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GENE KNOCKOUTS THAT CAUSE FEMALE INFERTILITY: SEARCH FOR NOVEL CONTRACEPTIVE TARGETS

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1. ABSTRACT

The gene knockout technology has revolutionized the fertility/infertility field. It has revealed several essential previously undiscovered molecules, new insights and novel mechanisms involved in steps of the fertility cascade in females. Using database and literature search, knockouts of at least 83 genes were discovered that demonstrated an effect on fertility of female mice. These effects ranged from abnormality in reproductive structure, ovarian function, oocyte, fertilization, embryonic and fetal development, implantation and pregnancy to delivery. However, only a few of these knockout of genes such as encoding oocyte glycoprotein coat comprised of zona pellucida (ZP) 1, ZP2 and ZP3 and oocute plasma membrane specific proteins showed a specific and exclusive target infertility effect without concomitant effects on the non-reproductive organ system. These molecules will provide novel targets of contraception including contraceptive vaccine development.

2. INTRODUCTION

Infertility is failure to conceive after one year of unprotected intercourse. Female infertility can be attributed to defect in ovarian development, proliferation and maturation of oocytes, differentiation of granulosa and theca cells, ovulation, tubal transport of oocyte, timed coital delivery of sperm, cervical mucus receptivity, fertilization and/or the implantation of embryo into the uterine endometrium. These defects can result from deficiency or abnormality of several intragonadal and extragonadal factors (1). These factors are involved in initiation of follicular growth and development of the oocyte, granulosa cells and theca cells both in early and late stages of folliculogenesis, and in regulating the physiology of various reproductive organs (2). Among the female factors responsible for infertility, the ovulatory problems constitute the majority (40%), which could in turn result from lack of required number of follicles within the ovary and/or to irregular hormonal concentration. Over 30% of infertile females have disorders in the tubal transport and another 3% have infertility associated with endometriosis. Coital/cervical problems constitute ~13% of female infertility and 10% are attributed to unexplained causes (http://medstat.med.utah.edu /kw/human_reprod).

Physiological, biological and molecular studies have helped us to understand the process of gonadogenesis, folliculogenesis and other aspects of infertility. The advent of gene knockout technology has provided a great impetus to study the role of various genes and proteins in fertility

and infertility (3-6). This technology provides a phenotype to examine resulting from the functional deletion of a gene. In addition to the "classic gene-knockouts", the lox/cre system has now been used to get conditional gene-knockouts that prevent formation of lethal phenotypes which might be produced by the classical method due to inactivity of key biologically essential enzymes (7). The aim of this review is to identify the gene knockout mice that have been shown to have an effect on female fertility. Using search in the database (http://www.humgen.nl/orgspecdatabases.html#Mouse) and studies on reported gene knockouts in the literature (http://www.ncbi.nlm.nih.gov/entrez), a total of 83 genes were identified as of January 14, 2005 whose knock out (KO) caused female infertility in mice (table 1). The knockout can be broadly divided into five broad categories depending upon which parameter(s) of female fertility is affected by a particular gene deletion. These categories are described below.

3. DISCUSSION

3.1. Gene knockouts affecting gonadogenesis and ovarian function

A total of seven genes were found in this category (table 1). The development of the ovary occurs during the early fetal life from an extraembryonic mass of cells that migrate to gonadal ridges of developing embryo. The germ cells form important association with somatic cells of the gonad for the duration of germ cell development and female reproductive life span. The ovarian development is controlled by several transcriptional factors, nuclear multi-protein core complex and hormonal receptor mediated factors. The knockout of *Zfx* gene, encoding for an X-chromosome located zinc-finger transcription factor, demonstrates a role of this protein in sex differentiation and in premature ovarian failure (8). The knockout of *WT-1* gene, another transcription factor gene, affects the urogenital development during sex determination resulting in ovarian agenesis (9). *Sox 3* is an X-chromosome coded transcription factor gene of high mobility group (HMG) family expressed in brain during early development and gonads. The mutants of this gene develop follicle atresia and defective oocyte formation and are infertile (10). Cytokine-activated signaling molecule, *Stat 3*, is expressed in brain during development and growth, and affect transcription by direct binding to DNA of target genes (11). Its non-expression leads to hypogonadotrophic hypogonadism and several reproductive organ disorders due to gonadotrophic abnormalities. Gonadal agenesis has been reported in gene knockouts of nuclear receptor superfamily steroidogenic factor coding gene, *Ftz-F1* (12). Another gene responsible for hypogonadism is the nuclear multi-protein core complex protein Fanconi anemia complementation group, *Fanc* (13-15). Since these genes have varied roles in development of other tissues, their deletion leads to a variety of organ defects.

3.2. Gene knockouts affecting folliculogenesis and germ cell development

A total of 33 genes were found in this category (table 1). An ovarian follicle consists of an oocyte surrounded by granulosa and theca cells. The folliculogenesis involves co-coordinated steps of initiation of oocyte growth from primordial follicles, granulosa and theca cell differentiation and proliferation, and finally development of the antral follicle. These well-knit patterns of events result in a continuous recruitment of pre-ovulatory follicles during each ovarian cycle. The development of a viable and fully functional oocyte is one of the crucial events in reproduction.

Three coat proteins of the oocyte, the zona pellucida (ZP) proteins, ZP1, Zp2 and Zp3 (coded by *Zp1*, *Zp2* and *Zp3*, respectively) are very important for the structural integrity of the zona pellucida and thereby for the functional viability of oocyte. The knockouts of these three genes have been shown to have a drastic effect on fertility (16-19). The proliferation of germ cells and their migration to the genital ridges are controlled by a protein called the proliferation of germ cells (Pog) encoded by *Pog*, the deletion of which results in the depletion of follicles in the developing ridges (20). This protein is expressed widely in the embryo and its deletion causes germ cell deficient phenotype. Dazla protein is a cytoplasmic protein that controls the translation in maturing oocyte by localization or packaging of mRNA (21). Deletion of the *Dazla* gene, leads to lack of germ cells due to a prenatal degeneration. This protein is shown to have an effect on both male and female gonads. An oocyte-derived growth factor, GDF-9, is transcribed only in the oocyte. The *Gdf-9* KO mice show a block in the follicular development at the one-layer stage leading to complete infertility (22). A similar protein GDF9b (Bone morphogenetic protein 15) encoded by *Bmp 15* gene, is also secreted by the oocytes and its deletion affects ovarian folliculogenesis, cumulus physiology and ovulation (23). *Gja 4* encodes for an intracellular signaling protein Connexin 37, that is present in the gap junctions between oocyte and granulosa cells. The *Gja 4* KO mice result in a block in maturation of Graffian follicles beyond meiotic competence and hence there is no ovulation (24). Similar arrest of the oocytes at the pachytene stage is seen in KO mice deleted off *Cpeb* gene, a cytoplasmic polyadenylation element binding protein that regulates translation during the oocyte maturation (25). Factor in the germline α (*Figla*), is a germ-cell specific factor that co-ordinates expression of the zona proteins. A KO of this gene results in sterility (26).

Besides these, there are several other gene knockout of female mice (27-52) that have a reduced fertility as shown in table 1. Majority of these knockouts not only have an effect on fertility but also affect growth and development of somatic cell function in a variety of organs.

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

A total of 24 genes were found in this category (table 1). The normal endpoint of a developing oocyte is either the apoptotic demise or ovulation resulting from the LH surge. This process is followed by morphological and physiological changes in the ovary called luteinization. The oocyte release and formation of the corpus luteum are important events in the process of conception and

Table 1. Gene knockouts that affect female fertility

No.	GENE	PROTEIN			INFERTILITY TARGET	PHENOTYPE	EFFECTS ON OTHER TISSUES	REF.
		NAME	FUNCTION	LOCALIZATION				
I. GENE KNOCKOUTS AFFECTING GONADOGENESIS AND OVARIAN FUNCTION								
1.	*Zfx*	Zinc finger transcription factor	Role in sex differentiation, spermatogenesis, Turner's syndrome	Expressed in several tissues	Ovary	Premature ovarian failure, shortened reproductive life span.	Critical role in embryonic growth and germ cell development.	8
2.	*WT-1*	Transcription factor	Early urogenital development	Expressed in developmental stages	Ovary	Gonadal agenesis	Failure of urogenital ridge development. Abnormal heart, lung development.	9
3.	*Ftz-F1*	Steroidogenic factor-1	Nuclear receptor superfamily protein.	All primary steroidogenesis tissues.	Ovary	Complete gonadal agenesis.	Affects several tissues and functions requiring steroid receptors. Lethal.	12
4.	*Fanc/c/g*	Fanconi anemia complementa -tion group A, C, G	FA proteins form a nuclear multi-protein core complex	Expressed in several tissues especially hematopoetic cells	Ovary	Hypogonadism	Not reported	13-15
5.	*Lhcgr*	Lutenizing hormone receptor	Activity of Lutenizing hormone	Present in several tissues	Sex organs	Grossly underdeveloped external and internal genetalia	Not studied	99
6.	*Stat 3*	Signal transducer and activator of transcription	Cytokine activated signaling molecules binding to DNA	Expressed in CNS during development and growth	Ovary	Hypogonadotrophic hypogonadism, reduced uterine horn size, no ovulation	Obesity, diabetes and thermal dysregulation	11
7.	*Sox 3*	Single exon X chromosome gene	HMG family transcription factor	Developing gonads and brain	Ovary	Excess follicular atresia, defective oocytes ovulated	Mental retardation and short stature. Testis also affected in males	10
II. GENE KNOCKOUTS AFFECTING FOLLICULOGENESIS AND GERM CELL DEVELOPMENT								
1.	Acvr2	Activin receptor-type IIA	Activin signaling in pituitary gonadotrophs	Expressed in several endocrine and exocrine tissues.	Ovarian follicles	Antral follicle block	Skeletal and facial abnormalities; FSH suppression	27
2.	*Gcd/Pog*	Proliferation of germ cells	Normal germ cell proliferation when they migrate to genital ridges	Widespread expression in embryos and adult gonads	Normal primordial germ cell proliferation	Drastic depletion of follicles in developing genital ridges	Not reported	20
3.	*Zp1*	Zona pellucida glycoprotein 1 (Zp1)	Required for the structural integrity of the zona pellucida	Forms the first coat over the egg below the Zp2 and Zp3	Structural integrity of ova leading to subfertility.	Precocious hatching and reduced fecundity	Not reported	16
4.	*Zp2*	Zona pellucida glycoprotein 2 (Zp2)	Required for structural integrity and maintaining interaction between granulocytes and oocyte.	Intermediate egg coat between Zp1 and Zp3.	Severe structural defect of ova causing infertility.	Significant decrease in number of antral stage follicles in ovaries. No live birth although fertilization does occur *in vitro*	Not reported	17
5.	*Zp3*	Zona pellucida glycoprotein 3 (Zp3)	Required for formation of zona pellucida matrix and organized corona radiata.	Outermost egg coat	Oocytes lack zona pellucida matrix	Completely sterile due to lack of a visible zona pellucida, with disrupted cumulus oocyte complex	Not reported	18,19
6.	*Atm*	Ataxia telagiectasia gene	Nuclear protein with role in cell cycle and DNA repair	Expressed in a variety of tissues	Germ cells	Lack of primordial or maturing ovarian follicles and lack of estrous cycle	Neurologic degeneration, growth retardation, lymphocyte maturation defects	28
7.	*Dazla*	Dazla protein	Translational control by localization or packaging of mRNA	Cytoplasmic protein in maturing oocyte and in zona pellucida of adult follicles	Germ cells	Lack of germ cells by prenatal degeneration of germ cells	Affects germ cells in both testis and ovary	21
8.	*Steel panda*	Steel factor	Ligand for tyrosine kinase encoding *c-kit* protooncogene	Expressed in a variety of tissues during embryonic development and adult life	Early ovarian follicles	Reduced germ cells and folliculogenesis defects	Defects in melanogenesis and hematopoiesis at several stages of development	29
9.	*GDF-9*	Growth differentiatio n factor-9	Oocyte derived growth factor regulating somatic cell function	RNA synthesized only in the oocyte	Oocyte	Block in follicular development beyond the primary one-layer follicle stage leading to full infertility	Not reported	22
10.	*Cyclin D2*	Cell cycle regulating D-cyclin	Cellular proliferation control	Expressed in proliferating tissues. Expression specifically induced in granulosa cells by FSH stimulation	Granulosa cells	Infertile due to inability of ovarian granulosa cells to proliferate	Hypoplastic testis	30

11.	*c-mos*	c-mos protooncogene product MOS	Essential component of the cytostatic factor (CSF)	Expressed in oocytes, testes and several somatic cells	Oocytes	Develop ovarian teratomas by oocyte maturation from second meiotic metaphase without activation	Not reported	31
12.	*MLH 1*	Mismatch repair enzyme	Responsible mismatch repair and for normal meiosis	Expressed in several tissues	Oocytes	Sterility due to absence of viable oocyte that does not complete meiosis II	Sterile males due to lack of viable sperm arrested at pachytene stage	32
13.	*Ambp*	α1-microglobulin (Ulinastatin)	A serine protease inhibitor maintaining homeostasis	Found in urine and blood	oocyte	Defects in oocyte maturation and ovulation. Oocytes lack zona pellucida	Several anti-inflammatory responses	33
14.	*Ahr*	Aryl-hydrocarbon receptor	Ligand activated transcription factor mediating toxicity against environmental contaminants	Present in several cells and tissues	Follicles	Early development of primordial follicles resulting in low number of antral follicles	Not studied	34
15.	*Bcl2/ Bclx*	B-cell leukemia/ lymphoma 2 / X	Anti-apoptosis protein controlling cell survival	Expressed widely during development	Germ cells	Loss of primordial germ cells and hence their depletion in post-natal ovary	Lethal	35,36
16.	*Bmp 15*	Bone morphogenetic protein 15 (Growth differentiation factor 9b)	Growth factor required for ovarian function	Secreted by oocytes	Oocyte	Defects in ovarian folliculogenesis, cumulus cell physiology and ovulation	Not studied	23
17.	*Pde4d*	c-AMP specific phosphodiesterase type 4	Feedback regulation of cAMP levels	Expressed in different tissues	Granulosa cells	Disruption of granulosa cell differentiation leading to increased estrogen level and reduced ovulation	Growth retardation	37
18.	*Casp 2*	Caspase 2	Intracellular death effectors	Expressed in a variety of cells	Germ cells	Significant increase in the primordial follicles in ovary due to absence of cell death	Effect on neurons and lymphoblasts	38
19.	*Cd9*	CD9 antigen	Metastasis suppressor molecules role in cell adhesion, motility, differentiation, signal transduction	Present in multiple tissues	Oocytes	Ovulated oocytes non-competent to sperm fusion resulting in reduced fertility	Not reported	39
20.	*Gja 4/Cx 37*	Connexin 37	Intracellular signaling at gap junctions	Present in gap junction between oocyte and granulosa cells	Oocyte	Prevents maturation of Graafian follicles beyond meotic competence and ovulation	Not studied	24
21.	*Gja 1/ Cx 43*	Connexin 43	Intracellular signaling at gap junctions	Present in oocytes and cumulus cells	Follicles	Smaller gonads due to germ cell deficiency caused by impaired folliculogenesis	Offspring die due to heart abnormality	40
22.	*Cpeb*	Cytoplasmic polyadenylation element binding protein	RNA binding protein regulating translation during oocyte maturation	Expressed in vertebrate oocytes	Oocytes	Vestigial ovaries without mature oocytes. Development arrested at pachytene	Not studied	25
23.	*Dmc 1*	Disrupted meiotic cDNA 1	Meiosis specific gene involved in recombination repair	Transcribed in mouse testis and ovary	Oocytes	Defects in oogenesis and subsequent death of oocytes lead to depletion of oocytes in adult ovary	Affects spermatid development.	41
24.	*Figla*	Factor in germline α	Germ cell specific factor co-coordinating expression of zona proteins	Transcripts detected in embryonic tissues and adult oocytes	Oocytes	Sterility due to inability of oocytes and granulosa cells to develop into primordial follicles	Not reported	26
25.	*FSHr*	FSH receptor	Target for the FSH activity	Located in several tissues	Follicles, ovary and uterus	Infertility due to aberrant gametogenesis with uterine and ovarian hypertrophy	Small testis	42,43
26.	*Ggtp*	γ-Glutamyl transpeptidase	Catalyze secreted glutathione into γ–glutamyl acid and cysteinyl glycine	Widely expressed in many mammalian tissues	Follicles	Hypogonadal due to follicular degeneration	Growth retardation, cataracts and others	44
27.	*Msh4/5*	MutS homologue 4/5	Post replicative DNA mismatch repair gene	Widely expressed	Germ cells	Disruption of ovarian development due to loss of the oocytes	Not reported	45

28.	*Madh1/ 5*	MAD homolog, Smad 1/5	Signal mediators for bone morphogenetic proteins	Widespread tissue distribution	Germ cells	Greatly reduced or completely absent primordial germ cells in mutant embryos	Lethal	46,47
29.	*Taf 4b*	TAF4B RNA polymerase II, TAFII 105	Transcription factor important in RNA polymerase II machinery	Low level in several tissues and high in testes and ovary	Follicles	Ovaries lack mature follicles due to impaired folliculogenesis	Not studied	48
30.	*Gp-130*	Shared receptor for IL-6 cytokine	Mediates IL-6 induced cell-signaling	Expressed in several tissues	Oocytes	Slight reduction in primary follicles and major defect in ovulation	Die due to cardiac/ hematologic disorders or at birth	49
31.	*Foxo 3a*	Foxo transcription factors	Metabolism, cellular stress response and aging	Expressed in several tissues	Follicles	Depletion of functional ovarian follicles by their early activation	Physiological and hematological abnormalities	50
32.	*Tnfip6*	Tumor necrosis factor induced protein 6	Anti-inflammatory role and also binds to hyaluronan in cumulus	Expressed in several tissues	Oocyte	Impaired cumulus expansion and mucification leading to infertility	Not reported	51
33.	*Ercc 1/ Xpf*	Nucleotide excision repair pathway proteins	Recombination, double strand break repair and repair of interstrand cross-links	Expressed in several tissues	Oocytes	Reduced number of oocytes and oocyte degeneration. No primary follicles formed	Lethal	52
III. GENE KNOCKOUTS AFFECTING OVULATION, FERTILIZATION AND POST FERTILIZATION EMBRYONIC DEVELOPMENT								
1.	*Il 11r*	Interleukin 11 receptor	Receptor for IL-11	Present in several tissues	Uterus	Infertility due to failure of implantation and decidualization	Not reported	59
2.	*Cox 2*	Cyclogenase II	Regulatory role in prostaglandin synthesis	Inducible isoform present in several tissues.	Ovary	Infertility due to ovulation defects and corpora lutea absence	Renal abnormalities, cardiac fibrosis, altered inflammatory responses	60
3.	*$p27^{Kip1}$*	Regulatory protein $p27^{Kip1}$	Cyclin dependent kinase inhibitory action	Present in several tissues	Follicles	Infertility due to absence of corpora lutea formation	Multiorgan hyperplasia, tumorigenesis	61
4.	*PR*	Progesterone receptor	Receptor for progesterone	Present in several tissues	All reproductive structures	Infertility due to absence of ovulation, uterine hyperplasia	Abnormality in sexual behavior, limited mammary gland development	62
5.	*PRLR*	Prolactin receptor	Receptor for prolactin hormone	Present in reproductive tissues	Multiple	Multiple reproductive abnormalities as irregular cycles, failure of implantation, reduced fertility rates	Not reported	53, 54
6.	*csfm*	Colony stimulating factor-1	Hematopoetic growth factor	Expressed on mononuclear phagocytes, preovulatory oocytes, decidual cells and trophoblasts	Ovary	Affects frequency and rate of ovulation. There is also severe lactational defects	Males have low libido	63
7.	*LIF*	Leukemia inhibitory factor	Cytokines	Expressed in several cells. Hyperexpressed in the uterine endometrial glands on fourth day of pregnancy	Uterus	Blastocyst fail to implant	Not reported	64
8.	*Srd5a1*	Steroid 5α reductase type-1 enzyme	Androgens converted to 5α reduced androgens	Expressed in liver and skin	Uterus	Embryonic death due to estrogen toxicity. Parturition defects lead to small litter size	Not reported	65
9.	*Inhbb*	Activin/inhibin β of TGF-β superfamily	Growth and differentiation of several cell types	Expressed in several tissues	Fetus	Impaired fetal development and female fecundity	Several defects in embryonic development.	66
10.	*Bsg*	Basigin	Glycosylated transmembrane protein involved in cell surface recognition	Expressed in several embryonic and adult tissues	Embryo	Failure of embryo to implant	Partially lethal	67
11.	*C/EPB b*	CCAAT/ enhancer binding protein β	Transcription factor acting as signaling molecule	Expressed in many tissues of adult mice	Granulosa cells	Defective granulosa cell function result in lack of corpora lutea. Impairment of ovulation.	Several developmental and immune defects	68
12.	*Dnmt 1o*	DNA methyltrans-ferase variant	Affect genomic methylation pattern	Expressed in oocytes and pre-implantation embryo	Embryo	Failure of maintenance of methylation pattern in embryo leads to embryo death	Not reported	56

13.	*Gdi 1*	Guanosine diphosphate dissociation inhibitor 1 (GDI α)	Important in actin cytoskeleton dependant cell functions	Expressed ubiquitously in most tissues	Embryo	Failure of embryo development in post implantation stage	Massive protenuria and renal failure	69
14.	*Hsf 1*	Heat shock transcription factor 1	Trans-activator of stress inducible genes.	Expressed in variety of tissues	Embryo	Embryos die at 1-2 cell stage due to failure to initiate zygotic transcriptional activity.	Prenatal lethality and growth retardation	70
15.	*Mater*	Maternal antigen that embryos require	mRNA or proteins that accumulate in egg during oogenesis	Expressed in oocytes	Embryo	Failure to develop beyond the two-cell stage	Not studied	55
16.	*Nos 3*	Nitric oxide synthase 3	Catalysis of the synthesis of NO from L-Arginine	Expressed in several tissues	Oocyte	Impaired oocyte meiotic maturation and reduced ovulation	Not studied	71
17.	*Nrip 1*	Nuclear receptor co-repressor RIP40	Expression inhibits activity of different nuclear receptors	Widely expressed	Ovulation	Failure to ovulate results in luteinized unruptured follicles	Not reported	72
18.	*Adcyap 1r1*	Adenylate cyclase activating polypeptide receptor 1	Stimulates adenylate cyclase in the pituitary gland	Located in pituitary gland, testis, ovary and other organs	Ovary	Delayed ovary response due to a longer irregular diestrous phase in estrous cycle	Affects several gonadotropin related functions	73
19.	*Ptx 3*	Pentraxin 3	Secretory inflammatory protein	Produced at several locations also in granulosa cells	Ovary	Reducedovulation and defects in cumulus cell-oocyte integrity	Variety of inflammatory effects.	74, 75
20.	*Ptgfr*	Prostaglandin F receptor	Mediate several physiological functions of prostaglandins	Located in several tissues	Parturition	Non-induction of labor and prolonged pregnancy leading to degeneration of placenta and fetal death.	Not studied	76
21.	*Psa*	Puromycin sensitive aminopeptidase	Putative extracellular enkephalinase	Cytoplasmic protein in brain cells	Ovary	Mating-induced surge of prolactin is prevented resulting in no corpus luteum formation	Impaired brain function results in behavioral changes	77
22.	*Arb 1*	Scavenger receptor class B1	HDL receptor on cell involved in lipoprotein metabolism	Expressed in several tissues including liver and uterus	Oocyte	Disturbed oocyte maturation and early embryo development	Lipid metabolism related disorders	78
23.	*Spam 1*	Sperm adhesion molecule, PH20	Inhibited hyaluronidase activity that helps to penetrate ova	Sperm surface	Fertilization	Decreased fertility	Not studied	57
24.	*Ptga*	N-acetyl glucosaminyl transferase	Involved in biosynthesis of glucosylphosphotidyl inositol (GP1)-anchored protein (AP) on egg surface	GPI-APs are egg surface proteins involved in sperm- egg interaction	Oocytes	No fertilization	Complete knockout lethal	58
IV. GENE KNOCKOUTS AFFECTING FEMALE REPRODUCTIVE STRUCTURES								
1.	*Wnt7a*	Secreted glycoprotein of Wnt family	Signaling molecules that regulate developmental functions	Expressed in dorsal ectoderm of developing embryos	Failure of regression of Mullerian duct	Abnormal development of oviduct and uterus	Several limb-patterning defects and Mullerian-duct associated sterility in both sexes	79
2.	*Wnt7b*	Secreted glycoprotein of Wnt family	Signaling molecules that regulate developmental functions	Expressed in extra embryonic membranes and chorionic villi	Fusion of chorion and allantois during placental development	Death of embryos at mid-gestation stages	Affects normal morphogenesis of chorionic plate	80
3.	*Abca1*	ATP-binding cassette transporter 1	Transmembrane protein involved in transport of vitamins, peptides and hormones	Expressed in several tissues	Placenta	Impaired steroidogenesis results in malformed placenta and embryo death	Aberrant lipid distribution and glomerulonephritis	84

4.	*Cenpb*	Centromere protein B	Binds to centromeres and is thought to be associated with their assembly	Abundantly present in a variety of tissues	Uterus	Severe disruption of uterine epithelium affecting its ability to support implantation and fetal growth	Lower body and testis weight	83
5.	*Cyp 40*	Cytochrome P450, 40	25-hydroxyvitamin D 1α-hydroxylase enzyme	Synthesized in several tissues, primarily in kidney	Uterus	Uterus hypoplasia, decreased ovarian size due to compromised folliculogenesis	Hypocalcemia, secondary hyperparathyroidism, retarded growth, and skeletal abnormalities	82
6.	*Hoxa 11*	Homeobox A11	Specify regional identity during development	Expressed in several developing tissues	Uterus	Defective uterine support of embryonic development	Dramatic malformation and alteration in body shape	81
V. GENE KNOCKOUTS AFFECTING ENDOCRINE MILIEU								
1.	*GnRH*	Gonadotropin releasing hormone	Thyroid and gonadal development and function	Hormone expressed in the pituitary gland	Gonads	Infertility since animals are hypogonadal	Dwarfism due to hypothyroidism	91
2.	*FSHb*	Follicle stimulating hormone β subunit	Important in folliculogenesis, spermatogenesis and Sertoli cell growth	Gonadotrophs in pituitary gland	Follicles, uterus and ovary	Infertility due to block in folliculogenesis prior to antral follicle formation; uterine and ovarian hypertrophy	Small testis size	43, 85
3.	*SOD1*	Copper/zinc superoxide dismutase	Dismutation of superoxide radicals to H_2O_2 and oxygen	Expressed in several tissues	Hypothalamus-pituitary axis	Post-implantation embryo death due to decreased serum gonadotrophin levels	Mice sensitive to paraquat administration	92, 93
4.	*IGF-I*	Insulin-like growth factor-1	Stimulation of granulosa cell proliferation	Selectively expressedin a subset of relatively healthy-appearing follicles	Follicles	Blocks folliculogenesis by affecting follicular responsiveness to FSH	Not reported	87
5.	*Esr 1*	Estrogen receptor α	Expressed in target sites of estrogen	Expressed in several target sites	Uterus and ovary	Hypoplastic uteri and hyperemic ovaries with no detectable corpus luteum	Affects testis, mammary glands, reproductive tract and skeletal tissue	94
6.	*Esr 2*	Estrogen receptor 2	Expressed in target sites of estrogen	Expressed highly in ovary, prostate, epididymis, lung, and hypothalamus	Follicles	Fertility compromised due to follicle arrest	Abnormal hyperplasia of prostate and bladder and abnormalities in several other tissues.	95
7.	*NGFI-A*	Transcription factor NGFI-A	Activate transcription by binding to GC rich sequences	Widespread expression in various tissues	Ovary	LH suppression results in absence of corpus luteum and abnormal ovary development	Abnormality in anterior pituitary development	96
8.	*PRL*	Prolactin	Development of post-pubertal changes	Hormone secreted from pituitary and few other local tissues	Multiple organs	Inferile due to multiple pre-implantation defect and irregular estrous cycle	Altered mammary gland development	86
9.	*Amhr2*	Anti-Mullerian hormone-2	Growth and differentiation factor of TGF superfamily	Produced by Sertoli cells and postnataly in ovary	Primordial follicles	Early depletion of primary follicles in ovary	Development of uteri in male causes infertility	88
10.	*Insl 3*	Insulin-like hormone 3	Growth and development of gubernaculum mediating testicular descent	Expressed in testis and ovarian tissues	Estrous cycle	Lower litter due to irregular estrous cycle lengths	More associated with infertility in the male than female	89

11.	*Ppp1r1 b*	Protein phosphatase 1 regulatory subunit 1B	Dopamine/cAMP regulated phosphoprotein, an intermediate in progesterone-mediated sexual receptivity	Expressed at several progesterone target sites	Sexual behavior	Modulation of reproductive behavior that is mediated by progesterone	Not reported	90
12.	*Tshb*	Thyroid stimulating hormone β	Release of thyroid hormones	Secreted from pituitary gland	Ovary	Continuous diestrous and fewer ovulated eggs, poor corpus luteum formation and no implantation	Several thyroid hormone related defects	97
13.	*Vdr*	Vitamin D receptor	Calcium homeostasis, cell differentiation and proliferation	In all calcium regulating tissues	Estrogen biosynthesis	Uterine hypoplasia and impaired folliculogenesis	Several effects	98

maintenance of pregnancy. The released oocyte is arrested at metaphase II. After ejaculation, the spermatozoa undergo capacitation in the female genital tract and then acrosome react to release proteolytic enzymes required to assist zona penetration and fusion of sperm membrane with oocyte membrane. The sperm cell entry induces a release of second polar body from oocyte nucleus to make it haploid. This is followed by fusion of two pronuclei to form a zygote that undergoes cleavage resulting in an embryo. The timed hormonal signals prepare the endometrium for receiving the embryo. The decidualization of the uterus is induced by signals from the blastocyst and several factors determine implantation and further growth of the embryo. Several genes are involved in these processes, some of these are discussed below.

Gene knockouts of prolactin receptors (*PRLR*) affect function of prolactin, resulting in multiple reproductive abnormalities including irregular menstrual cycles and failure of implantation, thus causing reduced fertility (53, 54). The embryo development is affected by deletion of genes such as *mater* (55) and *Dnmt 1o* (56), encoding for proteins that are important for the growth and differentiation of the embryo. The *mater* encodes for maternal antigen mRNA or proteins that accumulate in the egg during oogenesis. The absence of these proteins in gene knockout mice results in failure of the embryo to develop beyond the two-cell stage. *Dnmt 1o*, encodes a variant of DNA methyltransferase that is expressed specifically in the oocyte and pre-implantation embryo. It affects the genomic methylation pattern. The failure of maintenance of the methylation patterns lead to embryo death. One of the surface proteins of the sperm is a sperm adhesion molecule, PH 20 (*Spam 1*), a hyaluronidase bound to the inner acrosomal membrane, which helps sperm in penetration into oocyte. The knockout of *Spam 1* leads to decreased fertility (57). A similar GPI-anchored protein on the egg surface, N-acetyl glucosaminyl transferase, encoded by *Ptga* gene, is involved in the sperm-oocyte membrane interaction (58). Conditional mutants without the gene expression show no fertility due to lack of this protein on the surface of the oocyte.

There are several additional genes that affect ovulation, fertilization, and post-fertilization embryonic development (59-78). The effects of their knockout have been described in table 1.

3.4. Gene knockouts affecting female reproductive structures

A total of six genes were found in this category (table 1). Several genes that are involved in the development of non-reproductive organs are also involved in development of reproductive system. A family of signaling molecules, Wnt proteins, encoded by *Wnt* genes, has been shown to affect morphogenesis of embryonic layers. *Wnt 7a* KO causes Mullerian duct-associated sterility due to abnormal oviduct and uterus development (79). *Wnt 7b* KO causes embryonic death at mid-gestational stage (80). *Hoxa 11* are homeobox genes that specify regional specification during development (81). Their knockouts show defective uterine development that cannot support embryo growth. Also, in this group are cytochrome P450 (*Cyp 40*) (82), centromere protein B (*Cenpb*) (83) and transmembrane ATP-binding cassette transporter protein (*Abca 1*) (84). Their gene knockouts result in formation of malformed placenta, severe disruption of uterine epithelium and uterus hypoplasia, respectively. Their KOs have severe effects on embryonic development. They are either lethal or have severe structural deformities.

3.5. Gene knockouts affecting endocrine milieu

A total of 13 genes were found in this category (table 1). Hormones regulate every stage of reproductive growth and differentiation from gonadogenesis, folliculogenesis, ovulation, fertilization, embryo and fetal development, implantation, pregnancy and delivery. The gene KO of a hormone or its effector could have an effect on a specific fertility target. An interesting example among them is the knock out of gonadotrophin FSH β subunit (*FSH*). The infertility in this KO is due to block in folliculogenesis before the antral follicle formation with a uterine and ovarian hypertrophy. This gene also affects spermatogenesis and Sertoli cell function in males (43, 85). Prolactin (*PRL*) is responsible for several post-pubertal changes. Besides, the lack of PRL also leads to abnormalities in menstrual cyclicity. Defects are also seen in several other reproductive tissues involved in healthy fetal growth (86). Insulin-like growth factor (*IGF-1*) is required for granulosa cell proliferation by FSH. The *IGF-1* KO mice show no responsiveness to FSH that is required for growth of follicles (87). Anti-Mullerian hormone (*Amhr 2*), a growth and differentiation factor of TGF superfamily, is required for growth and differentiation of Mullerian ducts. The hormone is also synthesized, postnataly, by ovary and Sertoli cells. Lack of this hormone results in an early depletion of primary follicles (88). Insulin-like hormone, (encoded by gene *Insl 3)* is expressed both in ovary and testis. Its depletion results in irregular cyclicity thereby leading to severe lowering of the litter size (89) in females. The hormone also mediates the testicular descent in males. Another regulatory protein that

modulates progesterone-mediated reproductive behavior is protein phosphatase 1 subunit 1B, a dopamine- and adenosine 3', 5'-monophosphate (cAMP)-regulated phosphoprotein (DARPP-32) (90). The *Ppp1r1b* gene that encodes DARPP-32 constitutes an intermediate in the progesterone-mediated sexual receptivity in female rats and mice. The gene KO showed abnormal reproductive behavior. Other similar genes are described in table 1 (91-98).

4. CONCLUSION

The gene knockout studies have revealed several essential molecules, new insights and novel mechanisms involved in various steps of the fertility cascade in females. At the present time 83 gene knockouts were found in the database and literature that show an effect on female fertility. The fertility field has exploded with the advent of gene knockout technology. Almost every month there is a report on a new gene knockout with some effect on fertility. However, there are only a few genes whose knockouts have shown a specific effect on female fertility without a concomitant effect on non-reproductive systems. Among these specific molecules are, the oocyte coating zona pellucida proteins (ZP1, ZP2 and ZP3) involved in sperm recognition and binding and oocyte membrane proteins that are involved in fusion with sperm membrane. These proteins may provide novel targets for 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 and 3) accessibility and amenability of inactivation by inhibitors, antagonists or antibodies. These inhibitors/antagonists/antibodies should not affect follicullogenesis/endocrine milieu and should have a reversible effect on fertility without causing premature ovarian failure (POF) or menopause. The gene knockout technology is helping us to better understand female infertility and is opening up potential novel targets for contraceptive development.

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