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Gene knockouts that affect male fertility: novel targets for contraception

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Discussion
3.1. Gene knockouts affecting spermatogenesis
3.2. Gene knockouts affecting fertilization
3.3. Gene knockouts affecting mating behavior
4. Conclusions
5. Acknowledgement
6. References

1. ABSTRACT

There is an urgent need for a better method of contraception that is accepted, effective, and available, due the population explosion and unintended pregnancy. Various targets are being investigated that can be used for contraception. The ideal target should be non-steroidal, intercourse-independent, non-surgical, reversible, and non-barrier with no side effects. The gene knockout technology is a powerful approach to identify such novel targets. We identified at least 93 genes whose deletion demonstrated an affect on fertility in male mice till 2004 (1). In the present article, we found 71 additional gene knockouts in the database since the last report which demonstrated an affect on male fertility. The majority of these knockouts also demonstrated an effect on non-reproductive organs concomitant with an anti-fertility effect or effect on other organs was not examined. The knockouts of only a few genes/proteins induced a specific effect on fertility without a serious side effect. These genes/proteins may provide novel targets for contraception/contraceptive vaccine development.

2. INTRODUCTION

The population explosion and unintended pregnancies continue to pose major public health issues worldwide. The world population has exceeded 6.67 billion. Ninety-five percent of this growth is in developing nations. In the USA alone, half of all pregnancies are unintended, which results in >1 million elective abortions annually (2,3). These women use some type of contraceptive. This calls for a better method of contraception that is acceptable, effective and available both in the developed and developing nations. An ideal contraceptive method should be highly effective, safe, inexpensive, have a prolonged duration of action, be reversible, require infrequent administration, and can be used privately.

At present, there are limited options available for fertility control. Contraceptive options currently available to women include hormonal contraceptives e.g., birth control pills, contraceptive patch, hormone injections, natural methods such as abstinence, early withdrawal,

intrauterine devices (IUDs) such as the copper IUD, the progestin-releasing IUD, vaginal rings, diaphragm/spermicidal combinations, and sterilization (http://www.patientcareonline.com/patcare/article/articleDetail.)(4). The contraceptive options available for men are: vasectomy, condoms, and withdrawal prior to ejaculation. Vasectomy is a permanent procedure with low success rate of fertility reversal, and the condoms and withdrawal are either not readily acceptable or have high failure rate.

Antisperm contraception can target sperm production, disrupting sperm structure and function, interruption of sperm transport and deposition, and prevention of sperm-egg interaction. All these approaches involved identification of the genes and proteins that are involved specifically in these processes. A large number of genes are involved in reproduction. The gene knockout technology represents a very powerful approach to identify testes/sperm-specific genes/proteins that can provide novel targets for contraception. The study of a mutation in a particular gene that leads to an infertility phenotype in the mouse model can be extrapolated to humans and thus could lead to the identification of novel contraceptive targets.

A total of 93 gene-knockouts were found in the literature search till January 15, 2005 that affect fertility in male mice (4). This article discusses the articles on additional gene-knockouts that have shown to affect male fertility since then. We used NCBI (National Centre for Biotechnology Information) database, PubMed, and Google Scholar to search for these genes.

These genes can be categorized into following three major categories depending upon the fertilization/fertility parameter that is affected after their deletion, namely affecting spermatogenesis, fertilization, and mating behavior. We found 57 gene-knockouts affecting spermatogenesis, 10 affecting fertilization, and 4 affecting mating behavior (Table 1).

3. DISCUSSION

3.1. Gene knockouts affecting spermatogenesis

The process of spermatogenesis involves the development of spermatogonia into spermatocytes, which undergo meiotic divisions to form haploid round spermatids. The round spermatids differentiate into mature spermatozoa in a process known as spermiogenesis. A large number of genes are involved in the process of spermatogenesis and spermiogenesis and these could be potential targets for male contraceptives. A mutation in a gene involved in spermatogenesis can lead to a defect in the development of a mature spermatozoon.

A total of 57 genes were found in this category (table 1). They are described below:

Dmrt genes have been implicated in sex determination. DM domain containing genes (*Dmrt1, Dmrt2, Dmrt3* and *Dmrt7*) present on chromosome 9 cause XY sex reversal in humans. *Dmrt1* and *Dmrt7* are expressed only in adult testis. Deletion of D*mrt7*, like D*mrt1*, causes male infertility in mice with an arrest of spermatogenesis at the pachytene stage, suggesting an essential role of DM domains in sexual development and differentiation (5). *SCCRO* gene is an oncodevelopmental gene playing a critical role in early development and maintenance, and uncontrolled expression driving malignant transformation. It is an activator of cullin neddylation and ubiquitination, and the loss of *SCCRO* gene results in disruption of CUL3 neddylation, leading to abnormal sperm development and male infertility (6). The *Pms2* gene encodes for a Pms2 protein that is involved in repairing mistakes made during DNA replication. They are especially abundant in the male germline, and play a role in synaptonemal complex formation during prophase of meiosis I, indicating potential for abnormalities in chromosome synapsis. Disruption of the *Pms2* gene produces males with abnormal spermatozoa, resulting in infertility (7,8). Six MutS homologs, designated MSH1-6, and at least three MutL homologs, MLH1, PMS1and PMS2 have been identified. Mice homozygous for loss-of-function alleles of Msh2, Msh6, Mlh1, or Pms2 were healthy at birth but appeared to be highly predisposed to tumorigenesis. Recently the *Msh5* and *Mlh1*-deficient mice have been examined and both shown an infertile phenotype. Msh5 recognizes mismatches that arise by erroneous DNA replication and repairs these mistakes. The deletion of the *Msh5* gene results in an infertile male phenotype due to a disruption of spermatogenesis at the zygotene stage. *Mlh1*-deficient male mice did not produce spermatozoa at all (8,9). *Cyp17* is a bifunctional microsomal monooxygenase that mediates the 17α-hydroxylation of pregnenolone or progesterone, ultimately leading to the biosynthesis of cortisol and sex steroid biosynthesis. The deletion of *Cyp17* demonstrated reduced testosterone in blood serum and testis, and plays a critical role in the organization and structure of sperm mitochondria. The *Cyp17* knockout mice exhibited reduced mitochondrial function leading to altered sperm morphology, and infertility (10).

CD59 is a conserved gene universally expressed in most animal species. The m*CD59* gene encodes for a GPI-linked membrane proteins (mCD89a and mCD9b) that inhibits formation of the membrane attack complex of complement, and has a function in sperm maturation. The targeted deletion of *mCD59b* results in progressive loss of male fertility (11). The *Spem1* gene was recently discovered, and was found to encode for a protein that is exclusively expressed in cytoplasm of steps 14-16 spermatids. Its participation is required in the proper cytoplasm removal and subsequent maturation during the final stages of spermiogenesis. The lack of the *Spem1* gene in mice cause failure of the cytoplasm to become loose and detach from head and neck region of developing spermatozoa, resulting in sperm deformation and male infertility (12). The *MLH1* gene provides instructions for making a protein that plays an essential role in DNA repair. The MLH1 protein joins with other proteins to form an active protein complex that repairs mistakes made during DNA replication. The lack of the *Mlh1* gene in mice renders them infertile due to inability of the protein to interact with meiotic chromosomes at pachynema. *Mlh*-null

Table 1. Gene knockouts that affect male fertility

No.	GENE	PROTEIN NAME	SIZE	FUNCTION	LOCALLIZATION	INFERTILITY TARGET	PHENOTYPE	EFFECTS ON OTHER TISSUES	REF.
I. GENE KNOCKOUTS AFFECTING SPERMATOGENESIS									
1.	*Dmrt7*	DMRT7 (Doublesex and mab-3 related transcription factor 7)	39 kDa	Required for male sexual differentiation	Expressed in embryogenesis in gonads of both sexes, and only in testes of adult male mice	Pachytene stage of spermatogenesis	Smaller testes size and absence of sperm in epididymis	No other phenotypic abnormalities	5
2.	*SCCRO*	SCCRO (Squasmous Cell Carcinoma Related Oncogene)	30 kDa	Activator of cullin neddylatoin and ubiquitination	Widespread expression	Spermatogenesis	Dysfunctional spermatogenesis leading to abnormal sperm development	Smaller body size (33%)	6
3.	*Pms2*	Pms2 (Post-meiotic segregation 2)	40 kDa	DNA mismatch repair	Widespread expression	Spermatogenesis (Prophase of Meiosis 1)	Infertile, producing only abnormal spermatozoa	Prone to sarcomas and lymphomas	7,8
4.	*Msh5*	Msh5 (Muts homolog 5)	93 kDa	Heterodimeric protein complex component implicated in resolution of meiotic recombination intermediates	Widespread in testis, thymus, and immune system	Zygotene stage of spermatogenesis	Defect at seminiferous tubule epithelial stage, abnormally long zygotene stage and no progression into pachytene stage	Not Reported	9, 8
5.	*Cyp17*	Aromatase cytochrome P450 17α-hydroxylase/17	57 kDa	Role in organization and structure of sperm mitochondria	Liver and nonsteroidogenic tissues	Spermiogenesis	Reduced mitochondrial function leading to altered sperm morphology and infertility	Not Reported	10
6.	*CD59b*	mCD59b	14 kDa	Inhibit formation of membrane attack complex and plays role in sperm maturation	Seminal plasma and sperm plasma membranes	Spermatogenesis	Spermatogonia arrest inside seminiferious tubules	Hemolytic anemia	11
7.	*Spem1*	Spem1 (Spermatid maturation 1)	35 kDa	Role in proper cytoplasm removal during elongated spermatid formation	Cytoplasm of steps 14-16 spermatids	Spermiogenesis	Cause failure of cytoplasm to become loose and detach from head/neck region of developing spermatozoa	Not Reported	12
8.	*Mlh1*	Mlh1 (mutL homolog 1)	85 kDa	Role in genetic recombination, form active protein complex that repairs mistakes made during DNA replication	Meiotic chromosomes	Spermatogenesis	Spermatogenesis proceeded normally through pachynema but failed to progress through metaphase I	Strong cancer predisposition	13
9.	*Taf7l*	TAF7-like RNA polymerase II	50 kDa	Component of transcription factor TFIID required for most protein coding genes	Cytoplasmic in spermatogonia and early spermatocytes, translocates into nuclei of pachytene spermatocytes and round spermatids	Spermatogenesis	Abnormal sperm morpholoty, reduced motility, oligospermia	Using Cre-*loxP* strategy, specifically mutated in testis	14
10.	*LXRαβ*	LXRαβ (Liver X receptor α and β)	~51 kDa	Regulate lipid homeostasis is cells	Lower levels of testosterone, increased lipid accumulation in Sertoli cells and low proliferation rate of germ cells	Spermatogenesis	Infertile at 5 months of age due to high apoptotic rate of germ cells	Not Reported	15
11.	*Aurora-B Aurora-C*	Aurora-B Aurora-C	40(B); 48(C) kDa	Cell cycle-regulatory serine-threonine kinases	Somatic cells and testis	Spermatogenesis	Spermatogenic arrest, abnormal sperm, and subfertility	Not reported	16
12.	*JHDM2A*	JHDM2A (Jumonji C-domain-containing histone Demethylase 2A)	152 kDa	Regulate expression of transnuclear proteins	Post-meiotic stage of spermatogenesis	Spermatogenesis	Infertility due to oligospermia	Small testes size	17
13.	*GBA2*	GBA2 (β-Glucosidase 2)	105 kDa	Role in metabolism of bile acid-glucose conjugates	glycoprotein accumulation in liver, brain, and testis	Spermatogenesis	Infertility due to abnormal sperm morphology, abnormal acrosomes, and defective motility	Glycolipid storage disease	18
14.	*PDE11*	PDE11 (Phosphodiesterase 11)	105 kDa	Regulate normal sperm activation and spermiogenesis	Testis, prostate, developing spermatozoa	Spermatogenesis	Reduced sperm concentration, decreased forward motility, lower percentage of live spermatozoa, spontaneous/premature capcitation	Not reported	19
15.	*IP6K1*	IP6K1 (Inositol hexikisphosphate kinase 1)	53 kDa	Deposition of insulin and glucose	Nuclear protein	Spermiogenesis	Defect in spermiogenesis, no sperm in the epididymis	Smaller body size and lower circulating insulin	20
16.	*Bmal1*	Bmal1 (Brain and muscle Arnt-like protein-1)	70 kDa	Transcription factor regulating circadian rhythm	Defect in testicular Leydig cells	Spermatogenesis	Deficiency in steroidogenesis, low testosterone and high LH	Decreased expression of *StAR* (Steroidogenic acute regulatory protein) gene in testis	21
17.	*Ubb*	Ubb (Ubiquitin B)	11 kDa	Role in meiotic progression and gonad physiology	Cell death at pachytene stage of development	Spermatogenesis	Arrest during meiotic prophase, complete testicular degeneration by year 2	Not reported	22
18.	*Foxi1*	Foxi1 (Forkhead box 1)	41 kDa	Major proton secretory cells	Epididymal epithelia	Post-testicular sperm maturation	Insufficient post-testicular maturation, due to failure of proper acidification of epididymal luminal content	Not reported	23
19.	*CFTR*	CFTR (Cystic Fibrosis transmembrane conductance regulator)	168 kDa	Chloride channel regulated by cAMP	Transmembrane conductance regulator	Genital tract	Male infertility	Salty sweat, pancreatic insufficiency, intestinal obstruction, severe pulmonary disease	24
20.	*NPC1*	NPC1 (Niemann-Pick C1)	180 kDa	Regulate trafficking of LDL-mediated endocytosed cholesterol	Multiple membrane spanning protein	Spermatogenesis and fertilization	Partial arrest of spermatogenesis, impaired binding to ZP, and high frequency of sperm abnormalities	Niemann-Pick type C disease	25
21.	*GRTH*	GRTH (Gonadotrophin-regulated testicular helicase)	61 kDa	mRNA binding protein participating in posttranscriptional events	Present in nucleus, cytoplasm and chromatoid body of germ cells	Spermatogenesis	Azoospermia resulting from a complete arrest of spermiogenesis at step 8 of round spermatids that fail to elongate	Smaller testis size	26

22.	*Lama2*	Laminin α2 Chain	80 kDa	Role in basement membrane structure and function	Predominantly testicular basement membrane tissue	Spermatogenesis	Seminiferous tubules display defect in timing of lumen formation resulting in fewer spermatids	Congenital muscular dystrophy	27
23.	*LH-R*	LH-R (Luteinizing hormone - receptor)	51 kDa	Role in sexual development and reproductive function	Primarily in gonads	Spermatogenesis	Deficient spermatogenesis and cryptorchid testes	Not reported	28
24.	*FSHβ*	FSHβ (Follicle stimulating hormone-β)	39 kDa	Role in sexual development and reproductive function	Primarily in gonads	Spermatogenesis	Deficient spermatogenesis	Not Reported	29,30
25.	*SSTK*	SSTK (Small serine/threonine kinase)	33 kDa	Role in postmeiotic chromatin remodeling	Heads of elongated spermatids	Spermiogenesis	Impaired motility and morphology of spermatozoa	Not reported	31
26.	*Creb314*	Creb314 (cAMP response element-binding protein-314)	45 kDa	Role in male germ cell development	All organs, but high level of expression in testis	Spermatogenesis	Subfertility due to reduced number of spermatozoa and increased apoptosis of meiotic and postmeiotic germ cells	Not reported	32
27.	*Hanp1 / H1T2*	Hap1 (Haploid germ cell-specific nuclear protein-1)	44 kDa	Role in nuclear formation in spermatozoa and histone replacement during spermiogenesis	Nuclei of murine spermatids	Spermiogenesis	Infertility due to deficient motility and inability to fertilize eggs	Not reported	33
28.	*Brek/Lmtk2*	Brek (Brain-enriched kinase)/ Lemur tyrosine kinase 2	165 kDa	Essential for late stage spermatogenesis	Germ cells of testis	Spermiogenesis	Azoospermia, round-spermatid fail to become elongated spermatids	Not reported	34
29.	*PP1c gamma*	PP1c gamma (Protein phosphatase-1 catalytic subunit gamma)	59 kDa	Mediator of phosphatidic acid action in cells	Germ cells	Spermatogenesis	Male infertility due to severe impairment in spermatogenesis	Not reported	35
30.	*Ldhc*	LDHC (Lactate dehydrogenase-C_4)	116 kDa	Glycolysis and ATP production in sperm	Spermatocytes, spermatids, and sperm	Spermatogenesis	Rapid reduction in ATP resulting in decreased motility, unable to penetrate ZP	Not reported	36
31.	*pgk2*	PGK2 (Phosphoglycerate kinase-2	45 kDa	Glycolysis and ATP production in sperm	Selectively activated in primary spermatocytes	Spermatogenesis	Significantly reduced sperm motility	Not reported	37
32.	*LHβ*	LHβ (Luteinizing hormone β-subunit)	38 kDa	Promote steroidogenesis and gametogenesis	Primarily in gonads	Spermatogenesis	Spermatogenesis blocked at round spermatid stage	Decreased testes size, Leydig cell hypoplasia, defect in expression of genes encoding steroid biosynthesis pathway enzymes, and reduced testosterone levels	38
33.	*Hsf1/Hsf2*	Hsf1/Hsf2 (Heat shock factor-1/Heat shock factor-2)	~90 kDa	Major transactivators of heat shock proteins	All testicular cells	Spermatogenesis	Failure of meiotic prophase at pachytene stage	Reduced number of germ cells	39
34.	*Act*	Act (Activator of (CREM) cAMP-responsive element modulator in testis)	85 kDa	Enhances CREM-dependent transcription	Haploid nucleus of round and cytoplasm of elongated spermatids	Spermatogenesis	Reduced sperm in epididymis with severe abnormalities	None reported	40
35.	*Pank2*	Pank2 (Pantotenate kinase 2)	50 kDa	Role in energy metabolism	Mitochondria of retina and sperm	Spermiogenesis	Arrest in spermiogenesis and complete absence of elongated and mature spermatids	Retinal degeneration, decrease in body weight	41
36.	*RLX*	RLX (Relaxin)	6 kDa	Anti-apoptosis factor	Role in development and function of male reproductive tract and prostate growth	Spermatogenesis	Decreased/retarded sperm maturation, increased rate of apoptosis, decreased fertility	Increased collagen and decreased epithelial proliferation in the prostate	42
37.	*Plag1*	Plag1 (Pleomorphic adenoma gene 1)	55 kDa	Transcription factor protein	Reproductive organs and pituitary	Spermatogenesis	Major abnormalities during spermatogenesis resulting in impaired fertility	Significantly lower body weight at birth, and proportionally small organs, retarded growth; disproportionally small seminal vesicles	43
38.	*Sox8*	Sox8 (SRY [sex determining region Y]-box 8)	47 kDa	Transcription factor expressed during development	Sertoli cells	Spermatogenesis	Infertility due to deregulation of spermatogenesis	Weight loss	44
39.	*Ahr*	AhR (Aryl hydrocarbon receptor)	60 kDa	Ligand-activated transcription factor regulating xenobiotic-metabolizing enzymes	Role in germ cell devleopment	Spermatogenesis	Decreased fertility due to low sperm count in epididymis	Decreased serum testosterone concentration and expression of steroidogenic proteins in testicular Leydig cells	45
40.	*Galgt1*	Galgt1 (Galactoglycerolipid-Nac transferase)	~180 kDa	Modulate membrane receptors and ion channels	Present on extracellular leaflet of plasma membrane lipid bilayers and on topologically equivalent membrane sites of endocytotic and exocytotic organelles	Spermatogenesis	Arrest of spermatogenesis at haploid spermatid formation	None reported	46
41.	*Sycp1*	Sycp1 (Synoaptonemal complex 1)	114 kDa	synaptonemal complex assembly	Chromosome	Spermatogenesis	Spermatocyte arrest in pachynema	None Reported	47
42.	*Msy2*	Msy2 (Y-box-binding protein 2)	39 kDa	DNA/RNA-binding protein	Germ cells	Spermatogeneisis	Postmeiotic spermatogenesis disrupted with many misshapen and multinucleated spermatids, no spermatozoa detected in epididymis	Male sterility Female subfertility	48
43.	*SAFB1*	SAFB1 (Scaffold attachment factor B1)	103 kDa	DNA/RNA-binding protein	Involved in RNA processing, and RNA/DNA-binding	Spermatogenesis	Low circulating testosterone resulting in degeneration of germinal epithelium, increased apoptosis and Leydig cell hyperplasia	Exhibited prenatal/neonatal lethality, small testes, dwarfism, and low serum insulin-like growth factor (IGF1)	49
44.	*Sept4*	Septin	55 kDa	Cortical organization of annulus in sperm tail	Postmeiotic germ cells; cortical ring separating middle and principal piece	Spermatogenesis	Defective morphology and motility of sperm flagellum due to lack of annulus on	None reported	50

					of annulus on sperm tail		sperm tail		
45.	*AF5q31*	AF5q31 (ALL1 fused gene from chromosome 5q31)	127 kDa	Transcription regulator in testicular somatic cells	Preferentially in Sertoli cells	Spermiogenesis	Azoospermia with arrest of germ cell at spermiogenesis	87% died *in utero* and neonatally with impaired embryonic development and shrunken alveoli and impaired expression of protamine 1, protamine 2, and transition protein 2	51
46.	*MFP-2*	MFP-2 (Multifunctional protein-2)	79 kDa	Peroxisomal beta-oxidation metabolism	Germ cells, Sertoli cells, and Leydig cells	Spermatogenesis	Infertile with accumulation of neutral lipids resulting in complete testicular atrophy	None reported	52
47.	*MDC1*	MDC1 (Mediator of DNA damage checkpoint protein 1)	227 kDa	Role in checkpoint activation and DNA repair following damage	Directly mediates interaction between H2AX and ATM and regulates downstream signaling events	Spermatogenesis	Male infertility due to defective spermatogenesis	Smaller testes, growth retardation, immune defects, chromosome instability, DNA repair defects, and radiation sensitivity	53
48.	*TAL*	TAL (Transoladase)	37 kDa	Mitochondrial maintenance in germ cell	Testis, caput, cauda epididymis	Spermatogenesis	Male sterility with defective forward motility in spermatozoa due to loss of mitochondrial membrane integrity	None reported	54
49.	*Hspa4*	Hspa4l (Heat shock 70 kDa protein 4-like)	70 kDa	Role in spermatogenesis and osmotic tolerance	High in testis, moderate in other tissues	Spermatogenesis	Low sperm count and abnormal motility	Unilateral hydronephrotic kidneys, increased susceptibility to osmotic stress	55
50.	*Pax8*	Pax8 (Paired box 8)	48 kDa	Role in gonadal development	Epihelia of epididymis and efferent ducts	Spermatogenesis	Abnormal development of post-testicular ducts leading to atrophy of testes and complete lack of spermatozoa	None reported	56
51.	*CR16*	CR16 (Corticosteroids and regional expression 16)	45 kDa	Binding protein involved in inducing actin polymerization	Actin filaments at Sertoli cell-spermatid junction	Spermatogenesis	Male-specific sterility due to abnormal head morphology and reduced fertilization ability	None reported	57
52.	*Gpr54*	Gpr54 (G protein-coupled receptor 54)	43 kDa	Key role in regulating sexual maturation	Germ cells	Spermatogenesis	Hypogonadotropic gonadism, males lack preputial separation, low FSH levels	Females have delayed vaginal opening and smaller ovaries in uteri, absent estrous cycle	58
53.	*Kiss1*	Kiss1 (Kisspeptin 1)	15 kDa	Derives the Gpr54 ligand, metastin	Germ cells	Spermatogenesis	Abnormal sexual maturation, smaller testes, males lack preputial separation, low FSH levels	Female have delayed vaginal opening, absent estrous cycle	58
54.	*Psme4*	PA200 (Proteasome activator 200)	211 kDa	Activates proteasomal cleavage of peptides in an energy- independent manner	Broadly expressed nuclear protein	Spermatogenesis	Male infertility due to defect in meiotic spermatocytes during postmeiotic haploid spermatid maturation	None reported	59
55.	*G9a*	H3K9 (Histone H3 lysin 9)	138 kDa	Major H3K9 methyltransferase at euchromatin	Nucleus, associates with euchromatin regions	Spermatogenesis	Perturbation of synchronous synapsis in meiotic prophase	Specifically mutated in germ cells	60
56.	*Trp73*	P73 Transactivation Domain	Tap73 73 kDa	Anti-apoptotic function	Transcription factor	Spermatogenesis	Male and female sterility	Spontaneous and carcinogen-induced tumors and hippocampal dysgenesis.	61
57.	*PACRG*	Parkin-Co-Regulated Gene	33 kDa	Sperm axoneme formation and spermatogenesis	Axoneme of sperm and ependymal cilia	Spermiogenesis	Male infertility	Hydrocephalus	62
II. GENE KNOCKOUTS AFFECTING FERTILIZATION									
1.	*Tpst2*	TPST2 (Tyrosylprotein sulfonotransferase 2)	54 kDa	Plays role in growth and reproduction	*Trans*-Golgi network	Sperm-ZP binding	Impaired sperm motility and reduced ability to fertilize ZP-intact eggs	Impairs early growth from 2-10 weeks	63
2.	*ADAM3*	Cyritestin (a disintegrin and metalloprotease domain 3)	42 kDa	Possible ZP ligand	Sperm-surface protein	Sperm-zona pellucida binding	Cannot bind to egg ZP	No other phenotypic abnormality	64
3.	*PGAP1*	PGAP1 (post GPI attachment to proteins 1)	105 kDa	Transport of GPI-anchored proteins from the endoplasmic reticulum to the Golgi body	Endoplasmic reticulum membrane protein	Fertilization	Sperm unable to go into oviduct and weak attachment to ZP	Otocephaly	65
4.	*AC3*	AC3 (Adenylyl cyclase 3)	130 kDa	Glycosylated protein involved in the cascade required for detection of odorants	Postmeiotic germ cells	Fertilization	Decreased motility and increased spontaneous acrosome reaction	Not reported	66
5.	*Gapds*	GAPDS (Glyceraldehyde 3-phosphate dehydrogenase-S)	64 kDa	Glycolysis and ATP production in sperm	Fibrous sheath of sperm flagellum	Fertilization	Decreased sperm motility – sluggish movement without forward progression	None reported	67
6.	*Ace*	Angiotesin-converting enzyme	170 kDa	Cleaves angiotesin-1 and bradykinin that leading to upregulation of blood pressure	Widespread enzyme	Fertilization	Defective sperm-ZP binding	None reported	68
7.	*iPLA2β*	iPLA2β (Group VIA Phospholipase A_2)	88 kDa	Participates in arachidonic acid metabolism	Highest level of expression in testis and brain	Fertilization	Reduced sperm motility and impaired ability to fertilize ZP	None reported	69
8.	*ZPBP1*	ZPB1 (Zona pellucida binding protein 1)	38 kDa	Role in structural development during spermiogenesis	Acrosome	Fertilization	Prevent acrosome compaction resulting in fragmentation;	Abnormal sperm morphology and motility	70
9.	*ZPBP2*	ZPB2 (Zona pellucida binding protein 2)	29 kDa	Role in structural development during spermiogenesis	Acrosome	Fertilization	Deformed acrosomal membrane invagination and sperm with reduced ability to penetrate ZP	None reported	70
10.	*Tekt4*	Tektin-4	51 kDa	Regulates proper coordinated beating of sperm flagellum	Flagella of haploid round spermatids in testis	Fertilization	Abnormal motility with reduced forward progressive velocity and uncoordinated waveform propagation	None reported	71
III. GENE KNOCKOUTS AFFECTING MATING BEHAVIOR									
1.	*α_1-ADR*	α_1-ADR (α_1-adrenoceptor) ($\alpha_{1A,B,D}$)	42 kDa	Role in blood pressure and ejaculation	Contractile dysfunction of the vas deferens	Male sexual function	Infertility due to ejaculation	Blood pressure response decreased?	72
2.	*Acvr2*	Acvr2 (Adult activiin receptor type II)	58 kDa	Upstream regulator of nitric oxide synthase (NOS) activity within the medial	Brain	Sexual behavioral deficits	Delayed initiation of copulation, reduced mount and intromission frequencies,	None reported	73

				preoptic area (mPOA) of the forebrain			and increased mount, intromission and ejaculation latencies.		
3.	*Slo*	SLO Streptolysin-O	77 kDa	Responsible for pore-forming subunit of BK-channel which controls hyperpolarization of smooth muscle membrane of corpus cavernosa muscle	Corpus cavernosa smooth muscle (CCSM)	Sexual behavioral deficit	Erectile dysfunction	None reported	74
4.	*NOS1*	nNOS (neuronal nitric oxide synthase)	160 kDa	Required for central hormonal regulation of reproductive function	Brain	Sexual behavior dysfunction	Hypogonadism, males do not display mating behavior	Decreased gonad size, increased levels of plasma LH and FSH	75,76

mice also exhibit a strong predisposition for cancer (13). TAF7L is an X-linked germ cell-specific paralogue of TAF7, which is a generally expressed component of TFIID, a general transcription factor required for transcription of most protein-coding genes by most RNA polymerase II. The target mutation of *TAF7L* in the testes produces mice that are fertile, but have reduced litter size due to oligospermia involving the production of abnormal sperm with low motility (14). The LXRα and LXRβ genes encode for proteins that regulate lipid homeostasis in cells exposed to high amounts of cholesterol and/or fatty acids. A double-knockout of these genes results in lower level of testosterone, increased lipid accumulation in Sertoli cells and high apoptosis of germ cells ultimately leading to an infertile phenotype (15).

The Aurora kinases are cell cycle-regulatory serine-threonine kinases that have been implicated in the function of spermatogenesis. The localization of Aurora-B is tightly regulated during spermatogenesis, and Aurora-C expression appears to be testis specific. Knockouts of each molecule were performed separately, and the phenotypic expression of both included abnormal spermatocytes, increased apoptosis, spermatogenic arrest, and compromised fertility (16). The Jhdm2a protein is an important transcriptional activator that removes methyl groups from histones through a hydroxylation reaction. These proteins directly bind to and control the expression of nuclear proteins which are required for packing and condensation of sperm chromatin, thus are essential for successful spermatogenesis. The deletion of the *Jhmd2a* gene results in a significantly lower sperm count in males leading to infertility (17). GBA2 is a resident enzyme of the endoplasmic reticulum that plays a role in the metabolism of bile acid-glucose conjugates. It is expressed in the liver, brain, and testis. A knockout performed on the *GBA2* gene results in an accumulation of glycolipids in the Sertoli cells of the testis gave rise to misshapen sperm. These phenotypic defects result in infertility in the male mice (18). Eleven known families of phosphodiesterases (PDE1-PDE11) catalyze the hydrolysis of cyclic nucleotides to their corresponding monophosphates. PDE11, in particular, is highly expressed in the testis, and plays a role in normal sperm activation and therefore has an impact on fertilization rates. Studies reported on the targeted deletion of the *PDE11* gene reveal an infertile phenotype due to reduced sperm concentration, decreased forward motility, lower percentage of live spermatozoa, and spontaneous/premature capacitation (19). Inositol pyrophosphates have been implicated in a variety of physiologic functions including apoptosis, endocytosis, telomere length maintenance, and chemotaxis. The various mammalian IP6Ks serve diverse functions. Selective deletion of the *IP6K1* gene reveals its role physiological role in spermiogenesis. Knockout *IP6K1* mice were infertile due to defects in spermiogenesis, where there were very few advanced spermatids observed in the seminiferous tubules and no sperm in the epididymis (20).

The BMAL1 protein is a transcription factor known to regulate circadian rhythm, which in turn regulates the circadian clock and mammalian reproductive physiology. Recent knockout studies discovered that BMAL1 protein plays an important role in steroidogenesis. The deletion of the *Bmal1* gene produces infertile male mice with low testosterone and high luteinizing hormone levels, suggesting a defect in testicular Leydig cells (21). Ubiquitin is encoded in mice by two polyubiquitin genes, *Ubb* and *Ubc,* which are involved in for nearly all cellular processes. Studies reveal that loss of *Ubb* gives rise to profound defects in germ cell maturation that are surprising in view of the fact that mitotic cell cycle is not obviously affected. Mice with the targeted deletion revealed an infertile phenotype due to a block in the pachytene stage of spermatogenesis where they fail to enter the diplotene stage or progress to the first meiotic division phase, and eventually undergo cell death. Also, by two years of age, the $Ubb^{-/-}$ mice undergo complete testicular degeneration (22). An essential aspect of male reproductive capacity is the process of post-testicular maturation of spermatozoa in the epidydimis. The *Foxi1* gene encoding for the Foxi1 protein is essential regulator of epididymal epithelia facilitating epididymal sperm maturation. In *Foxi1*-null male mice, the spermatozoa fail to reach the female genital tract in sufficient numbers, resulting in male subfertility (23). The *CFTR* gene encodes for the Cystic Fibrosis transmembrane conductance regulator protein that functions as a chloride channel. Dysfunction of the CFTR protein results in salty sweat, pancreatic insufficiency, intestinal obstruction, severe pulmonary disease, and male infertility. Knockout *CFTR* mice were examined, and the exact mechanism of the CFTR protein role in male infertility is not known (24). The NPC1 gene encodes for a multiple membrane spanning protein, which regulates the trafficking flow-density lipoprotein-mediated endocytosed cholesterol. Analysis of the *NPC1* gene knockout revealed a partial arrest of spermatogenesis in testes, abnormal sperm morphology, and impairment in the binding of sperm to the egg zona pellucida, resulting in an infertile male phenotype (25).

The Gonadotrophin-regulated testicular RNA helicase (GRTH) belongs to the DEAD-box (Asp-Glu-Ala-Asp) protein family of RNA helicases. Members of this family play a regulatory role in many aspects of RNA functions. GRTH is a binding protein that participates in posttranscriptional events concerned with the translation of genes that are essential for the progression of

spermatogenesis. The deletion of the *GRTH* gene results in a sterile male phenotype due to complete arrest at step 8 of spermiogenesis (26). Laminins are important for basement membrane structure and function. The laminin α2 chains are a major component of testicular basement membranes, and are major chains in the basement membranes of seminiferous tubules. Laminin α2 chain-deficient mice displayed a defect in the timing of lumen formation, resulting in production of fewer spermatids, and therefore male infertility (27). Luteinizing hormone receptor (LHR), a member of the G protein-coupled, seven transmembrane receptor family, and is essential for normal sexual development and reproductive function. LHR are expressed primarily in the gonads. Null LH-R males are sterile due to cryptorchid testes and deficient spermatogenesis (28). The pituitary gonadotropin follicle stimulating hormone (FSH) interacts with its membrane-bound receptor to produce biologic effects. Traditional functions of FSH include follicular development and estradiol production in females and the regulation of Sertoli cell action and spermatogenesis in males. The targeted deletion of the *FSHβ* gene results in male infertility due to defective spermatogenesis (29, 30). Phosphorylation of serine, threonine, and tyrosine residues in substrate targets by protein kinases is a common posttranslational protein modification in eukaryotes and provides a fundamental mechanism for the control of cellular events. The SSTK protein belongs to a group of genes that are expressed at late stages of spermiogenesis, and plays a role in postmeiotic chromatin condensation. Targeted deletion of the *STTK* gene results in male sterility due to impairment of motility and morphology of spermatozoa (31).

Creb3l4 belongs to the CREB/ATF family of transcription factors that are involved in mediating transcription in response to intracellular signaling. This study shows that Creb3l4 is expressed at low levels in all organs and in different stages of embryogenesis, but is present at very high levels in the testis, particularly in postmeiotic male germ cells. In mice with a knockout *Creb314* gene, there was a significant reduction in spermatozoa in the epididymis, and evidence of increased opoptosis of meiotic/postmeiotic germ cells (32). The changes in the nuclear proteins occur in association with the displacement of general nucleohistones by transition proteins (TNP) and other proteins, including a number of testis-specific histones and nonhistone chromosomal proteins. A novel haploid germ cell-specific nuclear protein (HANP1) was recently characterized in the mouse testis that is involved in the histone-protamine transition of sperm chromatin and the subsequent production of functional sperm. Deletion of the *Hanp1* gene resulted in an infertile phenotype due to deficient sperm motility and inability to fertilize eggs (33). The Brek/Lmtk2 protein is a member of the Aatyk family of kinases, and plays a physiological role in germ cell differentiation and is essential for late stage spermatogenesis. The targeted disruption of the *Brek/Lmtk2* gene results in male infertility with azoospermia, where the round spermatids fail to undergo the normal change in morphology to become elongated spermatids (34). The PP1c gamma protein functions as a mediator of phosphatidic acid in cells. The targeted deletion of the *protein phosphatase-1 catalytic subunit gamma (PP1c gamma*) gene has been found to result in male infertility that is a direct result of severe impairment of spermatogenesis (35). The lactate dehydrogenase protein family members are typically distributed in tissue- and cell-type specific patters and serve as the terminal enzyme of glycolysis, catalyzing reversible oxidation-reduction between pyruvate and lactate. LDHC is a family member that is abundant in spermatocytes, spermatids, and sperm, and also in modest amounts in oocytes. The enzymatic activity is required for the process of glycolysis and ATP production in flagellum of sperm. Mice with an *Ldhc* knockout have an in fertile phenotype due to rapid reduction in ATP level and motility, where the sperm fail to penetrate the zona pellucida (36).

Transcription of the testis-specific *Pgk2* gene is selectively activated in primary spermatocytes to provide a source of phosphoglycerate kinase that is critical to normal motility and fertility of mammalian spermatozoa. Pgk2 is a glycolytic enzyme that is expressed only during spermatogenesis. The targeted deletion of *pgk2* gene results in severely reduced sperm motility, rendering male mice models infertile (37). Luteinizing hormone (LH) acts on gonadal cells to promote steroidogenesis and gametogenesis. These hormones are heterodimers consisting of a common α-subunit noncovalently linked to a hormone-specific β-subunit. The deletion of the *LHβ* gene results in male infertility due to block of spermatogenesis at the round spermatid stage, accompanied by other gonadal growth/functional defects (38). Heath shock factors are major transactivators of heat shock proteins, and are also involved in regulation of other genes active in embryonic development. They play a role in the cyclic process of spermatogonia cell-differentiation into mature spermatozoa. The targeted disruption of *hsf1* and *hsf2*, individually, only minimally affect male fertility. In contrast, the double knockout of both *hsf1* and *hsf2* genes results in severe male sterility due to arrest of meiotic prophase at the pachytene stage, suggesting that the additive transcriptional activity of both *hsf1* and *hsf2* genes are required for normal spermatogenesis (39). The ACT protein is expressed only in haploid round and elongated spermatids and functions as a transcription factor for CREM in postmeiotic male germ cells to enhance CREM-dependent transcription. The targeted disruption of the *Act* gene results in a drastically reduced amount of mature sperm in the epididymis causing a subfertile male phenotype. The sperm that are present display severe abnormalities, including fully folded tails and misshapen heads (40). Pantothenate kinase-2 is a morthologous murine gene that is localized in the mitochondria of the retina and sperm, and plays a role in energy metabolism. Mice with the targeted deletion of the *Pank2* gene show progressive retinal degeneration and male infertility due to azoospermia. This defect occurs in spermiogenesis, where there is a complete absence of elongated and mature spermatid in the mutant mice (41).

The relaxin-like peptide family belongs in the insulin superfamily, and affects collagen metabolism, inhibiting collagen synthesis and enhancing its breakdown

by increasing matrix metalloproteinases. It can be found in the arteries of male mice, and is an important factor in the development and function of the male reproductive tract in mice. Deletion the *Rlx* gene results in decreased rate of sperm maturation and increased rate of apoptosis, leading to male infertility (42). The pleomorphic adenoma gene 1 (*Plag*) proto-oncogene encodes a transcription factor that functions in tumorigenesis via ectopic overexpression. It is localized in the reproductive organs and pituitary and has been implicated in reproductive development. Targeted deletion of the *Plag1* gene results decreased fertility due to major abnormalities during spermatogenesis, and also includes significantly small seminal vesicles and ventral prostate size (43). The *Sox8* gene encodes a high-mobility group transcription factor that is widely expressed during development. The Sox8 protein is a product of the adult Sertoli cells. The deletion of the *Sox8* gene results in an age-dependent deregulation of spermatogenesis, characterized by sloughing of spermatocytes and round spermatids, spermiation failure and progressive disorganization of the spermatogenic cycle, which results in inappropriate placement and position of the germ cells within epithelium, ultimately resulting in infertility (44). The aryl hydrocarbon receptor is a ligand-activated transcription factor that mediates diverse dioxin toxicities, and is conserved among animal species, suggesting important physiological functions. Recent studies demonstrate that the Ahr plays an essential role in reproduction. Knockout mice exhibited abnormal vaginal plugs, low sperm counts in epididymis, and low fertility. Also, serum testosterone concentrations and expression of steroidogenic proteins in testicular Leydig cells was decreased (45). In mice, spermatogenesis is dependent on two types of glycolipids, seminolipid, a sulfated galactoglycerolipid, and glycosphingolipids (GSLs). GSLs are amphipathic cell membrane molecules present on the extracellular leaflet of the plasma membrane lipid bilayers and on topologically equivalent membrane sides of endocytotic and exocytotic organelles. They modulate membrane properties and receptors or ion channel functions. Loss of the *Galgt1* gene by systemic deletion renders male mice sterile. Their spermatogenesis arrests at the stage of haploid spermatid formation (46).

The *Sypc1* gene encodes for a transverse filament proteins that are responsible for meiotic chromosomes behavior and recombination. The synaptonemal complexes closely appose homologs along their length and are assembled from two axial elements. The mice with *Sypc1* knockout exhibited an infertile phnotype due to spermatocyte arrest in pachynema (47). *Msy2* is a germ-cell specific member of the Y-box family of DNA/RNA-binding proteins, and functions as a coactivator of transcription in the nucleus to stabilize and store maternal and paternal mRNAs in cytoplasm. The targeted deletion of the *Msy2* gene leads to sterility due to disrupted postmeiotic germ cells with many misshapen and multinucleated spermatids and no spermatozoa detected in the epididymis (48). Scafforld attachment factor B1 is a multifunctional protein that can bind both DNA and RNA and is involved in RNA processing and stress response. It also contains a transcriptional repression domain and can bind certain hormone receptors to repress activity. *Safb1* mutant mice with targeted disruption of the gene were infertile, with low levels of testosterone leading to progressive degeneration of the germinal epithelium, increased apoptosis of germ cells, and Leydig cell hyperplasia. Female mice null for *Safb1* also experienced subfertility. Both male and female mice exhibited dwarfism, low serum insulin-like growth factor (IGF1) (49). Septins are polymerizing GTP binding proteins required for cortical organization during cytokinesis and other cellular processes. It is expressed mainly in postmeiotic male germ cells and is responsible for cortical organization and cortical ring assembly which separates the middle and principles pieces of sperm flagella. The deletion of the *Sept4* gene renders male mice infertile due to defective morphology and motility of the sperm flagellum (50). AF5q31 was originally identified by its involvement in chromosomal translocation with the gene *MLL* (mixed lineage leukemia), which is associated with infant acute lymphoblastic leukemia. Its function as a transcriptional regulator in testicular somatic cells and germ cell differentiation has been uncovered. Mice with the *AF5q31* knockout show 87% death rate *in utero*/neonatally with impaired development and shrunken alveoli. However, the 13% that survived thrived normally, but with sterility due to azoospermia due to arrest of germ cell development during spermiogenesis (51).

The multifunctional protein-2, encoded by the *MFP-2* gene, has a role in metabolism of peroxisomal beta-oxidation, which is essential for lipid homeostasis in the testis and for male fertility. The targeted deletion of the *MFP-2* gene results in extensive accumulation of neutral lipids in Sertoli cells, beginning in prepubertal mice and evolving in complete testicular atrophy. This inactivation of peroxisomal beta-oxidation results in male infertility (52). MDC1 regulates many aspects of the DNA damage-response pathway, such as intra-S phase checkpoint, G2/M checkpoint and radiation-induced apoptosis. It also helps to maintain genomic stability by participating in the amplification of ATM-dependent DNA damage signals. The *MDC1* knockout renders male mice infertile due to defective spermatogenesis, along with growth retardation, immune defects, chromosome instability, DNA repair defects, and radiation sensitivity (53). Fertility of spermatozoa depends on the mitochondrial transmembrane potential, where Ca^{2+} levels function to control the forward motility of sperm. Transoladase plays an essential role in sperm-cell mitochondrial function and integrity. Male mice lacking the *TAL* gene are sterile due to defective forward motility of sperm (54). The Hspa4l gene belongs to the HSP110 heat shock gene family, which include three genes encoding highly conserved proteins. The *Hspa4l* gene is hyperosmotic and heat stress inducible. The targeted deletion of this gene reveals normal development and adulthood, however ~42% of male mice were infertile due to low sperm count in the epididymis and sperm motility was drastically reduced. Hydronephrosis development was also observed in *Hspa4l*-null mice (55). The *Pax8* gene is a member of the paired box (PAX) family of transcription factors. Members of this gene family typically encode proteins which contain a paired box domain, an

octapeptide, and a paired-type homeodomain. This nuclear protein is involved in thyroid follicular cell development and expression of thyroid-specific genes. The targeted deletion of the *Pax8* gene leads to infertility in male mice due to abnormal development of post-testicular ducts leading to atrophy of testes and complete lack of spermatozoa (56).

CR16 is a member of the Wiskott-Aldrich syndrome protein (WASP)-interaction protein (WIP) family. The CR16 protein binds to neutral WASP (nWASP) and induces actin polymerization. The CR16 and nWASP were localized to the actin filaments at the Sertoli cell-spermatid junctions. Transgenic mice with a *CR16* knockout resulted in male-specific sterility with sperm abnormal head morphology and greatly diminished fertilization ability (57). The G protein-coupled receptor Gpr54 and its ligand metastin, which is derived from the *Kiss1* gene product kisspeptin, are key gatekeepers of sexual maturation. Both mice with the targeted deletions of *Gpr54* and *Kiss1* exhibit similar reproductive phenotypes, including infertility due to abnormal sexual maturation. Males lack preputial separation, have significantly smaller testes, and have notably lower FSH levels. Both knockouts have abnormal sexual maturation consistent with hypogonadotropic hypogonadism, although *Kiss1* knockout mice appear to be less severely affected then the *Gpr54* knockouts (58). The *Psme4* gene encodes for the PA200 proteosome activator protein that is a broadly expressed nuclear protein. It has a role in activating proteasomal cleavage of peptides in an energy-independent manner. Transgenic mice were developed that lacked the PA200 gene, revealing male-specific infertility. This was found to be due to defects in spermatogenesis observed in meiotic spermatocytes and during maturation of postmeiotic haploid spermatids (59). Histone H3 lysine 9 (H3K9) methylation is a crucial epigenetic mark of heterochromatin formation and transcriptional silencing. G9a is a major mammalian H3K9 methyltransferase at euchromatin and is essential for mouse embryogenesis. Mice that are null for the *G9a* gene, specifically in germ cell lineage displayed sterility due to a drastic loss of mature gametes (60).

The *Trp73* gene encodes two major groups of protein isoforms, Tap73, and ΔNp73, with opposing pro- and anti-apoptotic functions. It has been found that the p73 transactivation domain, Tap73 isoform, exerts tumor-suppressive functions and plays a role in genomic stability. The targeted deletion of the *TaP73* isoform induces spontaneous and carcinogen-induced tumors, infertility, and hippocampal dysgenesis. These *Tap73*-null mice are thought to have deficits in various sensory and hormonal pathways which may result in these defects (61). Analysis of the novel Parkin co-regulated gene (*Pacrg)* reveals that it is localized to the axoneme of sperm and ependymal cilia, and plays a role in the formation and function of the axoneme and eventual motile cilia/flagella. The targeted ablation of *Pacrg* gene renders mice infertile with defective spermiogenesis, while also exhibiting hydrocephalus (62).

3.2. Gene knockouts affecting fertilization

Tpst genes (*Tpst1* and *Tpst2*) are responsible for mediating the post-translational modification of tyrosine *O*-sulfation, which plays an important role in the function of known TPST substrates by enhancing protein-protein interaction. It is suggested that tyrosine sulfation of unknown substrate(s) plays a crucial role in the processes of post-translational tyrosine sulfation in male fertility. The deletion of *Tpst2* renders male mice infertile by severely defecting sperm motility in viscous media (i.e. female genital tract), and inhibiting the ability of sperm to bind to and fertilize ZP membrane of ZP-intact eggs (63). The ADAM gene family members are expressed in a variety of tissues, about half of which are exclusively/predominantly expressed in testis. ADAM2 and ADAM3 are testis/sperm-specific members that are suggested to form a testicular ADAM2-ADAM3 complex on the sperm surface, which plays an essential role in sperm-egg zona pellucida binding. The deletion of ADAM2 and ADAM3 results in male infertility due to the inability of the sperm to bind to the egg zona pellucida (64). The *PGAP1* gene encodes for the protein PGAP1, identified as a GPI inositoldeacylase that removes palmitate from inositol, and is important for the efficient transport of GPI-anchored proteins from the endoplasmic reticulum to the Golgi body. GPI anchor abnormalities have been shown to cause a deleterious effect on development and sperm function. Male mice with deletion of *PGAP1* exhibit severely reduced fertility due to the inability of spermatozoa to travel into the oviduct, and also exhibit weak attachment to the zona pellucida (65). AC3 is a glycosylated protein involved in the cascade required for detection of odorants. It has been demonstrated that olfactory receptors, G protein, and cyclase involved in olfactory signaling are expressed in spermatids and are possibly retained in spermatozoa, suggesting that an olfactory-like signaling pathway including AC3 has a key role in spermatogenesis and spermatozoa function. The *AC3* knockout mice produce spermatozoa with decreased motility and increased spontaneous acrosome reaction that compromises fertilization, and results in an infertile phenotype (66). Glyceraldehyde 3-phosphate dehydrogenase-S is the product of a mouse gene expressed only during spermatogenesis, and is the sole GAPDH isozyme in sperm. The GAPDS enzyme regulates sperm glycolysis, and plays a critical role in spermatogenesis and sperm function. The targeted deletion of *Gapds* gene results in male infertility due to defects in sperm motility, exhibiting sluggish movement with no forward progression (67).

The angiotesin-converting enzyme (ACE) is a key regulator of blood pressure. It is known to cleave small peptides, such as angiotesin I and bradykinin and changes their biological activities, leading to upregulation of blood pressure. A new activity for ACE was recently uncovered, as a glycosyphosphatidylinositol (GPI)-anchored protein releasing activity (GPIase activity). The targeted deletion of the *Ace* gene results in the inhibited release of these GPI-anchored proteins on the sperm surface that result in ZP binding deficiency, therefore inhibiting fertilization and rendering males infertile (68). The Group VIA Phospholipase A_2 ($iPLA_2\beta$) is a cytosolic Ca^{2+}-independent PLA_2 that participates in arachidonic acid incorporation into glycerosphocholine lipids, cell proliferation, exocytosis, apoptosis, and other processes. The $iPLA_2\beta$

hormone also plays a specific functional role in spermatozoon maturation. Spermatozoa from *iPLA$_2\beta$*-knockout mice have reduced motility and impaired ability to fertilize mouse oocyte *in vitro* and *in vivo*, resulting in male infertility (69). Zona pellucida binding protein 1 is a spermatid and spermatozoon protein localized on the acrosome. Recently a novel paralog, ZPBP2, was discovered and is expressed only in the testes in both mice and humans. The functions of both ZPBP proteins were studied by disrupting each gene, where results revealed effects on fertility. The *Zpbp1* knockout resulted in male sterility, due to improper acrosome compaction resulting in fragmentation, leading to inability for sperm-ZP binding. The *Zpbp2* knockout revealed a subfertile phenotype demonstrating irregular acrosomal membrane invaginations, and produced sperm with reduced ability to penetrate ZP (70). Tetkins are conserved components of the flagellar proteome in evolutionarily diverse species and play essential roles in the mechanics of sperm motility. The mouse *Tekt4* gene is a germ cell-enriched gene most abundantly expressed in haploid round spermatids and localized to the flagella, and shares 77% homology with the nearest human homologue. It plays an essential role in regulating coordinated beating of the sperm flagella. The targeted deletion of the *Tekt4* gene reveals a subfertile phenotype with reduced forward progressive velocity and uncoordinated waveform propagation along the flagellum of sperm. Also, intracellular ATP consumption increases ten-fold in the transgenic species (71).

3.3 Gene knockouts affecting mating behavior

The α_1-Adrenoceptors are stimulated by catecholamines released from sympathetic nerves and are known to have an important role in regulating the various physiological functions of the peripheral tissues and in vas deferens contractibility. They have been classified into three subtypes, α1A, α1B and α1D. A triple knockout of these three subtypes affects male sexual function and results in reduced fertility due to impairment of vas deferens contractibility and ejaculation (72). Adult activin receptor type II functions as an upstream regulator of NOS activity within the mPOA of the forebrain. The targeted disruption of the *Acvr2* gene leads to multiple reproductive behavioral deficits in male mice, including delayed copulation, reduced mount and intromission frequencies, and increased mount, intromission frequencies and ejaculation latencies, resulting in male fertility defects. The impairment can be attributed to decreased NOS activity in the mPOA, but not the rest of the hypothalamus or cortex (73). The *Slo* gene is responsible for the pore-forming subunit of the BK channel. Activation of the BK channel hyperpolarizes smooth muscle cell membrane causing necessary relaxation of the arterial and corpus cavernosum smooth muscle that is required to increase blood flow into the corpora cavernosa, leading to penile erection. The *Slo*-knockout mice demonstrated erectile dysfunction due to loss of function of the BK channel, which is necessary for erectile function (74). Nitric oxide (NO) has been recognized as a modulator in reproductive functions. Neuronal NO synthase (nNOS) is required for central hormonal regulation of reproductive function. The *NOS1* knockout mice exhibited hypogonadism resulting in male infertility. Males also had no observed mating behavior, and had decreased levels of plasma FSH. Females exhibited decreased ovary weight and corpus luteum count (75, 76).

4. CONCLUSIONS

The utility of a protein as a target for contraception is contingent upon its: (1) tissue-specific expression in testis/sperm with limited to no expression in somatic cells, (2) role in fertility (spermatogenesis/spermiogensis/sperm function/fertilization/embryonic development), and (3) it should be accessible and amenable for binding with inhibitors/drugs and/or antibodies. Using gene knockout studies in mice, at least a total of 71 genes were identified by literature search in the database that showed an effect on spermatogenesis/fertilization/mating behavior, during the last four years. The majority of these knockouts also demonstrated an effect on non-reproductive organs concomitant with an anti-fertility effect or effect on other organs was not examined. The knockouts of only a few genes/proteins induced a specific effect on fertility without a serious side effect. These genes/proteins may provide novel targets for contraception/contraceptive vaccine development.

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