarian masculinization | chemotherapy | |
| *62. SRC-2* | **S**teroid **r**eceptor **c**oactivator **2** | Regulates uterine function and progesterone signaling | Brain | Selective knockout in cells that express prolactin receptor | Uterus | Lack of implantation sites in uterine horn for proper implantation, placental hypoplasia | Postnatal mammary morphogenesis, amplified in breast and ovarian cancer | 77, 78 |
| *63. FKBP52* | **Fk**506-**b**inding **p**rotein **52** | Binds to steroid receptors complexed with heat shock protein 90 involved in protein folding/trafficking and immunoregulation | Several tissues | Homozygous whole body knockout | Uterus | Failure of zygote implantation; possible uterine insensitivity to progesterone or estrogen | Needs to be investigated | 79 |
| *64. Bteb1* | **B**asic **t**ranscription **e**lement-**b**inding protein-**1** | Progesterone interacting protein to increase transactivation in endometrium and transcription factor | Several tissues, prominent in endometrial stromal cells during pregnancy | Whole body knockout | Uterus | Subfertility associated with uterine hyperplasia, decrease uterine receptivity sites, decrease in litter sizes, and progesterone resistance | Needs to be invesigated | 80 |
| *65. Ctnnb1* | **B**eta-**ca**t**e**n**in** | FSH requires this transcriptional coactivator for proper regulation of aromatase expression; component of Wnt signaling pathway | Mesenchymal cells | Multiple tissue knockouts | Oviduct & uterus | Dysfunctional development of the uterus and oviduct | Whole body knockout causes embryonic death | 81, 82 |
| *66. Ndph* | **N**orrie **d**isease **p**seudoglioma **h**omolog | Murine ortholog of human gene responsible for Norrie disease | Purkinje cells of cerebellum, olfactory bulb, cochlea, and inner nuclear and ganglion cell layers of retina | Homozygous whole body knockout | Uterus | Massive bleeding at uterine implantation sites leading to fetal loss | Needs to be investigated | 83 |
| *67. MK & PTN* | **M**id**k**ine and **P**leio**t**roph**in** | Together compose a family of growth factors involved in growth, differentiation, and mobility of cells | Several tissues; specifically high during embryogenesis | Double-knockouts of MK/PTN | Female reproductive tract | Smaller reproductive organs; fewer number of mature follicles; decreased estrus, longer proestrus and diestrus; vaginal malformations | Smaller body size | 84 |

protein 2 (RAMP2) for activation. It is highly expressed in endothelial cells, smooth muscle, placenta, uterus, and other highly vascularized tissues. It can affect many processes of the body including pregnancy. It may be involved in pregnancy complications such as preeclampsia, gestational diabetes, low birth weight, and spontaneous abortion (75). Homozygous *Adm* knockout mice are embryonic lethal while heterozygous ($Adm^{-/+}$) knockouts how decreased fertility due to reduced receptivity of the uterus because of defective pinopodes (75). Knockouts show normal ovulation and fertilization rates. (75) R-spondin1 (Rspo1) is a soluble protein similar to the Wingless-type MMTV integration site family (Wnt) ligands that can alter transcriptional activation regulated by the β-catenin/T cell factor signaling pathway. Rspo1 and Wnt proteins collectively stabilize cytoplasmic β-catenin and

activate downstream pathways. Rspo1 proteins are involved in development, such as mouse placental development, human nail morphogenesis, apical ectodermal ridge maintenance, and gonad differentiation (76). Infertility is due to masculinization in developing XX gonads. In *Rspo1*-null XX mice, subfertility is due to dysgenesis of female ducts and organs (76). This phenotypic pattern of infertility mimics that of *Wnt-4*$^{-/-}$ mice (76).

Steroid receptor coactivator 2 (SRC-2) belongs to the steroid receptor coactivator/p160 family which enhance nuclear receptor (NR)-mediated transactivation by activation 2 domain in the C-terminal regions of nuclear receptors. The three main protein products of the SRC-2 family play a role in progestin-induced signaling. SRC-2 regulates signaling inputs from the dispatched ligand-bound nuclear receptor to the transcription complex (77). SRC-2 is more potent than other family members, SRC-1 and SRC-3, because progesterone receptor is solely dependent on SRC-2 for progesterone-induced physiological response pertaining to female fertility and postnatal mammary morphogenesis (77). $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ mice lack implantation sites which is the primary cause of infertility (77). FK506 binding protein 52 (*FKBP52*) can bind with steroid receptors in specialized complexes containing heat shock protein 90. The protein is expressed in several tissues throughout the body. *FKBP52* knockout mice were generated to determine the role of FKBP52 in reproduction (79). *FKBP52*$^{-/-}$ female mice display normal reproductive organ histology and estrus cycling, concluding that *FKBP52* is not needed for reproductive development (79). Homozygous whole body knockout mice display sterility due to implantation failure (lack of estrogen and progesterone) (79). Basic transcription element-binding protein-1 (*Bteb1*) is a transcription factor of the Sp/Krüppel-like family (KLF) which can bind to both isoforms of progesterone. This mediates the sensitivity of target genes in endometrial epithelial cells to progestin. *Bteb1*, which is found in several tissues, has consequential effect on female fertility when deleted. Although *Bteb*$^{-/-}$ mice have normal ovarian histology, they display uterine hyperplasia which causes subfertility (80).

Beta-catenin (Ctnnb1) is a transcriptional coactivator that is needed by FSH to regulate aromatase expression. It is also a component of the *Wnt* signaling pathway. Knockout mice were generated to assess the physiological role of beta-catenin in granulosa cells of developing follicles. The whole body knockout of the gene experiences embryonic death due to ectodermal cell layer defects during gastrulation (81). The *Ctnnb1* gene was knocked down in Mullerian duct derivatives, granulosa cells, brain, pituitary, heart, liver, and tail (81). Although females reach sexual maturity at the expected time and the ovaries are normal, female mice are infertile due to dysfunctional development of the uterus and oviduct (81). The oviduct is partially formed and lacks normal coiling concomitant effect on non-reproductive systems. Among them, these proteins involved in the fertilization cascade are especially interesting and may provide novel targets for (81). The uterus is thinner with more fat accumulation and less tone (81). The Norrie disease pseudoglioma homolog gene (Ndph) is the murine gene that has greater than 96% similarity to the human *NDP* gene, which is known to cause X-linked recessive Norrie disease. Expression of the gene is localized to the Purkinje cells in the cerebellum, olfactory bulb, cochlea, and inner nuclear and ganglion cells of the retina (83). *Ndph*$^{-/-}$ knockout mice display symptoms of the disease similar to humans (83). *Ndph*$^{-/-}$ females are unable to become pregnant (83). The most significant phenotypic attribute is the massive bleeding at implantation sites causing fetal loss (83). Trophoblasts are disrupted and spongiotrophoblasts are reduced (83).

Midkine (MK) and pleiotrophin (PTN) are growth factors which can independently cause mild reproductive and developmental abnormalities. Midkine is highly expressed during embryogenesis, and decreases in adulthood only to resume expression during times of tissue repair. It is involved in growth, survival, and movement of many cells. The expression of pleiotrophin often coincides with the expression of midkine during embryogenesis. Pleiotropin is involved in developmental processes and its deficiency can lead to nervous system abnormalities. To investigate a possible synergistic effect of both growth factors, a double knockout was created (84). The double knockout of *MK* and *PTN* (DKO mice) is partially embryonic lethal (84). DKO mice are smaller with smaller reproductive organs, having fewer mature follicles (84). Other abnormalities include prolonged diestrus and proestrus periods with a shortened estrus period, altered expression of the growth factors in the uterus during estrus, and vaginal malformations which all contribute to sterility (84).

## 4. CONCLUSIONS

The gene knockout technology has revealed several molecules, novel insights and mechanisms involved in various steps of the fertility cascade in females. In the previous review, 83 gene knockouts were reported that affect female fertility. In the present review, 67 additional gene knockouts were found using the database search; seven of these are updates of the genes that were reported in the previous review. Considering these two reviews, there are at least 143 genes that have an effect on some aspect of female fertility in the mouse. Their effect on female fertility in humans needs further investigation. The mouse genome is 2.5 billion DNA letters long. It is about 14 percent shorter than the human genome, which is 2.9 billion letters long. The human genome is filled with more repeat sequences than the mouse genome [85]. The mouse and the human genomes each seem to contain approximately 30,000 protein coding genes. A majority of them are evolutionarily conserved and some families of genes have undergone expansion /multiplication in the mouse lineage [85]. The fertility field has exploded with the advent of gene knockout technology. Almost every month there is a report on a new gene knockout that has some effect on fertility. However, there are only a few genes whose knockout demonstrates a specific effect on female fertility without contraception and/or for contraceptive vaccine development. The utility of a protein in contraception is contingent upon its 1) oocyte specificity, 2) essential role in gamete/ovarian function, 3)

accessibility and amenability of inactivation by inhibitors, antagonists, or antibodies. These inhibitors/antagonists/antibodies should not affect folliculogenesis/endocrine milieu and should have a reversible effect on fertility without causing premature ovarian failure (POF) or menopause. The gene knockout technology is help is helping us to better understand female infertility and is providing potential novel targets for contraceptive development.

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