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NOVEL TESTIS/SPERM- SPECIFIC CONTRACEPTIVE TARGETS IDENTIFIED USING GENE KNOCKOUT STUDIES

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1. ABSTRACT

The population explosion and unintended pregnancies resulting in elective abortions continue to be the major public health issues in spite of availability of current methods of contraception. There is an urgent need for a better method of contraception that is accepted, effective, and available. Various targets are being investigated that can be used for contraception. The gene knockout technology is a powerful approach to identify such novel targets. Using search in the database, at least 93 genes were identified in the literature whose deletion demonstrated an effect on fertility in male mice. However, majority of these knockouts also demonstrated an effect on non-reproductive organs concomitant with an effect on fertility. The knockouts of only a few genes/proteins induced a specific effect on fertility without a serious side effect. The potential role of these novel genes/proteins in contraception/contraceptive vaccine development is discussed.

2. INTRODUCTION

Presently, very few options are available for fertility control. There is a need for more and improved contraceptive options that can be made available and used in both the developed and developing countries. The need for contraception varies for different couples from postponing childbearing, spacing childbirth, limiting family size, to absolute no childbearing. 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) (1). Currently, contraceptive options available for men are: vasectomy, condoms, and withdrawal prior to ejaculation. Vasectomy or male sterilization is the most effective of male contraceptive methods currently available. However, vasectomy is a permanent procedure with low success rate of fertility reversal even after successful surgical re-anastomosis. Therefore, for men who wish to father children at a later time, condoms and withdrawal are the only contraceptive options, which are either not readily acceptable or have high failure rates.

Immunocontraception offers another approach towards contraception for both men and women. Over the last 20 years, a large number of strategies have been employed for the development contraceptive vaccines and a number of antigens have been identified as potential targets for immunocontraception. Research in this area has focused mainly on raising antibodies against gonadotropic hormones such as luteinizing hormone-releasing hormone

(LHRH), follicle-stimulating hormone (FSH) and human chorionic gonadotropin (hCG) (2), their receptors (3); sperm surface antigens such as fertilization antigen-1 (FA-1), sperm protein (SP)-10, sperm protein (SP)-17, and sperm surface PH-20 antigen (4) and epididymal proteins such as rat epididymal glycoprotein DE (5, 6). Besides the concerns of safety and efficacy, the major problem associated with the development of an effective contraceptive vaccine is variability of immune response against a particular antigen, which will have major effect on the predictability and reliability of the vaccine (7, 8). Chemical analogs and protein repressors have also been explored to inhibit/block the reproductive cell synthesis, development, maturation and/or function, which can be used to develop novel contraceptive methods (9).

Various strategies employed for contraceptive development targeting sperm include inhibition of sperm production, disrupting sperm structure and function, interruption of sperm transport and deposition, and prevention of sperm-egg interaction. All these approaches involve identification of the genes and proteins that are involved specifically in these processes. A large number of genes are involved in reproduction. In the past these genes/cDNAs have been identified by using biochemical methods, hybridization technology, and several molecular and immunological approaches. The advent of 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.

In this article, we will review the information about the genes that have been identified using the gene-knockout technology, whose deletion causes an affect on some parameters of fertility. A total of 93 gene-knockouts were found in the literature search carried on January 15, 2005 in NCBI (National Centre for Biotechnology Information) database that affect fertility in male mice (Table 1).

These genes can be categorized into following four major groups depending upon the fertilization/fertility parameter that is affected after their deletion:

3. DISCUSSION

3.1. Gene-knockouts that cause defect in sperm structure, function, and/or motility

The literature search revealed a total of 21 genes whose disruption can lead to sperm abnormalities and infertility (Table 1). Some of these genes are discussed below.

Recently, a family of sperm-specific cation channels has been identified, designated as Catsper1, 2, 3, and 4, respectively, which are localized on the principal piece of sperm flagellum and act as regulators of sperm motility (10), (11, 12). It has been observed that mice with targeted disruption of *catsper1* and *catsper 2* are infertile due to the failure to acquire hyperactivated motility, which renders the spermatozoa incapable to penetrate the oocyte (10, 11). The animals are otherwise phenotypically normal and show no other defect.

Another protein, known as A kinase anchoring protein 4 (AKAP4), seems to be a major component of the sperm flagellum. It is a scaffold protein required for the organization and integrity of the fibrous sheath and thus regulates flagellar function. AKAP4 is a testis-specific protein expressed specifically in the spermatids. In sperm cell, AKAP4 binds to the regulatory subunit of protein kinase A (PKA) leading to its compartmentalization thus ensuring the specificity of its function. Targeted deletion of the *Akap4* gene in mice results in the male infertile phenotype (13). Sperm numbers are not reduced but the spermatozoa fail to show progressive motility, rendering the male mice infertile. There was an incomplete development of the fibrous sheath leading to shortening of the flagellum and abnormal tail development, and the animals show no other phenotypic defect.

Casein kinase II (Ck2) is a c-AMP and calcium-independent serine-threonine kinase that has two isoforms Ck2a and Ck2a′ encoded by *Csnk2a1* and *Csnk2a2*, respectively. Csnk2a2 is preferentially expressed in the later stages of spermatogenesis. Disruption of *Csnk2a2* leads to male infertility due to oligospermia and globozoospermia (14). Hook1, a cytoskeletal linker protein is predominantly expressed in the haploid male germ cells. Immunohistochemical analysis revealed that Hook1 is responsible for the linkage of the microtubular manchette and the flagellum to cellular structures. Disruption of *hook1* gene result in ectopic positioning of microtubular structures within the spermatid leading to abnormal sperm head shape and infertility (15). Absence of another protein SSTY1, expressed exclusively in spermatids, leads to abnormal sperm development and abnormally-shaped sperm heads and thus infertility (16).

Epididymis represents an alternative target organ for male contraception. Spermatozoa produced in the seminiferous tubules of the testis are transported to the epididymis via rete-testis where they undergo a maturation process and acquire their capabilities for forward motility and oocyte fertilization at least in the mouse model. Recently, gene knockout strategies have been employed to identify various epididymal proteins such as c-ros (17) and HE6 (18) that can serve as non-testicular target for male contraception. In mice with targeted disruption of *c-ros*, sperm exhibit severe tail angulation at midpiece-principal piece junction leading to infertility (17). Mutant mice with deletion of the epididymal receptor HE6 show dysregulation of fluid reabsorption in the efferent ductules, leading to a backup of fluid accumulation in the testis and a subsequent stasis of spermatozoa within the efferent ducts leading to infertility (18).

3.2. Gene-knockouts that cause defect in spermatogenesis

The process of spermatogenesis involves the development of spermatogonia into spermatocytes, which

Table 1. Gene Knockouts That Affect Male Fertility

No	GENE	PROTEIN				INFERTILITY TARGET	PHENOTYPE	EFFECTS ON OTHER TISSUES	REF
		NAME	SIZE (approx.)	FUNCTION	LOCALIZATION				
I	DEFECT IN SPERM STRUCTURE, FUNCTION AND/OR MOTILITY								
1.	*HE6*	HE6 (GPR64)	180 kDa	Role in monitoring and controlling extracellular milieu	Expressed within the efferent ductules and proximal epididymis	Spermatozoa	Fluid accumulation in testis and stasis of spermatozoa within the efferent ducts	Not reported	18
2.	*Tektin-t*	Tektin-t	49 kDa	Participates in dynein inner arm formation or attachment	Forms filaments in flagellar, ciliary, and axonemal microtubules	Spermatozoa	Abnormal sperm morphology and function	Functionally defective tracheal cilia, impaired motility of both flagella and cilia, and one of the causal genes for immotile-cilium syndrome/primary ciliary dyskinesia	50
3.	*Pmca4*	PMCA4 (P-type Ca^{2+}-ATPase of the plasma membrane)	133 kDa	Maintenance of Ca^{2+} gradients across cellular membranes	Localized to principal piece of the sperm tail	Spermatozoa	Null mutant sperm are unable to achieve hyperactivated motility, cannot traverse female genital tract and fertilize the egg	Ca^{2+} overload and apoptotic cell death under some conditions	51
4.	*Catsper1*	CatSper1	79 kDa	Ca^{2+} channel controlling Ca^{2+} concentrations in the sperm tail	Localized to principal piece of sperm tail	Spermatozoa	Loss of hyperactivated motility	No other phenotypic abnormality	10
5.	*Catsper2*	CatSper2	69 kDa	Ca^{2+} channel controlling Ca^{2+} concentrations in the sperm tail	Localized to principal piece of sperm tail	Spermatozoa	Loss of hyperactivated motility	No gross abnormalities	11
6.	*Cnot7*	CNOT7	33 kDa	Transcriptional regulation	Strongly expressed in Sertoli and Leydig cells and weakly expressed in early round spermatids, spermatocytes, and spermatogonia	Unsynchronized maturation of spermatids	Low sperm number and motility, and abnormal sperm morphology	Normal health, size and behavior (No phenotypic abnormality)	52
7.	*c-ros*	c-ROS	262 kDa	Receptor tyrosine kinase	Expressed in embryonic epithelial structures of the kidney, lung, intestine, and the Wolffian duct. Expression declines in other tissues but is upregulated in proximal epididymis during prepubertal development	Epididymis	Lack of initial segment of the epididymis and sperm exhibit severe tail angulation at the midpiece/principal piece junction associated with swollen cytoplasmic droplets	Not reported	17
8.	*Smcp*	SMCP (sperm mitochondria-associated cysteine-rich protein)	28 kDa	Involved in the maternal inheritance of mitochondrial DNA by targeting proteolytic degradation of paternal mitochondria after fertilization	Localized in outer mitochondrial membranes and intermitochondrial spaces of the mitochondrial sheath	Spermatozoa	Reduced sperm motility (asthenozoospermia)	Not reported	53
9.	*sAC*	sAC (soluble adenylyl cyclase)	186 kDa	Produce cAMP, second messenger in various signal transduction pathways	Expressed in various tissues but high expression levels detected in testis	Sperm motility	Impaired motility	No pathological abnormalities	54
10.	*Akap4*	AKAP4 (A-kinase anchoring protein 4)	94 kDa	Act as a scaffold for protein complexes involved in regulating flagellar function	Expressed in spermatids	Sperm motility	Incomplete development of fibrous sheath, shortening of flagellum and loss of sperm motility	Not reported	13
11.	*ApoER2*	ApoER2 (Apolipoprotein E receptor-2)	106 kDa	Has a role in endocytosis and signal transduction	Expressed in post-mitotic neurons in the brain, placenta, ovaries and epididymis	Sperm maturation	Cell volume dysregulation, abnormal sperm morphology and immotility	Brain formation defects	55
12.	*Csnk2a*	Ck2a' (Casein kinase II a' catalytic subunit)	42 kDa	cAMP and calcium-independent ser/thr kinase	Preferentially expressed in adult testis, sperm, and brain.	Spermatogenesis	oligospermia and globozoospermia ('round-headed' spermatozoa)	Not reported	14

13.	*Poll*	DNA polymerase ? (Pol ß2)	63 kDa	Participates in short- and long-patch Base Excision Repair	Nuclear protein detected in several tissues but abundantly expressedin pachytene spermatocytes in testis and also in ovary	Sperm motility	Immotile sperm	Hydrocephalus, high mortality rate after birth, situs inversus totalis, and chronic sinusitis	56
14.	*Dnahc1 (Mdhc7)*	MDHC7 (Mouse dynein heavy chain 7)	155 kDa	Component of inner dynein arm that has a role in ciliary and flagellarmotility	Expressed in testis and somatic tissues containing ciliated epithelia	Sperm motility	Immotile sperm, asthenozoospermia	Decrease in beat frequency of tracheal cilia, no gross abnormalities	57
15.	*Dnahc5 (Mdnah5)*	MDHC5 (Mouse axonemal dynein heavy chain)	527 kDa	Has a role in ciliary and flagellar motility	Expressed in tissues containing ciliated epithelia	Sperm motility	Immotile sperm	Hydrocephalus, growth retardation, situs inversus, ciliary immotility, chronic infection of respiratory tract and otitis	58
16.	*Gtrgeo22*	GTRGEO22	33 kDa	Involved in intracellularsorting of transmembrane proteins	Widely expressed	Sperm motility	Defective spermflagellar development	Absence of inter-male aggression and reduced body fat	59
17.	*Hook1*	Hook homolog 1	84 kDa	Cytoskeletal linker protein	Expressed predominantly in testis	Spermatogenesis	Abnormal sperm head shape and fragile attachment of flagellum to sperm head	Not reported	15
18.	*Hrb*	HIV-1 Rev binding protein	58 kDa	Component of the vesicle-to-vesicle docking and/or fusion machinery that forms the acrosome vesicle	Expressed widely but expression levels were very high in testis and thymus	Spermiogenesis	Lack of acrosome, severely reduced motility and several structural abnormalities; globic head with a round nucleus and a tail, midpiece lacking mitochondrial sheath	No effect on other tissues studied	60
19.	*Ssty*	SSTY 1	32 kDa	Unknown	Expressed exclusively in testis during spermatid stages	Sperm	Stage IX testis tubule showed continued retention of pockets of mature sperm, abnormally shaped sperm heads	Not reported	16
20.	*Sir2a*	SIR2a	80 kDa	Has NAD+-dependent histone deacetylase activity and plays a role in growth and maturation of embryo andin gametogenesis	Nuclear protein widely expressed in various tissues	Spermatogenesis	Abnormal sperm morphology with small, rounded, or smudged cell bodies and blunted or absent hooks, and sperm are immotile	Early postnatal lethality	61
21.	*Spag6*	Sperm associated antigen 6	55 kDa	An axonemal protein important for flagellar motility and stability of axoneme central apparatus	Expressed only in testis and lung	Sperm motility	Sperm showed motility defect and morphological abnormalities- fragmentation of midpiece, truncated flagella, and loss of sperm head	Reduced growth, and hydrocephaly	62
II	**DEFECT IN SPERMATOGENESIS**								
1.	*Atm*	ATM	350 kDa	Cell cycle regulation	Nuclear protein expressed in brain, skeletal muscle, testis, spleen, lung, kidney, heart, liver, and thymus.	spermatocyte or maturation arrest	Disrupted spermatogenesis, and seminiferous tubules show reduced number of cells, are devoid of spermatids, show evidence of cellular degeneration, and many are barren of all cell types except Sertoli cells. Epididymis is also devoid of spermatozoa.	Growth retardation, neurological dysfunction, immunologic abnormalities, lymphoreticular malignancies, chromosomal instability, and extreme sensitivity to ionizing radiation	63
2.	*crem*	CREM (Cyclic AMP response element modulator)	37 kDa	Binds cAMP response element (CRE), a sequence present in many viral and cellular promoters	Nuclear protein expressed in various tissues	Spermatids	Spermatogenesis interrupted at stage of early haploid germ cells (round spermatids)	Not reported	29, 30
3.	*rxrb*	RXRB (Retinoic X receptor-ß)	45 kDa	Mediate action of a RA metabolite, 9-cis retinoic acid	Nuclear protein expressed ubiquitously	Spermatogenesis	oligo- astheno-teratozoospermia, failure of spermatid release within germinal epithelium, spermatozoa exhibit abnormal acrosome and tail, and progressive accumulation of lipids within the mutant Sertoli cells	Partial embryonic and perinatal lethality	64

4.	*AR* (Sertoli cell-specific AR knockout)	AR (Androgen Receptor)	98 kDa	Involved in the regulation of eukaryotic gene expression. Affect cellular proliferation and differentiation in target tissues	Nuclear protein	Spermatogenesis	Spermatogenic arrest predominantly at the diplotene premeiotic stage, and lower serum testosterone levels, which result in azoospermia and infertility	Not reported	20
5.	*PCI*	PCI (Protein C inhibitor, nonspecific, heparin-binding serpin (serine protease inhibitor)	46 kDa	Inhibits anticoagulant activated protein C, plasminogen activator urokinase, and sperm protease acrosin	In humans, PCI circulates as a plasma protein but is also present in high concentrations in organs of male reproductive tract	Spermatogenesis	Destruction of Sertoli cell barrier, abnormal spermatogenesis and sperm malformation	Mice are healthy, effects on other tissues not reported	19
6.	*Tnp2*	TNP 2 (Transition nuclear protein 2)	13 kDa	Participate in initial condensation of spermatid nucleus	Nuclear protein	Spermatogenesis	Teratozoospermia	Not reported	32
7.	*Tnp1*	TNP1 (Transition nulcear protein 1)	6.2 kDa	Role in histone displacement and chromatin condensation during spermatid development	Nuclear protein	Spermatogenesis	Impaired sperm motility	Not reported	31
8.	*Brca2*	BRCA2	371 kDa	Plays a role in dynamics of meiotic recombination and/or chromosome pairing and synapsis during spermatogenesis	Nuclear protein expressed in various tissues	Spermatogenesis	Early meiotic prophase arrest of spermatocytes	Embryonic lethality, growth retardation, neural tube defects, and mesoderm abnormalities	65
9.	*Adamts2*	ADAMTS-2 (**a** **d**isintegrin **a**nd **m**etalloproteinase with **t**hrombos**p**ondin repeats)	135 kDa	Procollagen N-proteinase	Expressed at high levels in all tissues that are rich in type I collagen such as testes, lung, spleen and kidney	Spermatogenesis	Decreased spermatogenesis	Triangular faces with shorter snouts, less dense hair with thinner hair follicles, and the very thin and soft skin that tear with slight trauma	66
10.	*Apaf1*	APAF1 (Apoptotic protease-activating factor 1)	141 kDa	Has a role in cytochrome c-mediated apoptosis	Cytoplasmic protein	Spermatogonia	Spermatogonial degeneration	Perinatal lethality and development neural lesions	67
11.	*Bsg*	Basigin	44-66 kDa	Belongs to immunoglobulin superfamily	Integral plasma membrane glycoprotein, expressed in various embryonic and adult tissues	Spermatogenesis	Spermatogenesis arrested at the metaphase of the first meiotic division (Azoospermia)	Periimplantation lethality, infant pneumonia, and impaired neural-glial interactions	68, 69
12.	*Bax*	Bcl2-associated X protein	21 kDa	Accelerates programmed cell death by binding to, and antagonizing the apoptosis repressor BCL2	Membrane bound protein, expressed in a wide variety of tissues	Maintenance of reproductive germ cells	Germ cell death and disorganization of seminiferous tubules	Lymphoid hyperplasia	70
13.	*Bcl212 (Bcl w)*	Bcl2-like 2	21 kDa	Promotes cell survival	Cytoplasmic protein expressed in a wide range of tissues, with highest levels in brain, colon, and salivary gland	Maintenance of reproductive germ cells	Progressive loss of germ cells, Sertoli cells and Leydig cells	Not reported	71
14.	*Camk4*	CaMKIV (Ca^{2+}/calmodulin-dependent protein kinase	52 kDa	Ser/thr protein kinase	Expressed in brain, and spermatids in testis	Spermiogenesis	Impaired spermiogenesis in late elongating spermatids, defective chromatin packaging	Cerebellar ataxia	72, 73
15.	*Cit-k*	CIT-K (Citron kinase)	146 kDa	Rho-interacting, ser/thr kinase	Expressed in brain, spleen, lung, kidney, and testis	Spermatogenesis	Testicular impairment, with embryonic and postnatal loss of undifferentiated germ cells and complete absence of mature spermatocytes	Complex neurological syndrome caused by cytokinesis block and apoptosis of specific neuronal precursors	74
16.	*Cks2*	CKS2 (mammalian homolog of the yeast Cdk1-binding protein)	10 kDa	Binds to the catalytic subunit of the cyclin dependent kinases and is essential for their biological function	Widely expressed	Spermatogenesis	Germ cells fail to progress past the first meiotic metaphase	Not reported	28

17.	*Cldn11*	Claudin-11/ Oligodendrocyte-specific protein (OSP)	22 kDa	Component of specialized tight junctions	Transmembrane protein found in CNS myelin and testis	Spermiogenesis	Loss of tight junctions between Sertoli cells, hypogonadism, abnormally narrow seminiferous tubules, absence of spermatozoa and disruption of the blood–testis barrier	Hind limb weakness and decrease in conduction velocity in the central nervous system	75
18.	*Cpeb*	CPEB (Cytoplasmic polyadenylation element binding protein)	62 kDa	Sequence-specific RNA binding protein that regulates translation, and controls germ cell differentiation by regulating formation of synaptonemal complex	Nuclear protein expressed widely	Spermatogenesis	Germ cells arrested at pachytene stage of meiosis	Not reported	22
19.	*Ccna1*	CyclinA1	48 kDa	Cell cycle regulation	Expressed in germ cell lineage, testis and ovaries	Spermatogenesis	Arrest of spermatogenesis prior to first meiotic division	Not reported	27
20.	*Cyp19*	Aromatase cytochrome P450	58 kDa	Catalyze the final step in estrogen biosynthesis	Membrane bound enzyme expressed in brain, placenta, testis, and ovary	Spermiogenesis	Disruption of spermiogenesis, degeneration of round spermatids and multi-nucleated cells, abnormal acrosomes, and Leydig cell hyperplasia/hypertrophy	Progressive increase in adiposity	76
21.	*Ahch*	Dax1	52 kDa	Transcriptional factor that has a role in sex determination and gonadal differentiation	Expressed in developing urogenital ridge, ovary, testis, adrenal cortex, hypothalamus and anterior pituitary gland	Spermatogenesis	Progressive degeneration of testicular germinal epithelium, complete loss of germ cells and Leydig cell hyperplasia and hypertrophy	Adrenal hypoplasia congenita (AHC) and hypogonadotropic hypogonadism (HH)	77
22.	*Daz1*	DAZL (Deleted in azoospermia like)	33 kDa	RNA binding protein essential for spermatogenesis	Expressed only in testis	Spermatogenesis	Spermatogenic arrest (block spermatogonial differentiation), clustering of Sertoli cells in seminiferous tubules	Not reported	25
23.	*Dmc1*	DMC1 (**d**isrupted **m**eiotic **c**DNA)	37 kDa	Has a role in meiotic recombination	Nuclear protein expressed specifically in testis and embryonic ovary	Spermatogenesis	Arrest of gametes in meiotic prophase, with failure of homologous chromosomes to undergo synapsis	Not reported	21
24.	*Prnd*	Doppel	35 kDa	Unknown	Expressed at high levels in adult testis and heart, but is detectable in brain only during embryogenesisand in neonates	Spermiogenesis	Partial blockade of spermiogenesis, spermatozoa have reduced motility and display both sperm head and flagellar morphological abnormalities, Mutant spermatozoa show increase in levels of DNA strand breaks and altered chromatin structure	Not reported	78
25.	*Eif2s3y*	EIF2S3Y (Eukaryotic translation initiation factor 2, subunit 3, structural gene Y-linked.)	51 kDa	Role in early steps of protein synthesis	Nuclear protein	Spermatogenesis	Impairment of spermatogonial proliferation	Not reported	79
26.	*Egr4*	EGR-4 (Early growth response protein 4), (NGFI-C, pAT133)	49.6 kDa	Zinc finger transcription factor involved in cellular growth and differentiation	Expressed in brain, central nervous system and within male germ cells during meiosis	Spermatogenesis	Incomplete block of germ cell maturation at early-mid pachytene stage, leading to oligozoospermia characterized by the production of a comparatively small number of spermatozoa with abnormal morphology (teratozoospermia)	No gross phenotypic abnormality	80
27.	*Fkbp6*	FK506 binding protein 6	37 kDa	Component of synaptonemal complex essential for sex-specific fertility and for fidelity of homologous chromosome pairing in meiosis	Nuclear protein expressed widely	Spermatogenesis	Complete block in spermatogenesis and cell death of meiotic spermatocytes	Not reported	81

28.	*Tls/Fus (translated in liposarcoma)*	TLS/FUS	68 kDa	RNA-binding protein that contributes N-terminal half of fusion onco-proteins implicated in development of human liposarcomas and leukemias	Nuclear protein expressed widely	Spermatogenesis	Increased number of unpaired and mispaired chromosomal axes in pre-meiotic spermatocytes leading to the apoptosis of the affected spermatocytes	Defective somatic growth and increased sensitivity to ionizing radiation	82
29.	*Rho Gdia*	Rho GDIa (Rho protein GDP dissociation inhibitor)	23 kDa	Regulates GDP/GTP exchange reaction of Rho proteins by inhibiting dissociation of GDP from them	Ubiquitously expressed	Spermatogenesis	Impaired spermatogenesis with vacuolar degeneration of seminiferous tubules	Massive proteinuria, renal failure	83
30.	*Gopc*	GOPC (Golgi-associated PDZ- and coiled-coil motif-containing protein)	58 kDa	Role in vesicle transport from golgi apparatus	Widely expressed	Spermatogenesis	Globozoospermia, complete lack of acrosomes	No gross abnormalities	36
31.	*Hspa2*	HSP70-2 (Heat shock-related 70 kDa protein 2)	70 kDa	Chaperone protein that is involved in protein folding	Expressed at high levels exclusively in pachytene spermatocytes	Spermatogenesis	Spermatogenic arrest, spermatocytes fail to progress to metaphase	Not reported	23
32.	*H2afx*	Histone H2A.X	15 kDa	Facilitates assembly of specific DNA-repair complexes on damaged DNA	Expressed widely but most abundant in testis, thymus and spleen	Spermatogenesis	Spermatocytes arrest at pachytene stage of meiosis I	High radiation sensitivity, growth retardation, and immune deficiency	84
33.	*Inpp5b*	Inositol polyphosphate-5-phosphatase B	75 kDa	Regulates function of phosphoinositides by hydrolyzing the D5 phosphate	Ubiquitously expressed	Spermatogenesis	Severe disruption in spermatogenesis, characterized by complete loss of spermatocytes and spermatids, Sertoli cell vacuolization, and abnormal germ cell adhesion	No effect on other tissues studied	85, 86
34.	*c-Abl*	c-Abl	125 kDa	Non-receptor protein tyrosine kinase	Widely expressed	Spermatogenesis	Absence of round and elongated spermatids	Increased perinatal mortality, thymic and splenic atrophy, and T and B cell lymphopenia	87, 88
35.	*jund*	JunD	35 kDa	Component of the AP-1 transcription factor complex	Nuclear protein expressed widely in many tissues and cell lineages.	Spermatogenesis	Reduced expression of a subset of spermatogenesis-related genes such as *calspermin*, *RT7* and *BMP8* leading to impaired spermatogenesis with abnormalities in head and flagellum of sperm	Growth retardation	89
36.	*Gamt*	GAMT (guanidinoacetate N-methyltransferase)	26 kDa	Role in creatine biosynthesis	Expressed widely in many tissues	Spermatogenesis	Impaired spermatogenesis was observed at the level of spermatid development	Increased postnatal lethality; reduced body weight, muscle tension, and creatine concentrations	90
37.	*Slc19a2*	SLC19A2	56 kDa	Thiamine transporter	Expressed widely in various tissues on cell surface and intracellularly except placenta	Spermatogenesis	Arrest in spermatogenesis prior to meiosis II- no mature sperm found in the tubules or epididymis	Reticulocytopenia and erythroid hypoplasia of the marrow	91, 92
38.	*Fanca*	FANCA (Fanconi anemia, complementation group A)	163 kDa	Role in linking the FA nuclear complex with other pathways	Detected both in the nucleus and cytoplasm in various tissues	Maintenance of reproductive germ cells	Elevated frequency of mispaired meiotic chromosomesand increased apoptosis in germ cells	Growth retardation, microphthalmia, craniofacial malformations and hematological changes	93
39.	*Pog (proliferation of germ cells)*	Pog	43 kDa	Role in normal primordial germ cell proliferation and early embryonic development	Detected both in nucleus and cytoplasm in various tissues	Maintenance of reproductive germ cells	Defects in primordial germ cell proliferation	Reduce embryonic body weight and embryonic lethality	94

40.	*Tiar*	TIAR	43 kDa	RNA recognition motif / ribonucleoprotein-typeRNA-binding protein involved in the splicing, transport, translation, and stability of mRNA	Highly expressed in primordial germ cells	Primordial germ cell development	Decrease in survival of primordial germ cells at genital ridge, absence of spermatogonia, spermatids, and mature spermatozoa in testes	Partial embryonic lethality and reduced postnatal survival, reduced embryonic and postnatal body weight	95
41.	*Miwi*	Miwi (murine homolog of piwi) (piwi- P-element induced wimpy testis)	99 kDa	Essential for spermatogenesis	Cytoplasmic protein specifically expressed in spermatocytes and spermatids	Spermatogenesis	Spermatogenic arrest at beginning of round spermatid stage	Not reported	33
42.	*Tlf / Trf2*	TLF/TRF2 (TATA box binding protein-like factor)	21 kDa	Physiological function of mammalian TLF is not known	Nuclear and cytoplasmic protein expressed ubiquitously	Spermiogenesis	Arrest in spermiogenesis at transition from round to elongating spermatids; round spermatids undergo apoptosis at step 7	Not reported	35
43.	*Msh4*	MSH4 (MutS homolog 4)	107 kDa	Member of mammalian mismatch repair gene family involved in DNA mismatch repair and meiotic recombination	Expressed predominantly in testis	Spermatogenesis	Abnormal chromosome pairing during zygotene phase of meiotic prophase I leading to induction of apoptosis in spermatocytes	Normal development with no disease phenotype	26
44.	*Scp3*	SCP3 (synaptonemal complex protein 3)	29 kDa	Component of the axial/lateral element of the SC and is associated with the centromeres in meiotic metaphase I cells	Nuclear protein expressed in testis and ovary	Spermatogenesis	Disruption of Spermatogenesis at the Zygotene Stage of Meiosis I-massive apoptotic cell death during meiotic prophase	Normal development	24
45.	*HR6B*	HR6B	17 kDa	Ubiquitin-conjugating DNA repair enzyme	Nuclear protein	Spermatogenesis	Abnormal postmeiotic condensation of spermatid chromatin	Not reported	34
46.	*Hsl*	HSL (Hormone-sensitive lipase)	130 kDa (HSL_{tes}), 84 kDa (HSL_{adi})	Mediate the hydrolysis of triacylglycerol stored in adipose tissue and heart, and cholesterol esters in the adrenals, ovaries, testes, and macrophages	Expressed widely in various tissues	Spermatogenesis	Oligospermia	Adipocyte hypertrophy	96
47.	*Nectin-2*	Nectin-2	57 kDa	Component of cell-cell adherens junctions	Expressed in general columnar epithelia	Spermiogenesis	Sperm head and midpiece malformation, reduced migration to the oviduct, impaired zona binding, and sperm-oocyte fusion, and lack of oocyte penetration	Viable and without any gross morphological or behavioral phenotype	97
III	**DEFECT IN TESTIS DEVELOPMENT AND DISRUPT ENDOCRINE MILIEU**								
1.	*Dhh*	DHH (Desert hedgehog)		Regulation of mammalian spermatogenesis	Expressed in the Sertoli cells, Schwann cells, vascular endothelium, endocardium, and seminiferous epithelium of the embryonic mouse	Testis development and spermatogenesis	Small testis, anastomotic seminiferous tubules, pertitubular cell abnormalities, and absence of adult-type Leydig cells. No mature sperm either in testis or epididymis	Not reported	37, 38
2.	*Amh*	Anti-Mullerian hormone (Mullerian inhibiting substance, MIS)		Initiates regression of mullerian ducts during sexual development of male	Secreted by fetal sertoli cells during mammalian male sexual differentiation	Reproductive organ development	Development of female reproductive organs, which interfered with sperm transfer into females and Leydig cell hyperplasia	Not reported	39
3.	*Amhr2*	Anti-Mullerian hormone type 2 receptor	61 kDa	AMH receptor	Expressed in Mullerian duct cells, and in Sertoli and granulosa cells of fetal male and adult female gonads	Reproductive organ development	Development of female reproductive organs, with blockage for sperm transfer	Not reported	40

4.	*Cutl1*	CDP/Cux (CCAAT displacement protein)	200 kDa	Transcription factor involved in regulation of cell growth and differentiation-relatedgenes	Expressed in brain and all major internalorgans except in adult liver	Needs further study	No gross anatomical and histological abnormalities are observed, except low serum testosterone levels	Stunted growth, high postnatal death rate, and sparse abnormal coathair	98
5.	*Dmrt1*	DMRT1 (Doublesex- and mab-3-related transcription factor 1)	28 kDa	Has a role in testis development	Expressed in genital ridges of both sexes, becomes testis-specific at end of sex-determining period	Testis differentiation	Disorganized seminiferous tubules, absence of germ cells, degeneration of interstitial (Leydig) cells with infiltration by macrophages	Not reported	41
6.	*Krox-24*	Krox-24 (NGFI-A, Egr-1)	82 kDa	Zinc finger phosphoprotein, functions as a sequence-specific transcriptional activator	Expressed in endothelial system, thymus, muscle, cartilage, bone,and part of the central and peripheral nervous systems	Gonadal development	Deficiency of leutinizing hormone leading to severely reduced testes,seminal vesicles and prostate	Abnormal development of anterior pituitary and ovary, and reduction in body size and weight	99, 100
7.	*Emx2*	EMX2 (Empty spiracles homologue 2)	28 kDa	Homeobox protein involved in embryonic development	Nuclear protein expressed in embryonic tissues	Gonadal development	Complete absence of gonads and genital tracts	Complete absence of kidney and ureters	101
8.	*Ggt1*	GGT (?-glutamyl transpeptidase)	62 kDa	Mobilizes cysteine from glutathione pool and makes it available to many tissues	Widely expressed in many mammalian tissues	Hormonal imbalance	Reduce testis and seminal vesicle size, and cause severe oligospermia	Cysteine deficiency leading to growth retardation, excessive glutathione secretion in urine, coat color defects, and cataracts	102, 103
9.	*Cga*	CGA (Glycoprotein hormones a chain)	14 kDa	Common alpha chain of TSH, LH, and FSH hormones	Expressed in pituitary	Hormonal deficiency	Hypogonadism	Dwarfism, hypothyroidism, and hypertrophy and hyperplasia of pituitary cells	104
10.	*Great*	GREAT (**G**-protein-coupled **re**ceptor **a**ffecting **t**estis descent)		Seven-transmembrane receptor which mediates hormonal signals that affect testicular descent	Expressed in testis, brain and skeletal muscles, with highest expression in gubernaculum	Hormonal imbalance	Intra-abdominal cryptorchidism	Not reported	43
11.	*Gdf7*	GDF7 (Growth / differentiation factor 7)	48 kDa	Involved in mammalian development	Expressed in mesenchymal cells	Organ development	Seminal vesicles are small, lack any sign of folding, and are more darkly colored than wild-type due to lack of secretions in lumen	Hydrocephalus	105, 106
12.	*HNF-1a*	HNF1a (Hepatocyte nuclear factor 1 alpha)	67 kDa	homeodomain-containing transcription factor	Expressed in liver, kidney, intestine, stomach,and pancreas	Gonadal development	Underdeveloped reproductive organs, vestigial seminal vesicles and vas deferens, and decreased spermatogenesis	Growth retardation, and hyperglycemia	107
13.	*Hoxa10*	HOXA10 (Homeobox protein Hox-A10)	41 kDa	Sequence-specific transcription factor, role in establishing cellular patterns during development	Expressed in developing limb bud, gut, and urinogenital tract, and in kidney and skeletal muscle in adults	Gonadal development	Bilateral cryptorchidism	Anterior homeotic transformation of lumbar vertebrae	108
14.	*Hoxa11*	HOXA11 (Homeobox protein Hox-A11)	34 kDa	Sequence-specific transcription factor, role in establishing cellular patterns during development	Expressed in limbs, kidney and stromal cells surrounding Mullerian and Wolffian ducts	Gonadal development	Malformation of vas deferens that resembles a partial homeotic transformation to an epididymis, and cryptorchidism	Homeotic transformations affecting thoracic and sacral vertebrae, and forelimb defects	109
15.	*Hoxa13*	HOXA13 (Homeobox protein Hox-A13)	40 kDa	Sequence-specific transcription factor, role in establishing cellular patterns during development	Widely expressed	Gonadal development	Hypoplasia of proximal os penis	High degree of fetal lethality, and hypodactyly	110

16.	*Igf1*	IGF-1 (Insulin-like growth factor 1)	17 kDa	Structurally and functionally related to insulin and has a much higher growth-promoting activity	Secreted as plasma proteins, expressed mainly in liver, also expressed by other organs such as brain and gonads	Gonadal development and hormonal imbalance	Drastically reduced levels of serum testosterone; testes size reduced; vestigial vas deferens, seminal vesicles and prostate	Growth retardation	111
17.	*Insl3*	INSL3 (Insulin-like 3)	14 kDa	Hormonal factor with a role in testicular descent	Specifically expressed in Leydig cells of the fetal and postnatal testis and in theca cells of postnatal ovary	Gonadal development	Failure of gubernaculum development during embryogenesis leading to bilateral cryptorchidism with free moving testes and genital ducts	Not reported	44
18.	*Stat3*	STAT3 (Signal transducer and activator of transcription 3)	88 kDa	Belongs to a group of cytokine-activated signaling molecules that candirectly bind to DNA and activate or repress transcription oftarget genes	Expressed widely	Gonadal development	Hypogonadism	Obesity, Diabetes and thermal dysregulation	112
IV	**DEFECT IN FERTILIZATION**								
1.	*Nl1*	NL1 (Neprilysin-like peptidase 1)	90 kDa	Zinc metallopeptidase, degrades a broad variety of small peptides	Expressed mainly in testis as a secreted protein	Spermatozoa	Decreased egg fertilization and perturbed early development of fertilized eggs	No physicalor behavioral abnormalities	45
2.	*Cyrn*	Cyritestin	110 kDa	Involved in cell-cell adhesion through binding to integrins	Localized in acrosomal regions of spermatids and spermatozoa	Sperm-zona pellucida binding	Failure of mutant sperm to bind to zona pellucida	Not reported	46
3.	*ApoB*	ApoB (Apolipoprotein B)	550 kDa	Plays an important role in lipoprotein metabolism	Expressed in liver and lymph and circulates in blood plasma	Spermatozoa	Reduced sperm counts, motility, survival and penetration of zona pelucida and abnormal binding to the oocyte surface	Reduced cholesterol levels	113
4.	*Clgn*	Calmegin	93 kDa	Chaperone protein	Expressed only in testes, and is localized on ER membrane	Fertilization	Defective zona-pellucida binding	No developmental abnormalities	47
5.	*Adam2*	Fertilin ß (A disintegrin and metalloproteinase domain 2)	82 kDa	Sperm surface membrane protein, may be involved in sperm-egg plasma membrane adhesion and fusion during fertilization	Expressed specifically in testis	Fertilization	Impaired migration of sperm from uterus into oviduct, and binding to zona pellucida	Not reported	48
V	**DEFECT IN MATING BEHAVIOUR**								
1.	*Pea3*	PEA3	69 kDa	Member of the Ets family of transcriptional regulatory proteins	Nuclear protein expressed during embryonic development preferentially at sites of epithelium-mesenchyma interactions and widely expressed in various adult tissues	Neurotransmission defect	Normal spermatogenesis and spermiogenesis, normal mating behavior, but no copulatory plugs, no sperm in oviducts; Erectile and/or ejaculatory dysfunction	No abnormalities in other organs studied	49
2.	*Esr1*	Estrogen receptor a	67 kDa	Hormone receptor which regulates eukaryotic gene expression and cellular proliferation and differentiation in target tissues	Widely expressed	Low sperm count	Disruption of luminal fluid reabsorption in the head of epididymis resulting in oligospermia, behavioral defect – absent ejaculation	Female infertility, decreased bone mineral density	114

Molecular weight data for this table were retrieved from the TrEMBL link in Gene Detail Database, Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, Maine. World Wide Web (URL: http://www.informatics.jax.org). (Jan, 2005).

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 defect in the development of a mature spermatozoon. A total of 47 genes were found in this category (Table 1).

The generation of haploid spermatids from primary spermatocytes involves the meiotic cell division.

Several genes involved in meiotic process have been disrupted in mice leading to infertility. Protein C inhibitor (PCI) is a plasma protein, also present in high concentration in the male reproductive tract. PCI knockout mice exhibit abnormal spermatogenesis and sperm malformation leading to infertility (19). Defect in several genes leads to an arrest of spermatogenesis at a specific stage of meiosis leading to infertility. In a Sertoli cell specific androgen receptor (AR)-knockout, spermatogenesis was arrested at diplotene stage of meiosis I leading to azoospermia and infertility (20). Deletion of *dmc1* gene leads to an arrest of spermatogenesis in the meiotic prophase (21). Cpeb (cytoplasmic polyadenylation element binding protein) controls germ cell differentiation by regulating the formation of synaptonemal complex during meiosis I. In *cpeb* knockout mice, spermatogenesis is arrested at the pachytene stage of meiosis (22). Another protein HSP70-2 (heat shock protein 70-2) associates with the synaptonemal complex during meiotic prophase and is expressed at high levels exclusively in pachytene spermatocytes. Spermatocytes fail to progress past the metaphase in the mutant mice (23). Scp 3 (synaptonemal complex protein 3) is a component of the synaptonemal complex and its disruption leads to an arrest of spermatogenesis at the zygotene stage of meiosis 1 and massive apoptotic cell death during meiotic prophase (24).

Apart from the genes specifically involved in meiosis, mutations in other genes can also lead to spermatogenic arrest. *Dazl* knockouts are infertile with a spermatogenic arrest. There is almost complete absence of germ cells beyond the spermatogonial stage leading to infertility (25). *Msh4*, a member of the mammalian mismatch repair gene family, is expressed predominantly in testis. Mutant mice show abnormal chromosome pairing during the zygotene phase of meiotic prophase 1 leading to the induction of apoptosis in the spermatocytes and infertility (26). There is a group of proteins involved in cell cycle called cyclins and cyclin-dependent kinases (CDKs), which interact together to regulate cell division and growth. Cyclin A1 encoded by the *CcnA1* locus is expressed exclusively in the germ cell lineages of in testis and ovaries. Mice bearing a null mutation for the *CcnA1* gene are infertile due to arrest of spermatogenesis prior to the first meiotic division (27). The mice mutant in *Cks2* gene, encoding the mammalian homolog of the yeast Cdk1-binding protein, are infertile due to a block in spermatogenesis at the metaphase of meiosis I. CKS2 has a role in cell cycle regulation. It binds to the catalytic subunit of the cyclin-dependent kinases and is essential for their function (28).

Post-meiotic stages of spermatogenesis represent interesting targets for contraception. A targeted deletion in *crem* (c-AMP response element modulator) leads to an interruption in spermatogenesis at round spermatid stage (29), (30). The transition proteins (TNP1 and TNP2) are involved in the histone displacement and chromatin condensation during spermatid development. Mice with a disruption in *Tnp1* gene have increased abnormal head shapes, increased number of decondensed heads and impaired sperm motility (31). *Tnp2* knockout exhibit teratozoospermia and infertility (32). Absence of Miwi, a cytoplasmic protein specifically expressed in spermatocytes and spermatids, leads to spermatogenic arrest at the beginning of the round spermatid stage (33). Absence of HR6B, leads to abnormal postmeiotic condensation of spermatid chromatin (34).

The process of spermiogenesis, transition of round spermatid into elongated spermatid is susceptible to impairment. Absence of TATA-binding protein like factor (TLF) whose physiological function in the mammalian cell is still unknown, leads to a complete arrest in spermiogenesis at the transition from round and elongated spermatids inducing apoptosis of round spermatids at step7 (35).

3.3. Gene-knockouts that cause defect in testis development and disrupt endocrine milieu

The function of several differentially expressed genes is necessary for normal sexual differentiation. Although these genes do not represent good contraceptive targets, knockout studies will help in the understanding the process of testis development. A total of 18 genes were identified in this category (Table 1). DHH (desert hedgehog), expressed in Sertoli cells is one of the signaling molecules that regulate differentiation of peritubular myoid cells and the consequent formation of testis cords (36). In *Dhh* null mice, Leydig cells are absent and spatial organization of tubules is severely affected leading to anastomotic tubules and peritubular cellular abnormalities (37, 38).

Once testis development is initiated, cells in the testis secrete two hormones, testosterone, and anti-Mullerian hormone that control further sexual development. Testosterone stimulates the development of the male internal duct system (Wolffian ducts), including the epididymis, seminal vesicles, and vas deferens. Mullerian inhibiting hormone (MIH) inhibits development of female duct structures and causes degeneration of the Mullerian ducts. Mice mutant in *Amh* gene (anti-Mullerian hormone) (39) or *Amhr2* (anti- Mullerian hormone type 2 receptor) (40) develop female reproductive organs, which block sperm transfer into the vaginal tracts of females. Doublesex- and mab-3-related transcription factor 1 (*DMRT1)* is another gene involved in testis differentiation. In mice, *Dmrt1* is expressed in genital ridges of both sexes and then becomes testis-specific at the end of the sex-determining period. In testis, *Dmrt1* is expressed in germ cells and Sertoli cells (41). Male mutant mice for *Dmrt1* are viable but infertile with hypoplastic testes, because of disorganized seminiferous tubules and absence of germ cells. They also show degeneration of interstitial (Leydig) cells with infiltration by macrophages and abnormal sertoli cell morphology (41).

Cryptochroidism is the most common disorder of sexual development in the newborn leading to infertility (42). Mutations in the mouse genes, the *great* (G-protein coupled receptor affecting testicular descent) (43) and *Insl3* (insulin-like 3) (44) result in male infertility secondary to cryptochroidism. GREAT protein is a G-protein coupled

transmembrane receptor that mediates hormonal signals affecting testicular descent. It is highly expressed in the gubernaculum, the ligament that controls testicular movement during development (43). Insulin-like hormone 3 is specifically expressed in the Leydig cells of fetal and postnatal testis and is known to stimulate the gubernaculum outgrowth. Mice mutant in *Insl3* show failure of gubernaculum development during embryogenesis leading to bilateral cryptochroidism with free moving testes and genital ducts (44).

3.4. Gene-knockouts that cause defect in fertilization

A group of at least 5 genes have been delineated using the gene knockout studies, whose disruption leads to neurotransmission defect and male factor abnormality (Table 1). These genes represent excellent contraceptive targets since there is generally no other defect in any other organ associated with them. The production of sperm is normal with no apparent defect in their morphology and motility. NL1, a zinc metallopeptidase, is expressed mainly in the testis as a secretory protein. Mice mutant in *Nl1* show highly decreased fertilization rates and perturbed early development of the fertilized eggs (45). Other genes whose knockouts have been shown to produce sperm with an impaired binding to oocyte zona pellucida are *cyritestin* (46), *calmegin* (47), and *fertilin ß* (48). Cyritestin is a plasma membrane protein localized in the acrosomal region of spermatids and spermatozoa and is involved in sperm-oocyte membrane adhesion through binding to integrins. Sperm from the mutant mice are unable to bind the zona pellucida (46). Calmegin is a testis-specific chaperone protein localized in the endoplasmic reticulum. It binds to the nascent polypeptides synthesized during spermatogenesis. Calmegin null mice are infertile even though spermatogenesis is morphologically normal. The sperm from null mice show defective zona-pellucida binding (47). Fertilin ß is expressed specifically in testis. It is a sperm surface membrane protein that may be involved in sperm-egg plasma membrane adhesion and fusion during fertilization. Mice mutant in *fertilin ß* show impaired migration of sperm from uterus into oviduct, and defective binding to the oocyte zona pellucida (48).

3.5. Gene-knockouts that cause defect in mating behavior

Polyomavirus enhancer activator 3 (PEA3) is a member of the Ets family of transcriptional regulatory proteins. It is a nuclear protein expressed during embryonic development preferentially at sites of epithelium-mesenchyma interaction and is widely expressed in various tissues in adults. *PEA3* mutant mice are physiologically normal and live a normal life span. Gross and histological analysis of organs from *PEA3* null mice reveals no abnormality. Spermatogenesis and spermiogenesis also appear normal. *PEA3* mutant males are infertile due to erectile and/or ejaculatory dysfunction (49). It was observed that null males engage in normal mating behavior, but they do not induce formation of copulatory plug in females after intercourse and as a result sperm are not detected in the uteri of females that had mated with *PEA3* null males.

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/spermiogenesis/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 93 genes were identified by literature search in the NCBI database that showed an effect on male fertility. However, most of them demonstrated an effect on other cells/tissues. Knockouts of 46 of these genes specifically affected fertility with a limited deleterious effect on other organs and/or function. These genes /proteins can provide valuable targets for novel contraceptive development. Also, some of the genes/proteins whose knockout show an effect on fertilization in a tissue-specific manner can be used for the development of a contraceptive vaccine for both males and females.

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