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Factors Influencing Goat's Semen Fertility and Storage: A Literature Review

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ABSTRACT The present review paper gives an idea about the candidate genes for buck fertility, including genes encoding hormones, their receptors, proteins of the seminal plasma, proteins involved in spermatozoa-ovum binding and genes influencing sexual development. Buck reproductive performance is highly influenced by semen quality. The quality of sperm after freezing is very important for success of artificial inseminations. Adopting marker could assist selection of candidate gene for genetic improvement of the bucks' fertility. Decrease in fertility is a multifactorial condition which 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 sub-fertility can be explored and also other non-genetic factors can be identified. Molecular genetic tools enables to explore individual genes in animals. Identification of these genes will eventually lead to genome assembly and development of novel tools for analyzing complex genetic traits.

INTRODUCTION

Breeding buck selection is the most critical decision for improvement of a herd. Artificial Insemination (AI) is one of the most important techniques for increasing the rate of genetic improvement and breeding efficiency in livestock production (Anand and Yadav 2016). Male fertility is highly influenced by semen quantity and quality (Mittal et al. 2014). The quality of thawed frozen semen is very important for success of AI. Therefore, understanding the sources of variation in semen quality and identification of genetic markers for early detection of highly fertile bucks and with good quality thawed frozen semen would be of great interest to all livestock breeders (Arredondo et al. 2015). Although progress in these areas with reference to its application in the caprine species has not progressed fast as with cattle, the need is still there. With the major populations of goats residing in the developing countries, there is the obvious demand to accelerate the understanding in the fields with the goal to boost the productivity for these countries.

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Genetic Resource Banks

The Food and Agriculture Organization (FAO) has developed a global plan of action for animal genetic resources (2007) in order to fight against the loss of animal genetic diversity, being a priority for the *in situ* and *ex situ* preservation of zoo-genetic resources. In *situ* conservation, animals are preserved in their natural habitat, while *ex situ* conservation implies the maintenance of populations out of their natural environment. The creation of Genetic Resource Banks (GRB) is contemplated among the measures of *ex situ* conservation. The GRB allows the storage of semen, oocytes, embryos and other tissues indefinitely, being a key tool in order to ensure the genetic diversity of species (Roldan et al. 2006). The most widespread use of GRB has been the collection and freezing of semen (Watson and Holt 2001). However, it is necessary to know deeply the reproductive physiology of species or breeds which need to be preserved, as well as the reproductive advances on biotechnology in order to use those assisted reproductive techniques more accurately for each species (Yoshida 2000).

The Role of Artificial Insemination in Goat Production

Artificial Insemination (AI) has been used for many years and this technology is very in-

dispensable for reproductive biotechnology as well as for many other areas of animal science (Yotov et al. 2016). One of the most important advantages obtained when using AI is that one single ejaculate can be used to inseminate several females. Additionally, AI has demonstrated to be an effective reproductive technology that selectively allows the diffusion of desirable genes and accelerates the genetic progress in animal science. Farm animals, are usually selected for breeding programs based on breeding soundness examinations (Hart and Gipson 2007). Animals that do not meet certain criteria are excluded. Another advantage of AI is to ensure effective use of semen. A higher number of offspring from a superior sire can be produced when AI is used. Freezing bull semen can provide up to 200 straws of frozen semen from one ejaculate, corresponding to 200 AI doses. In goat breeding programs, AI represents an essential tool, since it promotes the efficiency of sire genetic evaluation as well as genetic improvements. However, AI is not extensively used in goats (Arrebola et al. 2012). In AI, cooled semen provides better results than frozen-thawed semen. Hormone treatments are used for fixed-time AI in order to stimulate oestrus and synchronize considerable number of goats rather than individually. This allows more goats to be inseminated over a shorter period, as well as enabling insemination and births to be carefully scheduled. It also helps increase the number of kids obtained by AI at the start of the kidding season, thus allowing a more efficient use of human resources (Nunes and Salgueiro 2011). Some factors have been implicated in affecting the success and effectiveness of AI, including nutrition, environmental conditions, breeding season, parity, farm, breed, depth of semen deposition, extender composition and hormone treatment (Nunes and Salgueiro 2011). Arrebola et al. (2012) reported that fertility was significantly influenced by the production system, season, buck, and semen deposition. The year on year variations in fertility may reflect the interaction of factors, including weather conditions, animal welfare, nutrition and animal breeding (Arrebola et al. 2012). Goat reproduction is affected by changes in day length over the year. Photoperiodic control of reproductive patterns is mediated through circadian rhythmic secretions of melatonin by the pineal gland during darkness, which influences gonadotropin-releasing hormone pulse generation and the hypothalamic-pituitary-gonadal feedback loop (Fatet et al. 2011). Reported goat conception rates range from fifty percent to seventy percent, depending on the season of insemination. The probability of pregnancy may be reduced when insemination taking place during the colder seasons (autumn and winter) (Arrebola et al. 2012).

The main objective of these investigations is twofold: firstly, to be able to know in advance the sperm quality and fertility of a male, and secondly, to design individual freezing/thawing protocols aimed at maximizing the performance of the technique.

SEMEN PRESERVATION

Cryopreservation

Cryopreservation is a way of preserving germplasm that have an agricultural application, aquaculture, biotechnology and conservation of threatened species (Andrabi and Maxwell 2007). Germplasm cryopreservation is used for genetic improvement of domestic species, to preserve rare breeds, well adapted to environmental changes, and in international germplasm exchanges. It was shown in ram that frozen semen stocked over 27 years did not affect fertility, becoming possible the preservation of genetic resources of the national breeds (Salamon and Maxwell 2000). Semen banks are currently more developed for domestic breeds (cattle, sheep, goats and pigs) than for non-domestic species, but the concept of using them to facilitate the management and conservation of endangered species is being promoted extensively. Nowadays, semen cryopreservation has many biotechnological applications. It can be used to solve problems of human infertility, life threatening diseases, preservation of semen and DNA from endangered species and conservation of biodiversity.

Buck Semen Cooling and Cryopreservation

Cryopreserved sperm is an important integral component for the advancement of goat production (Üstüner et al. 2015). The development of semen collection, storage and AI techniques depend on the donor males, since males have a great impact on reproductive success, due to the ability of their one ejaculate to insem-

inate many females. To increase the storage period of the semen collected, the development of optimal cryopreservation protocols is of great importance. Cryopreservation is a way of preserving germplasm that has applications in many areas of science (Barbas and Mascarenhas 2009). Cryopreservation is used to establish genetic resource banks for conservation of endangered species or breeds, and this allows maintaining genetic diversity (Špaleková et al. 2015). Moreover, cryopreservation of the sperm carrying desired genes is substantially important in commercial livestock production. However, the technique involves major changes in sperm cell environment, and their success to survive this process will depend largely on their ability to respond to these changes within the finite time period allowed by protocol (Mazur and Cole 1989). Several studies have been conducted to understand the changes that occur during cryopreservation of spermatozoa and development of protocol to ensure a successful storage (Benson et al. 2012). As a result of these studies, standard cryopreservation protocols were developed for many species, both domestic and wild animals. One important problem for standardizing sperm cryopreservation procedures is that spermatozoa from different individuals exhibit significant different responses to the same freezing treatment (Benson et al. 2012). Many researchers have already addressed this issue, and most of them have reported that different individuals exhibit significant different responses to the same freezing condition. These researchers have considered different sperm characteristics such as sperm morphology (Nunez-Martinez et al. 2007), sperm motility and velocity (Ramon et al. 2013), integrity of sperm membranes (Sancho et al. 2007) or DNA status (Hernandez et al. 2006) to evaluate the differences on male response. Others have reported the changes that occur in the dimensions of the sperm head during the cryopreservation process as way to predict fertility and freezability (Nunez-Martinez et al. 2007; Ramon et al. 2013). Most of them have used related measures of sperm head dimensions on fresh semen with measures of sperm quality at thawing. They have reported that males with smaller sperm heads in fresh semen showed better cryo-resistance than those with larger heads. These researchers have presented the important role of sperm head morphology on the characterization of spermatozoa of a male given its low intra-male variability. Male's semen can be classified into bad and good freezers depending on the ability of their spermatozoa to resist the cryopreservation process. Sperm motility in general, and characteristics of sperm motion in particular, could be some of the indicators of the quality of spermatozoa. Commonly, by evaluating the proportion of progressive motile percent at different stages, the quality of semen is monitored. However, evaluation of characteristics of sperm motion may provide valuable information on why certain samples despite containing good proportion of progressive motile spermatozoa pre-freezing poorly and less fertile. These findings suggested that it could possibly be the speed of sperm motility at pre-freeze stage (after 4 hours of glycerol-equilibration), which could have predominantly influenced the quality of frozen spermatozoa. Van Duijn (1962) has reported that more than the initial velocity of spermatozoa, the rates of velocity-decrease and the number of spermatozoa moving normally are more important with respect to motility characteristics. Therefore, the rate of velocity retardation of spermatozoa could be considered as an indicator for predicting livability and quality of sperm (Chakrabarti 1993). It has been observed in the evaluation of effect of freezing in Boer cross and Barbari goats that all the motility characteristics of spermatozoa has been found to be significantly influenced by freezing and thawing processes (Sundararaman and Edwin 2005). All the sperm motility and velocity parameters were significantly reduced in the post-thawing semen. Changes in the osmotic pressure during semen processing for cryopreservation critically affect the spermatozoa. This may be the most important deterrent to sperm survival during cryopreservation (Watson 1995). In the freezing of goat semen, the quality of sperm motility in terms of speed is important in determining the post-thawing survivability of the spermatozoa. It has been reported that Kilis goat semen had higher motility and viability at different glycerol concentrations after thawing, compared to Saanen and Angora goats. Motility and viability at five percent concentration of glycerol has been found higher than those at other glycerol concentrations. Furthermore, Saanen goat semen revealed higher percentage of abnormal spermatozoa after thawing compared to Angora and Kilis goats. This suggested that the different glycerol con-

centrations might play a major role in the success of cryopreservation of semen from different goat breeds (Kulaksiz et al. 2013). Further, the velocity measurements of sperm motility are useful in predicting the fertility of semen (Kirk et al. 2005). Eight distinct processing steps can be recognized in relation with changes affecting the sperm cryopreservation and they need to be examined in detail before a cryopreservation procedure can be initiated since these factors will influence the outcome of the process (Purdy 2006; Roldan et al. 2006). Among these factors include species and individuals, sperm collection method, collection season, extenders composition, cooling rate, equilibration time, freezing rate and thawing rate. Each of these has a relationship with membrane structure function and cell metabolism.

Buck Semen Extenders

The cryopreservation extenders used for goat and ram semen have either egg yolk or nonfat dried skimmed milk. In bucks there are some differences in the cryopreservation method compare to other domestic species, because of negative interactions between the phospholipids of the egg yolk or the milk based extenders and the bulbourethral gland buck secretions existing in seminal plasma. This situation does not exist in other domestic species such as bull, boar and ram (Purdy 2006). The addition of egg yolk based extenders to buck semen gives rise to its coagulation and sperm die due to the action of an enzyme secreted by the bulbourethral gland named egg yolk coagulating enzyme (EYCE) (Sen et al. 2015). Also, there is a protein secretion from this gland named *BUSgp60* that reduces motility and vitality of cooled and frozen sperm extended in milk based extenders. The EYCE was identified as phosholipase-A, acting as a catalyst that hydrolyzes egg yolk lecithin into fatty acids and lysolecithin, which are toxic to sperm cells. This hydrolysis stimulates the acrosome reaction, and chromatin decondensation (Purdy 2006). *BUSgp*60 is responsible for hydrolysis of the plasma membrane and skimmed milk triglycerides, resulting in fatty acid production (lysolecithin production from egg yolk and oleic acid from milk triglycerides), which causes damage to sperm cells (Rubio and Combarnous 1998). To overtake this problem, goat semen is diluted previously in a physiologic saline serum and centrifuged (Lebouef et al. 1998) to remove seminal plasma. This step is essential to produce frozen semen of good quality, increasing post-thaw motility and acrosomal integrity. Due to the importance of AI in livestock management and preservation of genetic material, many studies have been conducted to identify those characteristics that favor the quality and fertility of spermatozoa during cryopreservation (Gravance et al. 2009). Both goals support the idea that sperm cryo-survival and fertility can be under genetic control (Thurston et al. 2002). This review explores three hypotheses:

1) That consistent inter individual variation in sperm freezability is genetically determined.
2) That single nucleotide polymorphism (SNP) in the exon regions of the *Hsp70* gene and their effects on semen quality traits of pure Boer and Boer cross bucks.
3) That candidate genes have been shown to play a role in buck's reproduction and for which a relevant role in semen quality has been demonstrated.

Advances in the understanding of the causes of variation at the genetic level should offer some explanation for the phenomenon of good and bad freezers, and provide the basis for improving the quality of cryopreserved semen through selective breeding. It may be used as a way to select the parent stock at an early age and lead to novel and more widely successful methods of cryopreservation.

FERTILITY AND MOLECULAR GENETICS

Buck fertility is an economically important complex trait which 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 fertilization. However, the reports on genetic control of fertility in bucks are still at their infant stage and need extensive investigation to meet the future needs. Implementation of artificial insemination (AI) in modern breeding programs allows breeders to use a low number of males for improving the livestock genetics of economically important traits. At the same time, it stresses the meaning of the individual buck reproduction performance and requires consequent evaluation of

fertilization potential of a semen sample for AI in goat industries. This is because some males have a low fertility even when classical semen parameters (viability, motility, abnormal forms) are normal. Malmgren and Larsson (1984) proposed that semen evaluation could be used as an indicator of fertility in male animals. Sperm concentration, motility and normal sperm rate have usually been used as criteria for semen quality evaluation (Colenbrander et al. 1993). However, a number of laboratory assays that examine cellular attributes of sperm are still unable to predict the fertility of a semen sample consistently (Braundmeier and Miller 2001). It is thus important to develop new molecular tools to accurately estimate fertility levels. Genetic markers can be useful for selection of breeding bucks and ensuing improvement of goat population.

Spermatogenesis

Reproduction is an intricate process comprising sex differentiation, sexual maturation and gametogenesis. Development of spermatids in the testis from spermatogonia is known as spermatogenesis. During spermatogenesis meiosis occurs in male primordial germ cells (spermatogonia) producing a number of spermatids. In spermiogenesis, the spermatid develops a tail with a thickened mid-piece and severely condensed DNA. 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 semen quality and fertility traits. It has been proposed that candidate gene analysis can be used to identify individual genes responsible for traits of economic importance (Linville et al. 2001). Hormone and hormone receptors are presumed to be good candidate genes for the reproductive traits because they modulate limiting steps in many reproductive pathways (Vincent et al. 1998). Therefore, gonadotropin releasing hormone receptor (*GnRHR*), prolactin (*PRL*), prolactin receptor (*PRLR*), follicle stimulating hormone beta (*FSHβ*), luteinizing hormone beta (*LHβ*), follistatin (*FST*), inhibin alpha (*INHα*), inhibin beta α(*INHβα*) and inhibin beta β(*INHββ*) genes known for their function in male hormone pathways were selected to investigate their effects on sperm quality traits and boer fertility by Lin et al. (2006).

Gonadotropin-releasing Hormone (GnRH)

The *GnRH,* in conjunction with the *GnRH* receptor (*GnRHR*) is a main regulator of the reproductive system in mammals, produced from hypothalamus inducing the synthesis and release of *LH* and *FSH* in the pituitary. The *GnRHR* is a guanine nucleotide-binding protein coupled receptor with a characteristic seven trans-membrane domain motif. It transduces the hypothalamic message carried by gonadotropin-releasing hormone. At the adenohypophysial gonadotropic cell surface the hormone binds to the receptor, leading to pituitary synthesis and secretion of gonadotropins (Cohen 2000). *GnRHR* deficiencies and *GnRHR* mutations are associated with idiopathic hypogonadotropic hypogonadism or Kallmann's syndrome in humans (Abbas et al. 2003), because *GnRHR* mutations reduce *GnRHR* binding and/or activation of inositol triphosphate or phospholipase C (Achermann and Jameson 1999). The gene encoding *GnRHR* is located in the 6th chromosome of bovines and its haplotypes also showed a suggestive association with age at scrotal circumference (Lirón et al. 2012). *GnRHR* is associated with increased sperm motility and sperm volume (Sang et al. 2011).

Prolactin

Prolactin (*PRL*) is an anterior pituitary peptide hormone involved in many different endocrine activities. It is essential for reproductive performance, mammary development and lactation. The hormone exerts its physiological effects through an interaction with specific cell surface high-affinity *PRL* receptor (*PRLR*) (Omelka et al. 2008). The *PRL* is believed to mediate seasonal signals entraining reproductive and hair follicle growth cycles (Choy et al. 1997). The *PRLR* is a single membrane bound protein that belongs to class 1 of the cytokine receptor su-

perfamily (Marc et al. 2000). Bignon et al. (1997) reported the full-length coding sequences for short and long ovine *PRL* receptors (long form *PRL* receptor, *LPRLR*; short form *PRL* receptor, *S-PRLR*). Polymerase chain reaction experiments on ovine genomic DNA showed that the 39 bp insert was directly linked to the downstream exon. The same result was found in bovine and caprine genomes (Bignon et al. 1997). Although there are no differences in hormone binding activity between *L-PRLR* and *S-PRLR,* the two forms of the *PRLR* may have distinct signaling pathways (Nixon et al. 2002). 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 (Feysot et al. 1998). *PRLR* mRNA expression is almost consistent with *PRL* binding sites except for elongated spermatids and spermatozoa suggesting that *PRL* may have direct effects on spermatogenic cells (Hondo et al. 1995). Prolactin knockout mice showed delayed fertility in males, and the effects of *PRL* on testosterone production of Leydig cells and accessory reproductive glands can obviously be finally compensated by other regulatory factors (Feysot et al. 1998). The interaction between prolactin, gonadotrophins and *GnRH* is modulated by photoperiod and melatonin (Henderson et al. 2008).

Follicle Stimulating Hormone (FSH)

The *FSH* is essential for spermatogenesis during puberty, whereas spermatogenesis in adults is promoted mainly by testosterone. The *FSH* acts on the sertoli cells in the seminiferous tubules of the testis and regulates spermatogenesis up to the secondary spermatocyte stage, and later androgens from the testis support the final stages of spermatogenesis (Hafez 2000). The *FSH* influences sexual behavior and testicular morphology and function of the boar (Zanella et al. 1999). The *FSH* is a heterodimer that contains two polypeptide units, alpha and beta subunits that are coded by two distinct genes. The beta subunit varies, which confers its biologic action and is responsible for the receptors (Bernard et al. 2010). 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 (Dai et al. 2009). The expression of *FSHβ* gene in boar is positively associated with activin beta B-subunit (Li et al. 1998). Male homozygous *FSHβ* knockout mice had normal levels of serum testosterone but had small testes and oligospermia (Layman and McDonough 2000).

Luteinizing Hormone (LH)

The *LH* influences the sexual behavior and testicular function of the boar. The interstitial cells (Leydig cells) produce androgens after *LH* stimulation (Hafez 2000). The *LH* is a glycoprotein composed of an alpha and a beta subunit with a molecular weight of 30,000 Da and a biologic half-life of 30 minutes. The *LHβ* gene expressed during spermatogenesis and male sexual behavior and *FSHβ* may participate in spermatogenesis, whereas *LHβ* is more involved in spermatogenesis (Degani et al. 2003). A mutation causing inactivation of the *LH* beta subunit in human leads to absence of Leydig cells, lack of spontaneous puberty and infertility (Huhtaniemi et al. 1999).

Follistatin (FST)

Follistatin (*FST*) and activin were first isolated in the 1980s as peptides found in ovarian follicular fluids that could either inhibit or activate the production and secretion of follicle stimulating follicle stimulating hormone (*FSH*) from the pituitary gland, respectively (Ueno et al. 1987). The *FST* is a protein that has been also isolated in testis that may modulate a range of testicular actions of activin (Meinhardt et al. 1998). It does not only inhibit the secretion of *FSH* similar to that of inhibins (*INHs*) but also binds activin (*ACN*) and neutralizes its biological activity, thus it modulates the secretion of *FSH*. The *INH* and *ACN* belong to the transforming growth factor (TGF) β superfamily and act as markers of persistent spermatogenesis (Toulis et al. 2010). The *INH* regulates the secretion of *FSH* in conjunction with estrogen and testosterone (Bhardwaj et al. 2012). Genes encoding α and *βINHs* are potential candidates for fertility analysis and are located in chromosome 2 and 4, respectively. The *INHα* was reported to have a significant association with acrosome integrity and *INHβ* with semen volume per ejaculate and motility (Sang et al. 2011). Activin can directly stimulate *FSH* biosynthesis and release

from the gonadotrope cells of the pituitary gland or can up regulate *GnRHR* gene expression or can stimulate *GnRH* release from *GnRH* neurons in the hypothalamus and thereby affect *FSH* and *LH* secretion. *ACN*α receptor-IIα and *ACN*α receptor *II*β are located in chromosome 2 and 22, respectively (Mishra et al. 2013). The gonads are the main source of *INH* and related proteins, which contribute to the endocrine regulation of the reproductive system. Sertoli cells in the male produce *INHs*. *INHs* may partly be responsible for the differential release of *LH* and *FSH* from the pituitary. Hormone concentrations in the male and female and control of hormone receptors are recent traits under consideration. Genetics play an integral role in the control of the reproductive traits (Rothschild 1996). The expression of *INH*$\beta\alpha$ and *INH*$\beta\beta$, *FST* and *ACN*α receptor messenger RNA (mRNAs) in different stages of seminiferous epithelial cycle regulated spermatogenesis (Kaipia et al. 1992). Both levels of serum *INH*β and seminal plasma *INH*β could reflect testis spermatogenesis status, and levels of seminal plasma *INH*β could also reflect the function of seminiferous duct (Hu and Huang 2002).

Molecular Markers

Molecular markers are considered as tools to localize and visualize genetic variation among individuals. They can be used to associate the genetic variation with a trait of interest. The use of DNA markers to define the genotype of animal performance added a powerful tool in animal breeding program improvement (Vermerris 2008). At the DNA level, the types of genetic variation included base substitutions, commonly referred as single nucleotide polymorphisms (SNPs), insertions or deletions of nucleotide sequences within a locus, inversion of a segment of DNA within a locus, and rearrangement of DNA segments around a locus of interest. Through long evolutionary accumulation, many different instances of each type of mutation could exist in any given species, and the number and degree of the various types of mutations define the genetic variation within a species. The marker types include restriction fragment length polymorphism (RFLP), single strand conformation polymorphism (SSCP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) markers, single nucleotide polymorphism (SNP), and simple sequence length polymorphism (SSLP), variable number tandem repeat (VNTR), short tandem repeat (STR), restriction site associated DNA (RAD) and microsatellite markers. However, due to the existence of various molecular biology techniques to produce them, and to the various biological implications some can have, a large existing variety, from which choices will have to be made according to purposes. Thus, the well-designed studies using these markers will undoubtedly accelerate genes identification involved in quantitative trait loci (QTL) for marker-assisted selection (Vignal et al. 2002).

Molecular Markers for Male Fertility

Many researchers have generally ignored the male reproductive traits. However, as heritability estimates and suggests, selection progress for male traits may be quite effective. A moderately heritable trait, which could be measured in the male and which is positively correlated to improve reproduction in the female would be useful in selection to improve female reproductive ability. Sperm production requires the completion of a complex cascade of events. These steps are mediated by a number of proteins. Consequently, the genes encoding these proteins can be considered as potential candidate genes for male fertility traits (Leeb et al. 2005). The availability of genetic markers opens the possibility of detecting fertile and sub-fertile males from one selected line, and of establishing correlations with sperm parameters observed for fresh and frozen semen (Vicente et al. 2004).

Candidate Genes Associated with Sperm Fertility and Freezability in Goats

Candidate genes can be identified via their involvement in key processes of reproduction in goats and other species.

Heat Shock Proteins (HSPs)

Heat shock proteins (*HSPs*) are a group of proteins that provide thermo tolerance in cells and protect cells against apoptosis during injury and oxidative stress (Beere and Green 2001). Heat shock protein 70 (*HSP70*) is produced by the *HSP70* gene, and includes a family of HSPs, which range in size from 68 to 73 kDa. The *HSP70*

gene is encoded by a single exon. The open reading frame of the gene is 1926 bp and its protein includes 641 amino acids (Gade et al. 2010). *HSP70* plays a protective role in reaction to hyperthermia as well as other stress conditions (Santoro 2000) by providing a balance between synthesis and degradation of cellular proteins (Shi et al. 1998). It also acts as a molecular chaperone, which assists in the process of folding, transporting and assembling proteins in the cytoplasm, mitochondria and endoplasmic reticulum (Georgopoulos and Welch 1993). Elliott et al. (2009) found that *HSP70*, as a sperm binding oviductal proteins, increases longevity and viability of sperm in bull and boar. Lack of the *HSP70* gene leads to a significant increase in apoptosis (Dix et al. 1996). It was reported that semen quality might be influenced by levels of *HSP70* protein in boars (Huang et al. 2000). Govin et al. (2006) found an association between *HSP70* function and spermatid DNA packaging proteins during spermatogenesis. Knockout *HSP70* mice showed structural abnormalities in spermatocytes, arrested evolution of primary spermatocytes, and increased apoptosis of these cells (Christians et al. 2003). Previous studies reported five SNPs in the 5-flanking region of the *HSP70* gene (Huang et al. 2002). Polymorphism in this region showed association with sperm quality of boars (Huang et al. 2002), sperm characteristics in bull (Shrum et al. 2010) and calving traits. Two SNPs were detected in the *HSP70* gene at positions 74A>C (ss836187517) and 191C>G (ss836187518). These SNPs demonstrated significant association with quality traits of fresh and post-thaw semen (Nikbin et al. 2014). The ss836187517 locus showed a significant association with many of the fresh and post-thaw semen quality traits (Nikbin et al. 2014). However, allele C was identified as the favorable allele for the motility traits and ALH of post-thaw semen. In addition, the analysis of the additive effects showed allele C to have a positive effect on SCON and PROG of fresh semen ($P < 0.01$) (Nikbin et al. 2014). Association between the SNPs of *HSP70* and SCON may be attributed to the role of *HSP70* on spermatogenesis. Similarly, Huang et al. (2002) reported an association between total sperm number and SNP of 5-flanking region of *HSP70* gene in boar. *HSP70* as a chaperon protein involves in the formation of protein complexes (Bozidis et al. 2002). Since spermatogenesis is a thermo sensitive process (Bitto et al. 2008), normal spermatogenesis requires the testis temperature to be 4-5°C lower than the body temperature. The functions of *HSP70* may influence semen quality traits of goats in a tropical area such as Malaysia (Nikbin et al. 2014).

Follicle Stimulating Hormone (FSH)

The follicle stimulating hormone (*FSH*) is important for reproductive performance. *FSH* is a gonadotropin, which in combination with testosterone, which regulates the functions of Sertoli cell. *FSH* stimulates the germinal cells and is responsible for spermatogenesis up to the secondary spermatocyte stage (Hafez 2000). It is required for the initiation and maintenance of the quality and quantity in spermatogenesis (Ohta et al. 2007). *FSH* is composed of alpha and beta subunits, which are coded by two distinct genes. The beta subunit offers specificity (Lin et al. 2006). Two genotypes have been observed for *FSHβ* with AA genotype being of higher frequency and having higher sperm concentration in buck's fresh semen (Nikbin et al. 2011). Dai et al. (2009) reported three genotypes for *FSHβ3* in bulls. Similar to the present results, they found an association between *FSHβ3* and sperm concentration. However, this gene showed no association with sperm motility in bulls. Zhang et al. (2011) reported exon 2 of *FSHβ* showed association with reproductive traits in female goats. Kumar et al. (1997) reported that *FSH*-deficient mice had seventy-five percent lower epidermal sperm number and forty percent lower sperm motility compared to normal mice. Based on the above, it may be confidently assumed that the *FSH* gene affects sperm quality. Nikbin et al. (2011) suggested that *FSHβ* might be considered as candidate genes for male reproduction performance.

Neuropeptide Y (NPY)

Neuropeptide Y (*NPY*) is a peptide chemical messenger secreted by the hypothalamus (Keisler et al. 1996), that portion of the brain that controls hunger, thirst, fatigue, and body temperature. The *NPY* plays a role in various basic processes in the brain, including energy regulation, memory formation, and seizure activity. The main effect of *NPY* is to promote increased food intake and decreased physical activity in re-

sponse to a plummeting blood sugar level. In addition to its function in feeding behavior, several other physiological roles have been assigned to *NPY*, including involvement in circadian rhythms, sexual function, anxiety responses, it increases the percentage of calories stored as fat and blocks pain receptor signals to the brain and vascular resistance (Erickson et al. 1996). It has been proved that *NPY*, as a neurotransmitter acts in the regulation of gonadotrophic hormone secretion and indirectly affects reproductive performance (Gladysz et al. 2003). The *NPY* were chosen as the candidate genes for semen quality traits in Boer goats by Nikbin et al. (2011). Single strand composition polymorphism (SSCP) detects sequence differences in alleles based on the altered mobility of single-stranded conformers. This molecular technique is widely used in animals to find association between genes and production traits in livestock (Chu et al. 2007). This was the first attempt made by Nikbin et al. (2011) to find polymorphism in *NPY* and to consider it as a candidate gene from reproductive traits of male goats. Tao et al. (2009) investigated the association of *NPY* with prolificacy of female goats, but they found no polymorphism in the *NPY* regions. Using a molecular marker for evaluation of semen characteristic of bucks could increase the genetic response to selection and reduce production cost by enabling early selection of high performing bucks.

CONCLUSION

A very limited number of genes and their respective proteins involved in buck reproduction have been identified, more search and detailed studies on these components will help understand and diagnose cases of infertility and/or subfertility that enhance the accuracy for prediction of male reproductive performance. The identification and using of candidate genes as molecular marker for evaluation of semen characteristic of bucks, has the potential to advance research related to male fertility, in both goats and other mammalian species and could increase the genetic response to selection and reduce production cost by enabling early selection of high performing bucks. There is a lack of studies investigating candidate genes for semen characteristics in goats. Therefore, there is immediate need to develop a battery of accurate fertility tests to distinguish poor fertility samples from those of high fertility. These tests have to be not only accurate but also economically feasible to perform. Promising new molecular tests may serve as useful supplements to tradition methods used in semen analysis. As more fertility tests are developed, the subsequent challenge will be to determine the most economical tests that are adequate to predict fertility accurately. A minimal number of simple inexpensive tests are highly desirable. These tests could be used to identify males or individual semen samples with poor fertility, and results may suggest therapeutic measures to improve fertility.

RECOMMENDATIONS

The assessment of breeding bucks based on semen quality is important and provides the guideline to buck evaluation for reproductive performance. There is also a need to develop and implement a molecular marker technique (genetic markers), which could therefore facilitate earlier prediction of fertility and would speed up genetic improvement programs.

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