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REVIEW ARTICLE
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Candidate gene markers for sperm quality and fertility in bulls

Chinmoy Mishra[1], Tapan Kumar Palai[2], Laxmi Narayan Sarangi[3], Bikash Ranjan Prusty[4] and Biswa Ranjan Maharana[5]

1. Department of Animal Breeding and Genetics, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha-751003, India; 2. Division of Animal Biochemistry, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh-243122, India; 3. Department of Veterinary Bacteriology, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh-243122, India; 4. Division of Veterinary Biotechnology, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh-243122, India; 5. Department of Parasitology, Junagarh Veterinary College, Junagarh Agricultural University, Junagarh, Gujarat-362001, India
Corresponding author: Tapan Kumar Palai, email: tapan.palai@gmail.com
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Abstract

Fertility is one of the primary traits of reproduction in bulls. Decrease in fertility is a multifactorial condition and is very difficult to diagnose. Among various causes, genetic abnormality holds a major share. By identifying various genes that have effects on fertility, the genetic cause behind subferility can be explored and also other non genetic factors can be identified. Advancement of molecular genetic tools now easily enables us to explore individual genes in animals. Identification of these genes will eventually lead to genome assembly and development of novel tools for analysing complex genetic traits. This paper gives a brief idea about the candidate genes for bull fertility, including genes encoding hormones and their receptors, proteins of the seminal plasma, proteins involved in spermatozoa-ovum binding and genes influencing sexual development. The chromosomal location and gene structure are described, based on the bovine genome assembly.

Keywords: bull, fertility, gene, sperm.

Introduction

Bull fertility, an economically important complex trait, is controlled by genetic as well as environmental factors. Several studies conducted in different species highlighted the role of different genes during the process of male reproduction and the cascade of fertilisation. However, the reports on genetic control of fertility in bulls are scanty and needs extensive investigation to meet the future needs. Modern breeding programs use artificial insemination with a low number of males for improving the livestock genetics of economically important traits. However, some of the priced males have a low fertility even when classical semen parameters (i.e. viability, motility, abnormal forms) are normal. It is thus important to develop new molecular tools to accurately estimate fertility levels. Genetic markers can be useful for selection of breeding bulls and ensuing improvement of cattle population.

Bulls are half of the herds and often culled due to some reproductive anomalies even when having a good production index. Reproduction is an intricate process comprising sex differentiation, sexual maturation and gametogenesis (gametogenesis includes spermatogenesis, spermiogenesis, and sperm maturation). During spermatogenesis meiosis occurs in male primordial germ cells (spermatogonia) producing a number of spermatids. In spermiogenesis, the spermatid develops a tail with thickened mid-piece and severely condensed DNA. Sperm maturation which starts inside the testes is completed in the female genital tract. Capacitation process is initiated in the male genital tract by male accessory gland secretions and completed upon their passage through the female genital tract by interaction with the uterine membrane. After encounter with the egg, sperm cells bind to the zona pellucida, which triggers the acrosome reaction releasing membrane digesting enzymes and allow the sperm to penetrate the zona pellucida, so that the sperm and oocyte fuse to complete the process of fertilization. Each of these steps is guarded by a number of proteins. Hence, the genes encoding these proteins can be considered as potential candidate gene markers for male fertility.

Male sex determination

The primary male sex determination signal is produced by specific *SRY* (Sex determining Region of the Y chromosome) gene, located at the distal end of short arm of Y chromosome. The *SRY* encodes an HMG (High Mobility Group) protein that acts as a transcription factor for specific proteins for male sex determination. HMG protein binds to double stranded DNA and then bends the DNA causing increased transcription. Any functional mutations in *SRY* lead to the development of females (Swyer syndrome) with gonadal malfunction [1, 2].

Other genes like *SOX9* (19th chromosome), *DMRT1*, (18th chromosome) *WNT1* (5th chromosome), *AMH* (7th chromosome), *SF1* (29th chromosome), *DAX1* (X chromosome), *GATA4* (8th chromosome) and

aromatase (10th chromosome) are also involved in the sex-determining pathway [3] as mutation in these genes also affects sex differentiation and in some cases, it causes sex reversal. An elaborate discussion on these genes is beyond the scope of this review.

Male genital tract development

Androgen receptor: All androgens work through a single androgen receptor (AR) which, upon ligand binding, regulate the expression of androgen-responsive genes inside the nucleus and determine the expression of male phenotype and commencement of spermatogenesis. The *AR* in bovines is mapped to the long arm of X chromosome. *AR* is a ligand-dependent transcription factor that regulates testosterone signalling and plays a major role in spermatogenesis and fertility [4]. Any functional mutation in the *AR* disrupts testosterone signalling and leads to testicular feminization along with development of female secondary sex characteristics [5]. Although mutations in the *AR* do not inhibit testicular development, the testes fail to descend from abdomen.

Oestrogen: Oestradiol plays an imperative function in the maturation of epididymis at some stage in the pubertal transition. Along with major influence on female reproductive tissue, it also influences the development and function of testes [6] and prostate [7]. Oestrogen (estradiol/estriol) is the main agonist for oestrogen receptor. There are two isoforms known for estrogen receptor ESR1 and ESR2 each encoded by separate genes located in different chromosomes. *ESR1* is positioned on 9th chromosome and *ESR2* is located on 10th chromosome of bovine. The *ESR2* is localised in the epididymis independent of age, whereas *ESR1* localisation is regional and age dependent. Both subtypes bind estradiol-17β with differential DNA-binding affinity [8].

Relaxin: *Relaxin* (present on bovine 9th chromosome) encodes *relaxin* which plays key roles in the development of male reproductive tract, prostate gland and spermatozoa motility. The association between relaxin, sperm motility, capacitation and acrosome reaction in fresh and frozen-thawed bovine spermatozoa acts as an index for predicting bull fertility [9]. Among others, relaxin like peptides such as Relaxin-like factors (RLF) and insulin like 3 (INSL3) peptides (expressed in Leydig cells) are involved in male development and are accountable for descending of testicles. Hence, genetic targeting of the INSL3 causes failure of testicular descent leading to cryptorchidism [10].

Spermatogenesis: Development of spermatids in the testis from spermatogonia is known as spermatogenesis. Spermiogenesis is the differentiation of spermatids into spermatozoa. Several candidate genes were identified and can be used as markers.

Gonadotrophin releasing hormone (GnRH) and gonadotropins: GnRH, produced from hypothalamus, induces release of Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) from the anterior pituitary. FSH is essential for spermatogenesis during puberty, whereas spermatogenesis in adults is promoted mainly by testosterone. LH stimulates the release of testosterone and oestrogens from the leydig cells. FSH acts on the sertoli cells and regulates spermatogenesis up to the secondary spermatocyte stage. FSH protein dimer contains 2 polypeptide units, alpha and beta subunits. The alpha subunits of FSH and LH are identical [11]. The beta subunits vary which confers its specific biologic action [12] and is responsible for interaction with the receptor [13]. The genes encoding receptors for FSH and LH (*FSHR* and *LHR*) are mapped to the 11th chromosome in bovines. Functional mutations in the upstream region of beta subunits are associated with lower sperm concentration, lower percentage of acrosome integrity and a higher percentage of sperm deformities [14]. GnRH, in conjunction with the GnRH receptor (GnRHR) is the primary regulator of reproduction in vertebrates. GnRHR is located in the adeno-hypophysial gonadotropic cell membrane. The gene encoding *GnRHR* is located in 6th chromosome of bovines and its haplotypes also showed a suggestive association with age at scrotal circumference [15]. *GnRHR* is associated with increased sperm motility and sperm volume [16, 17].

Inhibin and activin: Inhibin and activin belong to the transforming growth factor (TGF) β superfamily and act as markers of persistent spermatogenesis [18]. Inhibin regulates the secretion of FSH in conjunction with oestrogen and testosterone [19]. Genes encoding *α* and *β inhibins* are potential candidates for fertility analysis and are located in chromosome 2 and 4, respectively. *Inhibin α* was reported to have significant association with acrosome integrity and *inhibin β* with semen volume per ejaculate and motility [20].

Activin can directly stimulate FSH biosynthesis and release from the gonadotrope cells of the pituitary gland or can upregulate *GnRHR* gene expression or can stimulate GnRH release from GnRH neurons in the hypothalamus and thereby affect FSH and LH secretion. *Activin A receptor- IIA* and *Activin A receptor-IIB* are located in chromosome 2 and 22, respectively.

Prolactin: The prolactin and growth hormone genes are produced from duplication of a common ancestral gene; hence they are structurally similar. *Bovine prolactin* (*bPRL*) is located on chromosome 23 [21] and consists of five exons [22] with 199 amino acids [23]. The gene coding for bovine prolactin receptor (PRLR) was mapped to 20th chromosome [24]. Two distinct (long and short) PRLR isoforms were identified in cattle which are produced by alternative splicing mechanism. The short PRLR is unable to mediate transcriptional activation via JAK-STAT pathway. Furthermore, short isoform can inhibit long PRLR activation of JAK2 and transcription via

formation of heterodimers [25]. The interaction between prolactin, gonadotrophins and GNRH is modulated by photoperiod and melatonin [26].

Seminal plasma proteins associated with bull fertility

Bovine seminal plasma (BSP) proteins: Bovine seminal vesicles secrete a family of similar proteins collectively called BSP proteins. Genes encoding BSP like *BSP1, BSP3, BSP4, BSP5, BSPH1, BSPH2* are located in 11th chromosome in bovines. These proteins consist of major and minor components in three main protein classes: fibronectin type II proteins, cysteine-rich secretory proteins (CRISPs) and spermadhesins. The primary structure of the BSP proteins contains two fibronectin type 2 (FN2) domains repeated in tandem [27]. The BSP family comprises BSP1, BSP3, and BSP5 previously called BSP-A1/-A2, BSP-A3 and BSP-30-kDa, respectively [28]. Functions of different BSPs are similar which can be expected from their three-dimensional structures which are identical [29]. BSP proteins bind specifically to phosphatidylcholine, plasmalogen and sphingomyelin on the sperm surface, interact with heparin and high-density lipoproteins (HDL), the capacitation factors in bovine and potentiate capacitation of sperm at epididymis [30]. Bioinformatics analysis revealed that N-terminal part of bovine BSP5 is intrinsically disordered suggesting its role in capacitation and/or sperm-egg interaction process. Epididymal sperm binding protein 1 (ELSPBP1) which is highly expressed in the caput and the corpus epididymis but present in lower expression levels in the testis and the cauda epididymis, characterises spermatozoa that are already dead at ejaculation [31].

Alpha-L-fucosidase: L-fucose is a common terminal residue of both *N*- and *O*-linked glycolipids and glycoproteins present on cell membranes. It is also reported to be involved during fertilization and has been identified as a component of gametes. α-L-fucosidases recognize and cleave terminal fucose residues thus playing a significant role in zona pellucida binding/penetration, sperm-egg membrane fusion, and post fusion events [32]. The *alpha-L-fucosidase* is located on 2nd chromosome of bovines.

Osteopontin: Osteopontin (component of sex gland fluid) is a highly acidic, phosphorylated glycoprotein detected in several species with moderate levels of conservation and is a fertility marker in bulls [33] as it affects sperm-oocyte binding and early embryo development. Gene encoding osteopontin is located in bovine chromosome no. 6.

Phospholipase A2 (PLA2): Bovine PLA2protein is secreted from seminal vesicles and exists in two isoforms (one with 15 and 16 kDa subunits and the other with 16 and 60 kDa) and have possible roles in sperm capacitation and the acrosome reaction. Bovine *PLA2* is present on 16th chromosome and activated in spermatozoa in response to progesterone and calcium. Male mice that do not express PLA2 produce spermatozoa with impaired motility and have greatly reduced fertility [34].

Prostaglandin D synthase (PGD): PGD is a fertility-associated protein and gene encoding it is located in chromosome 6 [35]. It is involved in binding, protecting and facilitating the uptake of retinoids within the male genital organs. Retinoids are required for normal spermatogenesis, cell growth, differentiation and maintenance of epithelial integrity [36].

Spermadhesin 2: Spermadhesin 2 also known as spermadhesin Z13 has adverse effect on sperm motility and is abundant in bulls of low fertility [37]. The gene encoding the protein is present in 26th chromosome in bovines.

Clusterin: Clusterin gene is located in bovine chromosome number 8. In bulls, it is produced by Sertoli cells and is associated with physiological processes like binding and agglutinating abnormal spermatozoa, preventing oxidative damage to the sperm, inhibiting complement induced sperm lysis.

Ubiquitin: Ubiquitin is an 8.5 kDa protein which degrades unrequited proteins. In the bull reproductive tract, it is secreted by epididymis and covalently links to the surface of defective mammalian spermatozoa [38]. So, increased ubiquitin levels in bull sperm are sign of both poor semen quality and fertility in bulls.

Sperm specific proteins

Phospholipase C zeta (PLCZ1): Ovulated mammalian oocytes are arrested at the second metaphase stage of meiosis. They are activated by sperm and complete meiosis after fertilization, a process called oocyte activation. Oocyte activation is triggered by rise in the intracellular concentration of free Ca^{2+} which is due to the production of inositol 1,4,5-triphosphate (IP3) following activation of the phosphoinositide signalling pathway [39]. The sperm factor, PLCZ1 is responsible for inducing the production of IP3 and Ca^{2+} release [40]. The gene encoding PLCZ1 is located in the 5th chromosome of bovines. The genetic variation in the promoter region of PLCZ1 is also associated with semen quality traits [41].

Zonadhesin: Zonadhesin is a mosaic type protein that localizes to the apical head of spermatozoa and binds to zona pellucida in a species specific manner. Bovine zonadhesin is present on its 25th chromosome and due to this binding property, it is one of the best investigated mammalian sperm ligands [42].

Calmegin: Calmegin (Clgn) gene is first expressed in meiotic prophase of primary spermatocytes and encodes a chaperon protein for other proteins in transition from endoplasmic reticulum to the spermatid surface. In Bovines, Clgn is located in chromosome 17. Loss of Calmegin gene leads to male sterility in mice with defects in migration into the oviduct and non

binding to the zona pellucida [43].

Fertilin: Fertilin, originally termed as PH-30, is a heterodimer (consisting of α and β) that plays an active role in mediating the binding and fusion of sperm-oocyte membrane. The α subunit, has a homology region to viral fusion peptides and the β subunit has a homology region to the disintegrin family of integrin ligands [44]. Fertilin is localized to the sperm domain, involved in binding to the egg membrane. Fertilin α and β subunits are members of a new family of transmembrane proteins, the ADAMs (A Disintegrin and a Metalloprotease domain) gene family. Approximately 30 numbers of ADAM family members are identified. Bovine fertilin α subunit is mapped to Adam1 (17^{th} chromosome) and the β subunit to Adam2 (27^{th} chromosome) [45]. It is suggested that disintegrin domain of fertilin β interacts with integrin receptor present on egg surface. Fertilin α also has been proposed to participate in fusion of egg and sperm membrane.

Serine/threonine phosphatase: Calcium and c-AMP regulated protein phosphorylation is essential for sperm capacitation. So, capacitation is always associated with an increase in tyrosine phosphorylation of a number of proteins and has been considered as the consistent biochemical marker for sperm capacitation [46]. Increase in c-AMP level stimulates sperm motility by protein kinase A (PKA) activation. Calcium can directly affect protein phosphorylation through calcium-activated protein kinases or phosphatases, or indirectly through changes in c-AMP levels [47].

Lactate dehydrogenase C: Lactate dehydrogenase may be the first testis specific glycolytic isozyme discovered in male germ cells [48]. The 3 different forms of LDH are encoded by 3 different genes: *LDHA, LDHB, and LDHC* which codes for A, B and C subunits. In knockout mice of LDHC gene disrupted male fertility was observed [48]. It may be due to reduction in sperm glucose consumption, ATP production and motility.

Angiotensin-converting enzyme (ACE): In bovines, two isoenzymes of ACE (somatic and germinative or testicular) are identified. Somatic ACE, primarily expressed in endothelial cells, contains a double domain produced by gene duplication. Germinative ACE is produced in the male reproductive system and contains a single domain [49]. Both isoforms are structurally related in that they are encoded by a single gene and arises from alternate transcription initiation and post-transcriptional splicing [50]. Primarily ACE converts angiotensin I to angiotensin II. However, it is a nonspecific peptidase that is capable of cleaving a wide range of substrates affecting many physiologic processes like blood pressure control, haematopoiesis, reproduction, renal and immune functions [51].

Testis-specific protein on Y chromosome (TSPY): The TSPY genes are found in groups (copy numbers may range up to 200 in bulls) on the Y chromosome. The bovine *TSPY* has seven exons with expression apparently limited to male germ cells which starts during fetal stage [52]. The protein product of the TSPY gene may interact with type B cyclins and activates cyclin B-CDK complexes. The activated complex promotes spermatogonial cell renewal, spermatocyte proliferation and differentiation [52]. Although Single Nucleotide Polymorphism was detected in fourth intron of TSPY gene in buffalo bull, no significant association was detected with the spermatogenic characters. Absolute copy number of TSPY gene is correlated with fertility rate [2].

Ubiquitin specific peptidase 9 (USP9Y): The USP9Y gene is classified under USP (ubiquitin-specific peptidases) family is positioned on the long arm of Y chromosome. It is thought to be associated with spermatocyte development, but its exact role is unknown. However, *USP9Y* mutation was correlated with severe spermatogenic failure and infertility with discovery of some novel SNPs [53]. *USP9Y* is more likely a fine tuner that improves efficiency of spermatogenesis, rather than a provider of an essential function [54].

Conclusion

A number of genes and their respective proteins involved in bull reproduction have been identified and more detailed studies on these components will help us to understand and diagnose cases of infertility and/or subfertility that will enhance the accuracy for prediction of male reproductive performance.

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