Chapter

Characterisation of Semen and Phenotypic Parameters in Relation to Male Goat Fertility

Fhulufhelo Vincent Ramukhithi, Tlou Caswell Chokoe, Thomas Ronald and Khoboso Christina Lehloenya

Abstract

The following are aspects were discussed in this chapter: Domestication of goats – checking on when the goats were first domesticated and for what purposes; Current goat industry - looking on the contribution of goats economically; Conservation of domestic animal diversity - looking on the importance of conserving indigenous animals since some of them are under threat of extinction; Male goat fertility – discussing male fertility indicators; Characterisation approaches - different approaches to be followed when characterising indigenous species; and Phenotypic and genetic characterisation of indigenous goats.

Keywords: Phenotypes, characterisation, goat, semen, seminal plasma

1. Introduction

It has been estimated 28% of farm animal breeds became eroded or threatened in the past century and this figure is now expected to be escalating. Therefore, conservation of these goat breeds is important to protect their diversity [1, 2], because once it is lost it cannot be replaced [2]. Conservation of genetic diversity provides protection against diseases, feed shortages, selection errors, unforeseen disastrous actions, social and climatic changes. The main aim of conservation is to keep different genes as pure as possible and to keep special genes of value [3, 4]. For the conservation and improvement of indigenous animals, phenotypic and semen characterisation can be a first step to be undertaken before the use of their genetic material [5–7]. Such characterisation provides information on the reproductive performance and identification of unique characteristics within different populations [8]. In small ruminants, it has been found that there is a positive correlation between phenotypic characteristics and semen parameters. For example: testicular circumference, body weight and condition score of a male goat were positively correlated to high sperm cell concentration [9, 10]. On the other hand, with all the technologies available, there is little information on what parameters can be used as indicators of male fertility [11]. As a result, the search for other male fertility markers such as seminal plasma constituents is needed [12]. Studying of seminal plasma constituents can be another method of evaluating the reproductive system functioning and semen quality in goats [13]. In other species,

evaluation of seminal plasma constituents such as proteins, sodium, potassium, magnesium, calcium, zinc, glucose, cholesterol, triglycerides, lipids and urea were found to be useful in determining male fertility [14, 15]. These seminal plasma constituents play a major role during sperm cell metabolic processes [16, 17].

2. Materials and methods

2.1 Phenotypic characterisation

Phenotypic quantitative characteristics is assessed with the aid of a flexible tape measure. Individual body weight is determined by using a weighing scale. Rectal body temperature is determined using a thermometer. The phenotypic qualitative characteristics is assessed through visual appraisal. Body condition score is assessed based on standard scale which ranges from 1 to 5 (1 = very thin, 2 = thin, 3 = satisfactory, 4 = fat and 5 = obese). The age of the males is estimated by counting the number of permanent incisors on the lower jaw of the mouth [9].

2.2 Laboratory evaluation

2.2.1 Semen volume

Semen volume is measured by reading the measurements on the collection tube [18].

2.2.2 Semen pH

Semen pH is determined using a pH meter, whereby pH electro-rode is washed with sterile water and wiped with sterile paper towel before being inserted into the tube containing the semen sample for about 30 seconds [18].

2.2.3 Semen colour

Semen colour is observed visually and categorised as clear/watery, cloudy, milky, creamy and thick creamy [19, 20].

2.2.4 Sperm cell motility

Sperm cell motility is determined using a Sperm Class Analyser [21]. Five hundred microliters of Ham's F-10 and 10 μL of semen are mixed in a 1 mL graduated tube and incubated for 5 minutes at 37°C. After incubation, 10 μL of extended semen is placed on a pre-warmed microscopic slide (37°C), mounted with a cover slip and examined (x 10) under a phase contrast microscope [18]. Sperm cell motility is categorised as follows:

2.2.4.1 Progression (%)

Total motility (TM) = is a sum of progressive and non-progressive motility; Progressive motility (PM) = sperm cells that are moving forward; Non-progressive motility (NPM) = sperm cells that are not moving forward [22].

2.2.4.2 Velocity (%)

Rapid = sperm cells moving rapidly (81–180 μm/s), medium = sperm cells moving at an average speed between 51 and 80 μm/s and Slow = sperm cells moving slowly at ≤50 μm/s [22].

2.2.4.3 Average values of velocity parameters

Curvilinear velocity (VCL) = average velocity which measures a sperm cell movement along its actual path (μm/s); Straight-line velocity (VSL) = average velocity which measures a sperm cell movement along a straight line from beginning to the end (μm/s); Average path velocity (VAP) = average velocity of the smoothed cell path (μm/s); Linearity (LIN) = linearity movement is a ratio of VSL/VCL (%); Straightness (STR) = straight line movement is a ratio of VSL/VAP (%) [11, 23] and Wobble (WOB) = wavering movement which is a ratio VAP/VCL (%) [11, 22].

2.2.5 Sperm cell concentration

Sperm cell concentration is determined with a spectrophotometer. A square cuvette is filled with 3 mL of sodium citrate solution and placed in a spectrophotometer for at least 30 seconds. Raw semen (15 μL) is added in a square cuvette containing the sodium citrate solution, again placed in a spectrophotometer in order to read the absorbance. The absorbance read is used to determine the final sperm cell concentration with the aid of a formula (201 X 25.97 X absorbance – 0.3) [21].

2.2.6 Sperm cell viability, morphology and abnormalities

For evaluation of sperm cell viability, morphology and abnormalities, 200 sperm cells per slide are counted in a smear stained with nigrosin-eosin. Nigrosin-eosin stain (60 μL) and 6 μL of semen are mixed, then 5 μL of the mixture is smeared on a slide [23], whereby a dragging slide is placed at an angle of 45° and slowly moved into contact with the semen sample, which runs alongside the edge of the slide until it produces a smear. The smeared slides are dried and evaluated (counting of the sperm cells) under a fluorescent microscope using an oil immersion objective (x 100) on a bright field, with the aid of a laboratory counter. Live sperm cells do not absorb stain (fluorescence) while dead sperm cells do (become purple). Live sperm cells are further evaluated for morphology and abnormalities. Abnormalities are categorised as primary (small, large or swollen head, double heads, abnormal acrosome, elongated and abaxial mid-piece, double and short tail), secondary (detached, loose or damaged acrosomes, bent and protoplasmic droplets of the mid-piece, bent and shoe-hook tail) and tertiary abnormalities (reacted acrosomes and coiled tails) [23].

2.2.7 Sperm cell acrosome integrity

Sperm cell acrosome integrity is determined on the slides smeared and stained with nigrosin-eosin stain, under a fluorescent microscope, oil immersion objective and bright field (x 100). At least 200 sperm cells are counted per slide [24].

2.2.8 Sperm cell membrane integrity

Sperm cell membrane integrity is determined using a hypo-osmotic test. A semen volume of 0.1 mL is mixed with 1 mL hypo-osmotic solution and incubated

at 37°C for 1 hour. Following incubation, 7 μL of semen is placed on a glass slide, then smeared and evaluated under a phase contrast microscope (x 40), at least 200 sperm cells per slide are counted. Sperm cells with swollen and coiled tail are considered intact [25].

2.2.9 Determination of seminal plasma constituents

Seminal plasma is collected from semen samples by means of centrifugation [25] at 1500 rpm for 5 minutes. Following centrifugation, seminal plasma is removed using 1 mL disposable plastic pipettes. The seminal plasma is transferred into 5 mL centrifuge tubes and stored at −20°C until analysis. Determination of seminal plasma constituents is done using commercial *kits*. For the determination of sodium, potassium, magnesium, zinc and calcium; seminal plasma is centrifuged at 1500 rpm (4°C) for 20 minutes. Then 1 M sodium hydroxide is added to form a coloured salt complex. The concentration elements in the coloured complex solution is determined spectrophotometrically using a digital fluorescent microscope [26].

Determination of osteopontin, metalloproteinases type-2 tissue inhibitor, fertility associated antigen and urea is carried out by the addition of 100 μL of standards and sample solution into the appropriate wells, shaken gently to mix the contents and then incubated at 37°C for 1 hour. The wells are washed 7 X with 400 μL of wash solution. After the final wash, the wells are firmly tapped on a lint free paper towel to remove any remaining wash buffer. A labelled antibody (100 μL), is pipetted into each well. The plates is sealed and incubated at 4°C for 30 minutes. The plates is emptied of their contents and washed 9 X with 400 μL of wash solution. After the final wash, the plates are emptied and the results are generated by the addition of 100 μL of TMB substrate to each well. The plates are incubated for 30 minutes at 25°C in the dark, the reaction is stopped with 100 μL of stop solution, and the absorbance read at 450 nm [26]. Lactose dehydrogenase is determined by the catalytical oxidation of lactate to pyruvate in the presence of NAD with subsequent reduction to NADH. The rate of NADH formation measured at 340 nm is directly proportional to serum LDH-L activity. The change in absorbance measured at 340 nm is directly proportional to the activity of lactate dehydrogenase in the sample and translated to the concentration of lactose dehydrogenase in the sample. Lactate dehydrogenase reagent 1 (4 X 40 mL) and lactate dehydrogenase reagent 2 (4 X 8 mL) are reacted with the sample and enzyme activity measured spectrophotometrically (Lactic acid + NAD + → pyruvate + NADH) [26].

For the determination of triglycerides, a spectrophotometer is set at a wavelength of 540 nm and the absorbance reading to zero with water as the reference. Free glycerol and triglycerides reagents are prepared and warmed up to assay temperature in the blank, standard and sample cuvettes. Free glycerol and triglycerides reagents (0.8 mL) is pipetted into each cuvette. Then 10 mL of water, glycerol standard and sample are added into the blank, standard and sample cuvettes and mixed by gentle inversion. The cuvettes are then incubated for 5 minutes at 37°C. Initial absorbance reading of blank, standard and sample at 540 nm versus water as the reference is taken. To each cuvette 0.2 mL of the reconstituted triglycerides reagent is added, mixed and incubated for 5 minutes at 37°C [26]. The final absorbance of the cuvettes is read at 540 nm versus water as the reference and the triglycerides concentration is calculated. For the determination of lipocalin-type prostaglandin D synthase, seminal plasma is incubated overnight at 48°C with 30 mg of anti-recombinant bovine lipocalin-type PGD synthase IgG. Samples are washed 3 X with PBS-BSA and re-suspended to 500 mL FC blocking medium. Five microliters (0.5 mg/mL) of fluorescein isothiocyanate-conjugated goat anti-rabbit IgG Fab fragment is added to 500 mL of seminal plasma suspension for 1 hour. After being

washed 3 times in PBS-BSA as before, the fluorescence of the samples (concentrations) is measured using a digital fluorescent microscopy. For lipids determination, seminal plasma lipids are extracted with 6 volumes of chloroform-methanol (2/1, V/V), centrifuged at 800 x g for 3 minutes and the resulting lower phase aspirated and dried under a stream of nitrogen. Total lipids are determined enzymatically and their quantities were measured colorimetrically [26].

3. Domestication of goats

Goats were the first ruminants to be domesticated [27] and they have been kept since 6000 BC [28]. They originated from Asia [29]. The main reasons were for the production of milk and meat [27, 30]. Bezoar goats were reported to be the first to be domesticated in Asia before spreading over North Africa and Southern Europe. These goats were raised under very harsh environmental conditions. Goats are active, sure-footedness and have a habit of eating any kind of plant material [28]. This provides motivation for the genetic improvement for this species [31]. Globally, there are about 800 million goats, which represents 12% of global mammalian breeds recorded. About 70% of the world's goats are found in Asia, with the largest number in China, India and Pakistan. Less than 15% are found in Africa [32].

4. Current goat industry

Goats contribute significantly to the livelihoods of poor farmers [5, 27] and they are well preferred due to their natural adaptation, lower maintenance requirements [9] and short reproductive cycle, with a high reproduction rate compared to large ruminants [5]. In South Africa, there are over 6 million goats, and some of the breeds were commercialised and have gained international recognition; for example: Boer, Kalahari Red and Savanna goats. These goat breeds are known for their fast growth rate and good carcass quality [33, 34]. The South African goat industry is dominated by three main products: meat (chevon), milk and fibre. Goat meat production contributed R 3.6 billion to the South African economy between 2001 and 2011 [33].

Worldwide, chevon consumption has increased by 63% over the years as an alternative to beef, pork and chicken because of its high nutritional value, lower fat content, cholesterol composition and its leanness [9, 31, 35]. There is also an increasing trend of socio-economic importance of goats [36, 37] and demand for goat's meat exceeds what is available [31]. Therefore, to keep up with the increasing demand for goat meat, goats' productivity needs to be increased. In South Africa and the other developing countries, most goats are farmed traditionally and a very small proportion is kept commercially [37–39]. The productivity of livestock such as cattle, sheep and goats in South Africa and other African countries is low, due to a low reproductive rate and poor management practices [9, 37, 40].

5. Conservation of domestic animal diversity

Indigenous animals provide necessary genetic diversity to modern agriculture, as a means to ensure stability and improve future breeding programmes. However, indigenous animals are under threat of extinction [5, 41, 42]. Extinction or erosion of farm animal genetic resources is escalating due to environmental conditions, human interference and a combination of genes [4], which is due to an increase in uncontrolled breeding and the introduction of

exotic breeds [40]. Factors such as breed dependence, changes in production systems and producer's preference because of socio-economic factors, political unrest, natural disasters (drought), increased disease incidence, single productive trait emphasis and intensive use of biotechnology also contribute to erosion genetic diversity [41, 42]. It is in the interest of the entire community to conserve livestock genetic materials and maintain their biodiversity [4] because once the diversity is lost it cannot be recovered [2].

So far, 22 to 28% of farm animal breeds have been reported to be eroded [32, 42] with one to two breeds disappearing every week [41]. The following threats could be faced due to this erosion: high rate of cross bred animals, development of new genotypes, expansion of intensive agriculture, economy changes, decrease in market demand and food security, disappearance of cultural and historical values, loss of indigenous knowledge [43] and diseases outbreaks [32]. Therefore, conservation of farm animal genetic resources is important to protect their diversity [2]. Conservation of genetic diversity provides protection against climatic change, diseases, food shortages, social changes, selection errors and unforeseen disastrous actions [4]. The main aim of conservation is to keep pure genes and special genes of value [3, 4]. Adequate identification and documentation of animal breeds simplifies conservation of genetic resources. According to Cardellino [41], the Food and Agriculture Organisation requested its members to implement a conservation strategy for the sake of animal genetic resources preservation, which might not be of interest to farmers now. Currently, *in-situ* and *ex-situ* conservation strategies (**Figure 1**) are currently used for the conservation of animal genetic diversity [4, 32].

5.1 In-situ conservation

In-situ conservation refers to the maintenance of endangered or rare live animals in their natural habitat through the enhancement of its production characteristics (conservation with utilisation) [4, 32]. This type of conservation allows the attainment of important information about ecological or historical-cultural value of a breed [32, 42] and it includes performance recording and breeding programmes, with emphasis on the maintenance of genetic diversity within the breed [32].

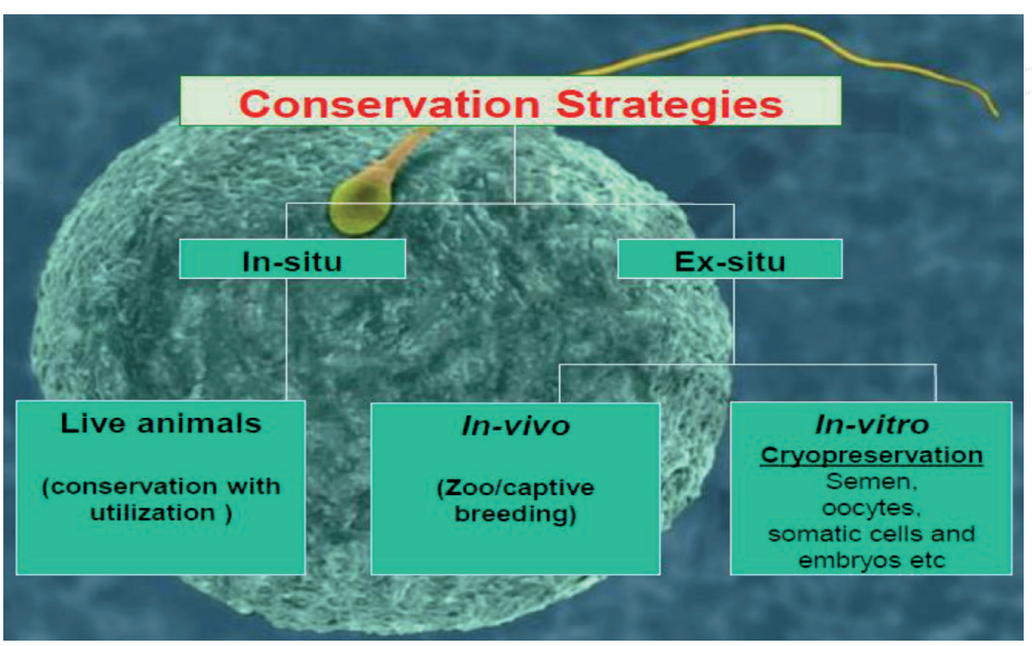

Figure 1.
Schematic representation of conservation strategies [44].

In-situ conservation would be the preferred approach for the short and long term, if there were maintenance and management of farm animal genetic resources (FAnGR). This is because in-situ conservation ensures that a breed is conserved in an active state [32, 43]. With the aid of appropriate genetic improvement techniques, the breed maintains its status to changes in the production, marketing and social environments. In general, conservation of FAnGR as live animals has many advantages compared to the in-vitro approach [43] such as enabling of breed development, adaptation to the environment, maintaining of indigenous knowledge to the keepers, sustainable utilisation in rural areas, contribution to nature management and financial viability [32].

5.2 Ex-situ conservation

Ex-situ conservation refers to the conservation of endangered or rare species in a designated area. This type of conservation strategy also plays a role in strategic breeding programmes [4, 43]. It protects against changes in the production conditions and helps with quick introduction of the breed with the aid of a limited number of recipients [32]. It comprises of in-vivo and in-vitro conservation. In-vivo conservation is the safeguarding of live animals in zoos, wildlife parks, experimental farms or any off-farm maintenance [4, 32, 43]. However, due to the high cost of this type of conservation, animals are usually kept in smaller numbers. It is therefore recommended that in-vivo conservation be complemented with in-vitro conservation. However, in-vivo conservation does not always give assurance of the preservation of the original genetic diversity of a breed, because animals are not preserved in their original habitat [32].

In-vitro conservation involves cryopreservation of genetic materials in haploid (semen, oocytes, somatic cells, testis and ovarian tissues) and diploid form (embryos) [4, 45]. This strategy was introduced because the in-vivo conservation strategy is costly. The cryopreserved genetic materials are used to regenerate a specific population in the future, even after the donors' death or can be used as a back-up [4, 43]. For the establishment of reinforced ex-situ conservation programmes: conservation priorities and goals need to be set, national and regional ex-situ conservation facilities need to be established, the use of genetic materials stored in ex-situ gene banks need to be facilitated, there should be a strict biosecurity measures and procedures must be followed for maintenance of cryo gene banks. Implementation of long-term regional and global conservation strategies is required for saving costs and avoiding duplication of conserved genetic materials [4].

6. Male goat fertility

Fertility is a complex term measured by conception rate [11], number of services, semen parameters, litter size, kidding interval, non-return and kidding rate. Fertility in male animals is an important characteristic that is used to select sires and it is more economically important than other production characteristics [46]. Potential fertility of mature males is a characteristic that has been evaluated based on phenotypic characteristics and it increases the probability of reproductive success during breeding season [39, 47]. Like in any other species, sub-fertile males prolong the kidding season due to delayed pregnancy, which will then lead to culling of does and loss of kids [11, 47, 48]. Male fertility is influenced by development of the testis, seminal plasma quality, libido, physical soundness [11] and the ability of sperm cells to penetrate the zona pellucida of the ova [49]. Therefore, it is important to manage male animals well in order to optimise their breeding performance [48].

6.1 Indicators for male goat fertility

Following physical examination and assessment of males' reproductive organs, semen samples needs to be collected. This is achieved by collecting semen samples with the aid of an approved artificial vagina or electro ejaculation method [50, 51]. Following collection, the semen samples are evaluated for semen volume, pH, sperm cell motility [52], semen colour [53], sperm cell concentration, acrosome and membrane integrity [54], live/dead and morphology [55]. Semen parameters evaluation is an accurate, objective, rapid, inexpensive and standard method of indicating fertility of breeding males, other than directly assessing their ability to make females pregnant [49, 52, 56].

6.1.1 Semen colour

Semen colour is evaluated subjectively by visual observation. It can also be used to predict sperm cell concentration [49]. Clear, cloudy, milky, thin-creamy, creamy and thick-creamy semen colours with average sperm cell concentration of <0.7, 0.7, 2.0, 3.0, 4.0 and 5.0 X 10^9/mL, respectively, are normally observed following semen collection. Semen samples with colour that ranges from milky to thick creamy were found to be good and highly fertile [49, 57]. Sometimes, pink, grey or brown semen colour can be observed because of blood presence, which can be due to injury of the penis or reproductive tract or diseases and infections. If there is urine contamination, the sample will be yellowish in colour, and such semen needs to be discarded, as it will affect sperm cell quality [49].

6.1.2 Semen volume

Semen volume is evaluated by taking the reading on the collecting tube [58] and varies according to the method of semen collection used [49]. Acceptable semen volume of good quality sample ranges from 0.5 to 3 mL, during the natural breeding season [59, 60]. Use of the electro-ejaculator for semen collection resulted in high semen volume when compared to the use of artificial vagina [49]. This was reported to be due to excessive accessory sex gland secretions and urine [61]. A good correlation was observed between animals' age and testicular size; young males produce lower semen volume when compared to old males [62]. Smaller testis produces lower semen volume when compared to bigger testis, and this is attributable to differences in semen production and storage capacity [63].

6.1.3 Sperm cell motility

Sperm cell motility is an essential parameter in the assessment of semen quality and different results have been reported on its effect on male fertility [64, 65]. Sperm cell motility can be determined using two methods, which are subjective and objective assessment [11, 53]. Subjective assessment involves the estimation of sperm cell motility and relies on the training and experience of the observer [53, 66]. Objective assessment mainly involves the use of a computer assisted sperm analyser to analyse sperm cell motility [11, 65]. Goat semen is regarded as good if it has a sperm cell motility of more than 70% [59, 67].

6.1.4 Sperm cell morphology

According to Hashida et al. [56], sperm cell morphology has a number of benefits, as one of the predictive factors in determining the positive outcomes of fertilisation.

A minimum of 70% normal sperm cells are needed for fertilisation to take place [68]. The main sperm cell abnormalities that hinder male fertility in livestock are: small, large or double heads, abnormal, detached, reacted or damaged acrosome, elongated and abaxial mid-piece, double, short, bent, shoe-hook and coiled tail, bent and protoplasmic droplets of the mid-piece [49, 55]. Diseases and stress, as a result of temperature, are the main contributors to high percentages of damaged sperm cells [53]. High ambient temperature and humidity may reduce males' fertility by 6 weeks, and during that period a high rate of abnormal sperm cells appears in the collected ejaculates during the recovery period. During the non-breeding season, abnormalities increase then they decline during the breeding season [49].

6.1.5 Sperm cell viability

Dead sperm cells lead to complete failure of fertilisation. For fertilisation to take place, goat semen samples should have less than 25% dead sperm cells [57, 63]. Temperature levels and infections are the main causes of dead sperm cells in goats [63, 69].

6.1.6 Sperm cell concentration

Males with acceptable semen quality should contain sperm cell concentration of $\geq 2 \text{ X } 10^9$ sperm cells/mL [59]. Sperm cell concentration is influenced by different factors such as age, breed, nutrients supply, climatic conditions [36], season, testicle size, semen collection frequency and method [63]. During the breeding season, sperm cell concentration increases [39, 63]. Larger testicles produce more highly concentrated sperm cells due to bigger site of production and capacity compared to smaller testicles [63]. The artificial vagina results in higher sperm cell concentration when compared to the electro-ejaculation method [70]. Excessive application of electric stimuli increases semen volume, due to unwanted secretions, which then leads to lower sperm cell concentration and dead sperm cells [63, 71].

6.1.7 Semen pH

Generally, semen pH of 7.0 to 7.2 has been reported as the best for optimum functioning of most of the enzymes in sperm cells [49], which is in turn favourable for sperm cell motility and viability [72]. Acidic and alkaline semen pH have been observed to be unfavourable for sperm cells to survive and leads to sperm cell damage and low fertilising ability [49, 71, 73]. Ramukhithi [52] also found low semen pH levels of 6.1 and 6.6 to result in total motility of 89.3 ± 1.8 and 76.9 ± 4.7%, respectively.

6.2 Characterisation or breeding soundness evaluation of male goat

Characterisation provides information on the reproductive performance, and identification of unique characteristics of value in different populations [8]. For optimum goat productivity, the farmers should select males that have the ability to serve many females during the breeding season, with the genetic potential for quick and efficient growth [73]. However, the importance of breeding with a highly fertile male is often neglected, especially under the communal set up. This is due to lack of management practices such as breeding soundness evaluation that needs to be done on males before using them for breeding. As a result, the pregnancy rate is compromised [74]. Breeding soundness evaluation is a useful tool to be used during the selection of males for breeding; this eliminates males with lower fertility [11, 30, 36, 73].

Breeding soundness evaluation consists of physical examination of body structure and reproductive organs, semen collection and evaluation, which were found to be the best indicators of breeding potential [30, 47, 73]. Three classes are used to rate breeding soundness, namely: satisfactory potential breeder, unsatisfactory potential breeder and deferred. A satisfactory potential breeder has good physical characteristics: ≥ 17 cm scrotal circumference, ≥ 70% sperm cell motility and normal morphology [11, 59, 73, 75]. Males failing breeding soundness evaluation (deferred breeder) can be rechecked after 1 or 2 months, to see if their fertility has improved [36, 47, 48, 73]. In a herd, about 15% of males kept are of unsatisfactory reproductive breeding quality [48, 73]. Although the method is accepted and highly utilised, it is very subjective and requires a highly trained technician [11].

6.3 Characterisation approaches

6.3.1 Exploratory approach

This approach is undertaken in a situation where there is very little information on the existence of recognised breeds, with the objective to examine the existence of distinct breeds in the study area. It is hypothesised that the target animal genetic resources (AnGR) population is the same and the hypothesis is tested by measuring and analysing the pattern of phenotypic diversity in the study area. This is followed by choosing the study area and sampling frame. For a large area, it is advisable to use stratified sampling based on the following criteria:

i. Geographical isolation of AnGR populations and their movement patterns;

ii. Known patterns of morphological and production characteristics in the AnGR populations;

iii. Indigenous knowledge on the origin of the AnGR

This approach also requires estimation of livestock populations and their keepers in the study area and primary characterisation falls within the exploratory approach [76].

6.3.2 Confirmatory approach

This approach is undertaken in a situation where there is little information on the breed identity. In such a case, the objective is to confirm breed identity and provide descriptions of the breeds, which depends on the national AnGR records, literature and local knowledge. In a situation where there is little information, which is not accurate for phenotypic characterisation, preliminary field data will need to be collected on the identity, geographical distribution, and relative significance of AnGR populations and hence to determine whether an exploratory or confirmatory approach is suitable [76].

7. Phenotypic characterisation (body assessment)

Animal phenotypic characterisation is a practice of documenting the physical appearance or characteristics of an animal. The information provided by phenotypic characterisation studies is crucial for planning management and the use of AnGR at local, national, regional and global levels [5, 76, 77]. This type of

characterisation is simple, non-invasive and inexpensive [2]. For the conservation and improvement of indigenous animals, phenotypic characterisation can be a first step to be undertaken before the use of their genetic material [6, 7]. It is necessary to guide decision makers in the development and breeding programmes of livestock [76]. Phenotypic characteristics have important socio-cultural and economic values to African people. As a result, most farmers have specific respect and choices for specific traits. For example: goat coat colours and body sizes are more favourable to the farmers [5]. Phenotypic characteristics are influenced by genetic and environmental factors such as nutrition, health practices and ambient temperature etc. [8]. The phenotypic characterisation tool gathers information on production environment, qualitative, quantitative and adaptive traits [77].

Evaluation of AnGR diversity is difficult because there are many animal populations that are not assigned to any known or registered breed. Even though some of these non-descriptive animals are known to be crosses of existing breeds, some animals may belong to the same population, which is unique from known populations on the basis of identifiable and phenotypic characteristics that need to be identified as separate breeds. Phenotypic characterisation is technically and logistically challenging. In order for it to be implemented in an efficient and cost-effective manner, it requires careful attention. For accurate phenotypic characterisation results, it is advisable to use standard practices and formats for describing animals' characteristics [76]. There are two types of characterisation; the first one is primary characterisation, which refers to the collection of information through single field visits. For example: measurement of animals' morphological features, gathering information from livestock keepers and mapping of geographical features. The second one is advanced characterisation, which refers to activities that require repeated visits. For example: measurement of productive traits such as growth rate, milk production and adaptive traits such as resistance to diseases [76].

7.1 Phenotypic qualitative characteristics

These characteristics include the external physical form, shape, colour and appearance of the animals, and are recorded as categorical variables. Phenotypic qualitative characteristics are based on a small number of genes. Some of the examples of phenotypic qualitative characteristics are coat colour, horn shape and ear length etc. [5, 8, 36]. These characteristics have less direct significance to the production and service functions of an animal. However, they may relate to adaptation to a specific environment. For example: hair coat, size of ears and presence of horns are known to be relevant to the dissipation of excess body heat [5, 76]. Other characteristics that may be relevant to the livestock keepers is hair coat colour. This is normally used in a situation where there is no proper identification of animals; some farmers use hair coat colour as an animal identification tool. As a result, they are as important as the phenotypic quantitative characteristics and hence they need to be included on the phenotypic characterisation studies [5, 76].

7.2 Phenotypic quantitative characteristics

Phenotypic quantitative characteristics are measures of animal body parts [34, 76] and are more directly associated to production characteristics when compared to phenotypic qualitative characteristics. For example; body weight and chest girth are directly associated to body size and production characteristics [5, 9]. These variables have continuous expression, due to numerous genes that influence their expression. Most of the phenotypic quantitative characteristics are dependent on animal age and the environment in which they are kept. Phenotypic quantitative

characteristics such as body weight, length and height, are used as an alternative indicator of production traits due to their strong relationship with production traits such as meat and milk [76].

7.3 Phenotypic characteristics correlated to male goat fertility

To evaluate the ability of a male to find, move to and mount a female on heat, a physical inspection is conducted. This includes observation and palpation of the penis, prepuce, sheath, testicles and epididymis, and measuring of the scrotal circumference [9, 73]. The main characteristics that are evaluated are: body condition score and structural soundness of the male. In addition, male history, age, temperature and reviewing the records of past breeding performance need to be done [9, 36].

7.3.1 Body condition score

Body condition scoring is an assessment of body fat, and is determined by feeling the ribs and spine of a male, as well as a visual assessment [9, 36, 73, 76]. The good thing about body condition scoring is that it is fast, simple and cheap, as it does not require special instruments [38]. Animal body condition is influenced by environmental factors, parasites and diseases etc. [78]. Normally, body condition score in males is assessed before mating, while in does it is assessed before mating, kidding and during lactation stage. This method involves allocation of scores to animals in relation to the amount of body fat and muscles. It is a rapid and economical method that shows energy reserves of the animals [38, 79]. A body condition score is assessed based on an arbitrary scale which ranges from 1 to 5, where 1 is very thin and 5 is obese [9, 38]. Body condition score of 3 to 3.5 is recommended for a male during the breeding season [9, 36, 73]. If a male is too thin, its breeding ability is negatively affected. On the other hand, overweight males may lack strength to breed large numbers of does [73]. Body condition monitoring is very important to minimise reproductive and productive losses [79].

7.3.2 Age

The optimum breeding age of a buck ranges from 6 months to 4 years. An increase in spermatogenic activities at a certain age results from a major development of the seminiferous tubules and sertoli cells differentiation [9, 36]. Age has an effect on testicular size. Old age arrives in males at different ages, depending on the health, environment and the use. Most old animals have adequate sperm cells, but most of them are abnormal and dead [49]. The best prediction of how long a male will be productive is to evaluate bloodlines [36].

7.3.3 Body weight

Body weight is an important economic trait in animal [9, 36] and it is influenced by several factors such as breed, age, nutrition and other environmental factors [30]. Body weight and size of a male are influenced by feeding and health care. Body weight can be estimated with the aid of body measurements, especially in the villages where there are no scales. It can also be determined objectively, with the aid of a weighing scale [36] or by visual appraisal [9]. Objective assessment enables farmers to recognise early and late maturing animals of different sizes. Body weight has a close relationship with age, breed and morphological characteristics. Sperm

cell concentration has also been shown to be positively correlated with body weight. It can be concluded that age and body condition of a male have a strong influence on body weight and conformation traits [36]. Body weight is also positively correlated to the production of viable sperm cells [9].

7.3.4 Body weight

Body size is an important phenotypic characteristic in meat producing animals [9]. Previously in goats, body size used to be visually assessed with a subjective method. Nowadays, body growth and development are objectively assessed. Delayed growth in body size of an animal leads to reproductive wastage and economic losses [36]. Goats are classified as dwarf (< 50 cm), small (51 to 65 cm) and large-sized animals (> 65 cm) based on their body height at the withers [5]. Large-sized animals are heavier and have bigger testicular measurements than small-sized animals [62]. Even though the body and testicular measurements of animals increase with age, the age at which domestic animals reach puberty in a commercial set up is postponed until they attain a required body size and weight [36].

7.3.5 Scrotal circumference

Scrotal circumference is determined at the widest part of the testis, when the testis is gently massaged and pulled to the bottom of the scrotum [9, 48, 73]. Scrotal circumference is known to be different among different breeds and individuals of the same breed, and it is highly heritable [11, 36]. An average of 17 and 25 cm of scrotal circumference in young and older males, respectively, is recommended to be ideal for breeding [73, 80]. Scrotal circumference varies with the season and body condition, and it is usually larger during the breeding season [39, 73] and can decrease by 2 to 3 cm during the non-breeding season [73]. Goat scrotal circumference also experiences some changes after reaching sexual maturity due to the influence of photoperiod, nutrition and temperature [30, 36].

Measuring of scrotal circumference during breeding soundness evaluation is very important, as it is strongly related to the semen production capacity of a male [9, 49, 73]. Breeders put great selection pressure on larger scrotal circumference [11, 36]. Large scrotal circumference is a reliable indicator of the reproductive maturity, good development of sperm cells and production of semen with greater quality [9, 11, 73]. When artificial insemination is to be conducted, semen samples from superior males with larger scrotal circumference could lead to insemination of many females. Testicular size is also influenced by breed, age, nutrition, genetics and other environmental factors [11, 30, 36]. Large-sized animals are heavier and have larger testicles measurements than small-sized animals [11, 62]. Large scrotal circumference was reported to be correlated with good semen quality and high sperm cell production in males [11].

7.3.6 Skeletal dimensions

Body height, length, depth, width, pelvic width, length hock length, tail length, heart girth and scrotal circumference are some of the characteristics that are measured in goats [34]. These characteristics have a good relationship with each other. For example, heart girth and body height are good indicators of body weight and condition score. Scrotal circumference is an indirect indicator of the testicular mass. It is a major element in breeding soundness evaluation, because it is easy to measure and reliable, as it provides an indication of size and growth [36].

8. Seminal plasma constituents and their relationship to male goat fertility

In many cases, female goats (does) are linked to flock infertility problems than males. Male fertility cannot however be taken for granted as males have a greater influence on a flock performance compared to females. Evaluation of males for breeding purposes is very important in the improvement of goat production as they supply half of the genetics to all the offspring [9, 48].

8.1 Seminal plasma proteins

Seminal plasma proteins have a great effect on the biological quality of semen samples as expressed by sperm cell motility, viability and morphology [81]. Evaluation of seminal plasma proteins were found to be useful in correlating male fertility in other species such as cattle [14], pigs [82] and fish [83]. Seminal plasma proteins are composed of non-protein nitrogen amino acids, peptides albumin, globulin [69] and other inorganic constituents that have effects on sperm cell quality [82]. Utilisation of two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) resulted in the recovery of four proteins in the seminal plasma, which were correlated positively to male fertility [14]. The functions of these proteins are to prolong sperm cell viability measured by its motility, protect sperm cells, supply enzymes of the metabolic processes [69], improve buffering capacity and semen quality [81, 84, 85], capacitation and acrosome reaction [86]. As a result, proteins are regarded as a male fertility marker in conjunction with standard breeding soundness evaluations [14, 47, 85]. However, the accurate mechanisms involved in their actions are not clear [86, 87].

According to Bozkurt et al. [83], protein concentration is normally low in most of the species and it decreases in the second phase of spermiation. Although protein concentration is normally low, it is normally higher in summer or during the breeding season, when compared to the other seasons. Low protein concentration in seminal plasma reduces its buffering capacity and negatively affects sperm cell characteristics [69]. Seminal plasma proteins were firstly associated with male fertility in dairy bulls, followed by beef bulls and horses. The first seminal plasma proteins to be associated with males' fertility in Holstein bulls were: lipocalin-type prostaglandin D synthase (LDH), osteopontin (OPN), albumin, transferrin, fertility associated antigen (FAA) and type-2 tissue inhibitor of metalloproteinases (TIMP-2) [14, 82, 86, 88].

8.1.1 Osteopontin

Osteopontin is an acidic protein and comes from mineralised bone tissue, which is rich in serine, aspartic, and glutamic acid [12, 89]. It is also known as secreted phosphoprotein-1, 2ar and bone sialoprotein [42]. Osteopontin belongs to a family of proteins called small integrin-binding ligand, N linked glycoprotein (SIBLINGS) [12]. Osteopontin (55-kDa) plays an indirect role in increasing fertility by actions in the male reproductive tract, without directly affecting sperm cells [47, 89]. In male animals, it is synthesised by sertoli and germ cells in the seminiferous tubules and expressed in the ampullae, seminal vesicles and epididymis. Its functions are for cell relocation, survival and adhesion, chemotaxis, intra-cellular signalling, macrophage activation and prevention of calcium crystal formation in the kidney [12, 14].

8.1.2 Lipocalin type prostaglandin D-synthase

Lipocalin type prostaglandin D-synthase was firstly identified in the cerebrospinal fluid in the early 1960s. This protein is abundant in compartments

beyond blood tissue barriers [90]. It is found in a large and different group of small extracellular proteins identified by their ability to bind hydrophobic molecules (retinoids, steroids, pheromones, odorants and fatty acids) [91]. Lipocalin-type prostaglandin D synthase has been reported to be more dominant in the seminal plasma of highly fertile bulls. It is composed by a series of four spots, which comprise 7.6% of all protein spots. In males with low fertility, they were found to be 2 to 3-folds. Lipocalin-type prostaglandin D synthase is a multifunctional calcium-binding protein, which is responsible for intracellular signalling pathways, interaction of cells, apoptosis [86, 92] and serves as a carrier for blood-derived substances across the blood-testis barrier, which is produced in the seminal plasma [47]. It is also responsible for sperm cell development [90]. Although, these proteins were reported to correlate with sperm cell quality or male fertility in several species, their mechanisms are not clearly understood [86, 92].

8.1.3 Fertility associated antigen

According to Dawson et al. [14], fertility associated antigen is a simple and non-glycosylated yielding a N-terminal 26 amino acid sequence which is 73% similar to human deoxyribonuclease (DNase) I-like protein [47, 82]. In total, fertility associated antigen is composed of 296 amino acids [93]. It is primarily located in the seminal vesicles and prostate glands. It was also labelled as one of fertility markers (26-kDa) for bull semen [47, 82]. For rapid determination of fertility-associated antigen in bulls, a lateral flow cassette has been designed [94]. Bulls that tested positive for fertility-associated antigen resulted in a higher pregnancy rate when compared to those that tested negative [14, 47, 94]. Ax et al. [93], summarised the findings of Bellin (1994, 1996 and 1998) which showed that pregnancy rate was 85 and 66%, when the fertility-associated antigen was present and absent, respectively. Dawson et al. [14], summarised the findings of Bellin (1994, 1996, 1998) and Sprott (2000), which indicated the pregnancy rate was 82.1 and 64.8%, when the fertility-associated antigen was present and absent, respectively. This was believed to be due to a lack of epitope [14, 47].

In South African unimproved indigenous male goats' fertility associated antigen showed a positive relationship with semen volume, sperm cell concentration, TM, NPM, rapid and medium velocity, VCL, VSL, VAP, intact membrane and live sperm cells [89]. Ramukhithi [89] results are in agreement with the literature that has indicated that when the fertility associated antigen is present in semen, the fertility level of that male is high [86, 93]. Contradictory, when South African unimproved indigenous and Tankwa goats' were used, fertility associated antigen did not have a positive relationship with TM and static sperm cells. Due to lack of a relationship, the importance of male fertility associated antigen in the sperm cell motility remains unclear [89].

8.1.4 Type-2 tissue inhibitor of metalloproteinases

Tissue-inhibitor of metalloproteinases are present at the same time as the matrix proteases [95] and it is another 24 kDa protein that has been isolated from accessory sex glands (bulbourethral gland, prostate and seminal vesicles) and found to be positively relevant in correlating bulls' fertility [14, 47]. However, their roles and mechanisms are still not clear, and are under investigation [14, 87]. On the other hand, they were assumed to provide energy and protect sperm cells as a complementary substance [95]. Like with fertility-associated antigen, bulls that tested positive for type-2 tissue inhibitor of metalloproteinases resulted in higher pregnancy rate when compared to bulls that tested negative [14].

8.1.5 Lactate dehydrogenase

Lactate dehydrogenase is an abundant and intracellular enzyme, which displays different kinetic parameters. Its roles are sperm cell metabolism, capacitation and fertilisation [16, 95, 96]. This enzyme penetrates the cell to generate a temporary oxygen in the form of accumulated lactate, which is later removed by the reoxidation of lactate [96]. Increased levels of lactate dehydrogenase in the seminal plasma may be an indication of good quality sperm cell membrane, acrosome integrity [95] and viability [88].

8.2 Other seminal plasma constituents

In addition to seminal proteins, other seminal plasma constituents have a great effect on the biological quality of semen samples as expressed by sperm cell motility, viability and morphology [81]. In other species, evaluation of sodium, potassium, magnesium, calcium, zinc, glucose, cholesterol, triglycerides, lipids and urea were found to be useful in the correlation of male fertility [15, 69]. These seminal plasma constituents play a huge role during sperm cells metabolic processes [16, 17] and ensure that the sperm cells are viable. Evaluation of seminal plasma constituents can be more useful during the preparation of species-specific diluents for short and long-term preservation of semen [81].

8.2.1 Sodium

Sodium is a soft, silver-white coloured and highly reactive metal. It is important for both plants and animals. Sodium is a major cation in the extracellular fluid and a major contributor to osmotic pressure. It is present in seminal plasma at a higher concentration than other seminal plasma constituents [97]. Sodium improves sperm cell motility [15] and it helps with the establishment of sperm cell osmotic balance [83, 95]. Low sodium concentration is associated with low sperm cell motility and may be caused by deficiency in the formation of seminal plasma [64].

8.2.2 Magnesium

Magnesium is the second most common intracellular cation after potassium [98]. It plays a role in enzyme activation [97, 99], energy metabolism, cardiac excitability, muscle contraction, synthesis of nucleic acids and relaxation of male reproductive muscles to delay ejaculation. In humans, a high magnesium concentration was found in the prostate gland and is released into seminal fluid [97]. In a normal situation, seminal magnesium is more than 70 mg/L while in blood serum it is 17–24 mg/L. Low level of magnesium cause premature ejaculation and erectile dysfunction [98]. However, if its concentration is too high it tends to affect sperm cell motility negatively [81]. When it is too low, it leads to disorders in male fertility [97]. There is a good relationship between low concentration of magnesium and human age [98]. In non-identified goat semen, 8.1 mg/dL magnesium with acceptable semen volume, sperm cell motility, concentration and normal morphology were detected [100]. In Granadina goat semen, 1.9 ± 0.3 mg/dL magnesium with unacceptable sperm cell motility, live sperm cells and acceptable semen volume and sperm cell concentration were detected [74].

8.2.3 Glucose

Glucose is a carbohydrate and an important simple sugar in animal metabolism. In humans, its normal concentration in the blood stream is 0.1%. This primary

molecule serves as an energy source for plants and animals [101]. Glucose also acts as the main energy source for sperm cell metabolism and provides osmotic balance [81, 84]. In the seminal plasma, glucose has been associated with the high-energy demand of the testis during spermatogenesis and lipids synthesis of sperm cells [81].

8.2.4 Calcium

Calcium is a soft greyish alkaline metal and the, which is fifth most-abundant element by its mass, and it is essential for all living organisms. Calcium contributes significantly to the ionic composition of a seminal plasma [83]. It is also important for sperm cell motility, physiology and acrosome reaction, where by the movement of calcium into and out of the cytoplasm serves as a signal for cellular processes [97, 102]. It is also responsible for muscle contraction of the vas deferens and corpus cavernosum [98]. However, the role of seminal calcium in sperm cell motility is not fully understood, as it inhibits acrosome reaction and at the same time has no effect on sperm cell motility [103]. In non-defined goat semen, 12.1 ± 0.6 mg/dL calcium with acceptable semen volume, sperm cell concentration, motility and normal morphology were detected [100]. On the other hand, in Granadina goat semen, 10.8 ± 1.86 mg/dL calcium with unacceptable sperm cell motility (63.5 ± 18%) and live sperm cells (69.8 ± 14.2%) were detected. However, the semen volume and sperm cell concentration were acceptable [74].

8.2.5 Potassium

Potassium is found in seminal plasma at high concentration when compared to other seminal plasma constituents [81]. It is for osmotic balance [83, 95]. The inhibition of sperm cell motility by potassium can be overcome by increasing the external calcium concentration. Sperm cell motility of *Salmo trutta* macro stigma was reported to increase with calcium and magnesium levels [83].

8.2.6 Zinc

Zinc is an essential mineral for domestic animals and has antioxidative properties [15, 97]. It is responsible for testicular and sperm cell development [15, 101], it stabilises membrane and nuclear chromatin of sperm cells. Shortage of zinc can damage the mechanism of DNA and make the sperm cells susceptible to oxidative damage. Zinc levels in mammalian semen are high and it has been found to be important for the development of sperm cells. When the zinc level decreases, sperm cell quality also decreases, which then leads to reduced chances of fertilisation [97, 103]. In Granadina goat semen, 1.2 ± 0.6 mg/dL of zinc with acceptable semen volume and sperm cell concentration was detected. However, unacceptable sperm motility and live sperm cells were also detected [74].

8.2.7 Cholesterol

Cholesterol is the precursor in the biosynthesis of sex hormones. Thyroid hormones stimulate cholesterol production and hepatic mechanisms that remove cholesterol from circulation. Seminal plasma cholesterol is higher during summer than in the spring season [69]. Like in the lipids, there is little information about the role of cholesterol in the sperm cells, but it has been assumed that it protects the sperm cells during temperature changes [81] and it is responsible for sperm cell capacitation [102]. According to Mellado et al. [74], there is a good relationship between blood cholesterol, body condition and level of nutrition. Blood cholesterol

increases with increased levels of nutrition during growth, which also have an effect on seminal plasma cholesterol levels. In rabbits, cholesterol was reported to contribute to infertility during the summer season, which might be due to the changes in biological functions caused by heat stress [69]. In Granadina goat semen, 80.5 ± 12.1 mg/dL cholesterol with unacceptable sperm cell motility (63.5 ± 18%) and live sperm cells (69.8 ± 14.2%) were detected. However, the semen volume and sperm cell concentration were acceptable [74].

8.2.8 Triglycerides

Triglycerides is a chemical compound derived from glycerol and three fatty acids. There are many triglycerides, some are highly unsaturated and some are less saturated. Triglycerides increases membrane fluidity [84] and provides energy to the sperm cells during regeneration after moving. Low levels of triglycerides are indicative of inadequate energy supply, which leads to low sperm cell motility and fertilisation capacity [64, 81].

8.2.9 Lipids

Lipids are naturally occurring molecules such as fats, waxes, sterols, fat-soluble vitamins (vitamin A, D, E and K), monoglycerides, diglycerides, triglycerides and phospholipids. They can be found in the whole cell, plasma membrane and head membrane [104]. There are different classes and levels of lipids in different species [81]. They were found to occur at higher levels in spring than in the summer season [69]. The goat sperm cell plasma membrane was found to be rich in phosphatidylcholine and phosphatidylethanolamine lipids. However, phosphatidylethanolamine lipids decrease during epididymal maturation of sperm cells [104]. Seminal plasma lipids play a role in sperm cell metabolism and capacitation, and it maintains membrane structure [105]. Lipid concentration has a good correlation with sperm cell concentration and motility. A decrease in seminal plasma lipids leads to a reduction in sperm cell concentration and motility [69, 105]. The changes in the lipids quantity and composition of sperm cell plasma membrane during maturation are believed to explain why ejaculated sperm cells are more sensitive to cold shock than testicular sperm cells [104]. In Granadina goat semen, 8.1 U/mL lipids with acceptable semen volume, sperm cell motility, concentration and normal morphology were detected [100].

8.2.10 Urea

Urea is an organic chemical compound, which is produced by the body after protein metabolism. It plays a role in the metabolism of nitrogen-containing compounds by animals. It is also responsible for sperm cell development [15, 101]. In Granadina goat semen, 19.7 ± 4.7 mg/dL urea with unacceptable sperm cell motility (63.5 ± 18%) and live sperm cells (69.8 ± 14.2%) were detected. However, the semen volume and sperm cell concentration were acceptable [74].

9. Conclusion

Collection of reproductive information from different indigenous goats to assist with future breeding plans and maintaining unique phenotypic characteristics and semen parameters of goat breeds is important. Evaluation of semen parameters is reliable and standard method of indicating fertility of breeding males, other

than directly assessing their ability to make females pregnant. However, evaluation of seminal plasma constituents and phenotypic characteristics, and their relationship to male fertility is still not well defined in smallstock. As a result, the intensive investigation of male fertility markers such as seminal plasma constituents is needed, as this can be another reliable method of evaluating the reproductive system functioning and semen quality in male goats.

Author details

Fhulufhelo Vincent Ramukhithi[1*], Tlou Caswell Chokoe[2], Thomas Ronald[1] and Khoboso Christina Lehloenya[3]

1 Agricultural Research Council, Pretoria, South Africa

2 Department of Agriculture, Land Reform and Rural Development, Pretoria, South Africa

3 University of Zululand, Empangeni, South Africa

*Address all correspondence to: ramukhithif@arc.agric.za

References

[1] Vallecillo A, Trigo PI, Delgago JV, Cabello A, Santos E, Tenorio, T. Effects of cryopreservation on sperm motility in Blanca Serrana Andaluza goat. South African Journal of Animal Sciences. 2004;34:116-118.

[2] Manzi M, Rutagwenda T, Kanuya N, Chatikobo P. Phenotypic characterization of goats raised under traditional husbandry systems in Bugesera and Nyagatare districts of Rwanda. Journal of Animal and Veterinary advances. 2011;10(24):3297-3302. DOI: 10.3923/javaa.2011.3297.3302

[3] Esquivelzeta C, Fina M, Bach R, Madruga C, Caja G, Casellas J, Piedrafita J. Morphological analysis and subpopulation characterization of Ripollesa sheep breed. Animal Genetic Resources. 2011;49:9-17. DOI: 10.1017/S2078633611000063

[4] Mara L, Casu S, Carta A, Dattena M. Cryobanking of farm animal gametes and embryos as a means of conserving livestock genetics. Animal Reproduction Science. 2013;138:25-38. DOI: 1016/j.anireprosci.2013.02.006

[5] Hassen H, Baum M, Rischkowsky B, Tibbo M. Phenotypic characterization of Ethiopian indigenous goat populations. African Journal of Biotechnology. 2012; 11(73):13838-13846. DOI: 10.5897/AJB12.2092

[6] Kunene N, Nesamvuni EA, Fossey A. Characterisation of Zulu (Nguni) sheep using linear body measurements and some environmental factors affecting these measurements. South African Journal of Animal Science. 2007;37(1):11-20. DOI: 10.4314/sajas.v37i1.4020

[7] Yakubu A, Raji AO, Omeje JN. Genetic and phenotypic differentiation of qualitative traits in Nigerian indigenous goat and sheep populations. Asian Research Publishing Network. 2010;5(2):58-66. DOI: 10.1.1.610.3565

[8] Yami A, Merkel RC. Sheep and goat production handbook for Ethiopia: Ethiopia sheep and goat productivity improvement program (ESGPIP). ed. Ethiopia: Branna printing enterprise; 2008. p. 38-62.

[9] Agga GE, Udala U, Regassa F, Wudie, A. Body measurements of bucks of three goat breeds in Ethiopia and their correlation to breed, age and testicular measurements. Small Ruminant Research. 2011;95:133-138. DOI: 10.1016/j.smallrumres.2010.09.011

[10] Mekasha Y, Tegegne A, Abera A, Rodriguez-Martinez H. Body size and testicular traits of tropically–adapted bucks raised under extensive husbandry in Ethiopia. Reproduction Domestic Animal. 2008;43:196-206. DOI: 10.1111/j.1439-0531.2007.00877.x

[11] Pepper-Yowell AR. The Use of Computer Assisted Semen Analysis to Predict Fertility in Holstein Bulls [Dissertation]. Colorado: Colorado State University; 2011.

[12] Moura AA. Seminal plasma proteins and fertility indexes in the bull: The case for osteopontin. Animal Reproduction. 2005;2(1):3-10.

[13] Teixeira AVC, Eloy AMX, Furtado JR, Pinheiro RR, Ponte MS. 1D mapping of seminal plasma proteins in Anglo-Nubian goats. Animal Reproduction. 2009;6(4):516-525

[14] Dawson GR, Oyarzo JN, Bellin ME, Zhang HM, Mccauley TC, Ax RL. Promising new technologies for semen evaluation. In: Proceedings of the applied reproductive strategies in beef cattle workshop; 5-6 September 2002; Manhattan, Kansas

[15] Massanyi P, Weis J, Lukac N, Trandzik J, Bystricka J. Cadmium, zinc, copper, sodium and potassium concentrations in rooster and Turkey semen and their correlation. Journal of Environmental Science and Health. 2008;43:563-565. DOI: 10.1080/10934520701796655

[16] Sirat MP, Sinha AK, Singh BK, Prasad L. Effect of cryoprotectants on release of various enzymes from buck spermatozoa during freezing. Theriogenology. 1996;45:405-416. DOI: 10.1016/0093-691X(95)00377-K

[17] Kasimanickam R. Application of technology in male reproduction. In: proceedings of Applied Reproductive Strategies in Beef Cattle, 30 September-1 October, 2011; Northwest. Boise

[18] Ramukhithi FV, Nephawe KA, Chokoe TC, Matabane MB, Mphaphathi ML, Lehloenya KC, Nedambale TL. Attainment of puberty in south African unimproved indigenous bucks. Small Ruminant Research. 2017;153:57-61. DOI: 10.1016/j.smallrumres.2017.05.009

[19] Mohlomie MH. Evaluation of Cryopreserved Ram Semen Following Fertilization in Vitro. [Dissertation]. Bloemfontein: University of the Free State; 2015

[20] Evans G, Maxwell WMC. Salamon's Artificial insemination of sheep and goats. Butterworth. Sydney; 1987. p. 194.

[21] Seshoka MM. Quality of Frozen-Thawed Nguni Bull Sperm Following Analysis Using the Computer Aided Sperm Analyser (CASA). [Dissertation]. Pretoria: Tshwane University of Technology; 2015.

[22] Mphaphathi ML. Cryopreservation of Semen from Venda Breed Chicken. [Dissertation]. Pretoria: Tshwane University of Technology; 2011.

[23] Loskutoff NM, Crichton EG. Standard operating procedures for genome resource banking. The Bill and Bernience Grewcock Center for Conservation and Research. Omaha's Henry Doorly Zoo; 2001. p. 1-16.

[24] Mafolo KS. Characterisation and Cryopreservation of Bapedi Ram Semen in Tris Egg Yolk Extender Supplemented with Phosphstidylcholine. [Dissertation]. Polokwane: University of Limpopo; 2018.

[25] Ramukhithi FV, Nephawe KA, Lehloenya KC, Seshoka MM, Jonker T, Kotze A, Chokoe TC, Nedambale TL. Characterization of seminal plasma constituents of unimproved indigenous and Tankwa goats. South African Journal of Animal Science. 2018;48(5):944-953. DOI: 10.4314/sajas.v48i5.14

[26] Gamanya R. (admin@carnatlab.com). 2015. Analysis. [E-mail to:] Ramukhithi, F.V. (ramukhithif@arc.agric.za) May. 14.

[27] Gaali EE, Satti M. Genetic characterization of two Sudanese goat breeds (Capra hircus) using RAPD molecular markers. African Journal of Biotechnology. 2009;8(10):2083-2087.

[28] Texas sheep & goat raisers' association. Learning About Goats. Secondary Level Packet [Internet]. 2015. Available from: http://www. tsgra.com/docs/secogoats. pdf. [Accessed: 2015-04-2015]

[29] Li MH, Zhao SH, Bian C, Wang HS, Wei H, Liu B, Yu M, Fan B, Chen SL, Zhu MJ, Li SJ, Xiong TA, Li K. Genetic relationships among twelve Chinese indigenous goat populations based on microsatellite analysis. Genetics Selection Evolution. 2002;34:729-744. DOI: 10.1186/1297-9686-34-6-729

[30] Gebre YM. Reproductive Traits in Ethiopian Male Goats, with Special

Reference to Breed and Nutrition [Thesis]. Uppsala Sweden: Acta Swedish University of Agricultural Sciences; 2007.

[31] Supakorn C. The important candidate genes in goats – A review. Walailak Journal of Science and Technology. 2009;6(1):17-36. DOI: 10.2004/wjst.v6i1.70

[32] Food and Agriculture Organization. In-vivo conservation of animal genetic resources. FAO animal production and health guidelines [Internet]. 2013. Available from: http://www.fao.org/docrep/018/i3327e/i3327e00.htm [Accessed: 2015-04-03]

[33] Mohlatlole RP, Dzomba EF, Muchadeyi FC. Addressing production challenges in goat production systems of South Africa: The genomics approach. Small Ruminant Research. 2015;131:43-49. DOI: 10.1016/j.smallrumres.2015.08.003

[34] Pieters A, van Marle-Koster E, Visser C, Kotze A. South African developed meat type goats: A forgotten animal genetic resource? Animal Genetic Resources Information, 2009;44:33-43. DOI: 10.1017/S1014233900002844

[35] Tardiff M. Goat meat nutrition facts [Internet]. 2015. .Available from: http://www.livestrong.com/article/330241 goat meat nutrition facts/ [Accessed: 2015-06-18]

[36] Akpa GN, Ambali AL, Suleiman IO. Body conformation, testicular and semen characteristics as influenced by age, hair type and body condition of red Sokoto goat. New York Science Journal. 2013;6(7):44-58.

[37] Sebei PJ. The Assessment of some Factors Influencing the Survival of Kids in a Small-Scale Communal Goat Production System [Dissertation]. Pretoria: University of Pretoria; 2005.

[38] Sodiq A. Doe Productivity of Kacang and Peranakan Etawah Goats and Factors Affecting them in Indonesia [Thesis]. Germany: University of Kassel; 2004.

[39] Webb EC, Dombo MH, Roets M. Seasonal variation in semen quality of Gorno Altai cashmere goats and south African indigenous goats. South African Journal of Animal Sciences. 2004;34:240-243.

[40] Nsoso SJ, Podisi B, Otsogile E, Mokhutshwane BS, Ahmadu B. Phenotypic characterization of indigenous Tswana goats and sheep breeds in Botswana: Continuous traits. Tropical Animal Health and Production. 2004;36:789-800. DOI: 10.1023/B:TROP.0000045979.52357.61

[41] Cardellino RA. Animal genetic resources conservation and development: The role of food and agriculture organization. Archivos de Zootecnia. 2003;52:185-192.

[42] Rodriguez CM, Day J, Killian GJ. Osteopontin gene expression in the Holstein bull reproductive tract. Journal of Andrology. 2000;21(3):414-420. DOI: 10.1002/j.1939-4640.2000.tb03396.x

[43] Gibson J, Gamage S, Hanotte O, Iniguez L, Maillard JC, Rischkowsky B, Semambo D, Toll J. Options and strategies for the conservation of Farm Animal Genetic Resources: Report of an international workshop; 7-10 November 2005; Montpellier. France

[44] Nedambale TL, Chokoe TC, Maiwashe A. Status of Farm Animal Genetic Resources in South Africa. In: Proceedings of International Strategic Program for Conservation of Animal Genetic Resources for Food and Agriculture [Internet]. 2010. Available from: www.usask.ca/wcvm/.../genetic.../workshop animal conserv emerging... [Accessed: 2014-08-12]

[45] Pieters A. Genetic Characterization of Commercial Goat Populations in South Africa [Dissertation]. Pretoria: University of Pretoria; 2007.

[46] Feugang JM, Kaya A, Page GP, Chen L, Mehta T, Hirani K, Nazareth L, Topper E, Gibbs R, Memili E. Two-stage genome-wide association study identifies integrin beta 5 as having potential role in bull fertility. BMC Genomics. 2009;10:176. DOI: 10.1186/1471-2164-10-176

[47] Gallino JL. Fertility Associated Antigen in Peripubertal Beef Bulls as an Indicator of Potential Fertility [Dissertation]. United States: Texas A & M University; 2005.

[48] Perry G, Patterson D. Determining Reproductive Fertility in Herd Bulls [Internet]. 2011. Available from: https://www.extension.missouri.edu/p/G2011 [Accessed: 2014-06-04]

[49] Bester N. Effect of Different Dietary Energy Levels on Productive and Reproductive Traits in Dorper Rams [Dissertation]. Bloemfontein: University of Free State; 2006.

[50] Leboeuf B, Restall B, Salamon S. Production and storage of goat semen for artificial insemination. Animal Reproduction Science. 2000;62:113-141. DOI: 10.1016/S0378-4320(00)00156-1

[51] Sundararaman MN, Kalatharan J, Edwin MJ. Attempts to achieve semen collections from incapacited Boer bucks by electro-ejaculation. Asian Journal of Animal and Veterinary Advances. 2007;2(4):244-246. DOI: 10.3923/ajava.2007.244.246

[52] Ramukhithi FV. Characterisation and Cryopreservation of South African Unimproved Indigenous and Boer Goat Semen [Dissertation]. Pretoria: Tshwane University of Technology; 2011.

[53] Hafez ESE, Hafez B. Reproduction in Farm Animals. 7th ed. USA, South Carolina, Kiawah Island: Blackwell Publishing; 2008. p. 1-509. DOI: 10.1002/9781119265306

[54] Naing SW, Wahid H, Azam KM, Rosnins Y, Zuki AB, Kazhal S, Bukar MM, Thein M, Kyaw T, San MM. Effects of sugar on characteristics of Boer goat semen after cryopreservation. Animal Reproduction Science. 2010;122:23-28. DOI: 10.1016/j.anireprosci.2010.06.006.

[55] Matshaba B. Characterisation and Cryopreservation of South African Unimproved Indigenous Goat Semen [Dissertation]. Bloemfontein: University of the Free State; 2010.

[56] Hashida NH, Abdullah RB, Rajikin MH, Noor MM. Ultrastructural studies of fresh, frozen-thawed and acrosome-reacted goat sperm. Biomedical Research. 2005;16(2):119-123.

[57] Maina VA, Chaudhari SUR, Williams A. Semen characteristics of the Brown ecotype of Sahel goats in the semi-arid zone of Nigeria. Journal of Applied Science. 2006;6(10):2329-2331. DOI: 10.3923/jas.2006.2329.2331

[58] Uysal O, Bucak MN. The role of different trehalose concentrations and cooling rates in freezing of ram semen. Ankara University Veterinary Faculty Derg. 2009;56:99- 103. DOI: 10.1501/VETFAK_0000002176

[59] Hidalgo M, Rodriguez I, Dorado J. The effect of cryopreservation on sperm head morphometry in Florida male goat related to sperm freezability. Animal Reproduction Science. 2007;100:61-72. 10.1016/j.anireprosci.2006.07.003

[60] Njenga MJ, Munyua SJM, Mutiga ER, Gathuma JM, Kang'etheb EK, Bwangamoi O, Mugera GM, Mitaru BN. Semen characteristics of goats with subacute, acute and chronic besnoitiosis. Journal of the South

African Veterinary Association. 1999;70(1):18-20. DOI: 10.4102/jsava.v70i1.745

[61] Jimenez F, Vicente JS, Viudes-De-Castro MP. Seminal plasma composition from ejaculates collected by artificial vagina and electro-ejaculation in Guirra ram. Reproduction Domestic Animal 2008;43:403-408. DOI: 10.1111/j.1439-0531.2007.00923.x

[62] AL-Ghalban AM, Tabbaa MJ, Kridli RT. Factors affecting semen characteristics and scrotal circumference in Damascus bucks. Small Ruminant Research. 2004;53:141-149. DOI: 10.1016/j.smallrumres.2003.10.003

[63] Dombo MH. Seasonal Effect on Semen Quality of Gorno Altai and South African Indigenous Goats [Dissertation]. Pretoria: University of Pretoria; 2002.

[64] Bozkurt Y, Ogretmen F, Secer FS, Ugur E. Effects of seminal plasma composition on sperm motility in Mirror Carp (*Cyprinus carpio*). The Israeli Journal of Aquaculture – Bamidgeh. 2009;61(4):307-314. DOI: 10.46989/001c.20570

[65] Verstegen J, Iguer-ouanda M, Onclin K. Computer assisted semen analyzers in andrology research and veterinary practice. Theriogenology. 2002;57:149-179. DOI: 10.1016/s0093-691x(01)00664-1

[66] Dorado J, Rodríguez I, Hidalgo M. Cryopreservation of goat spermatozoa: Comparison of two freezing extenders based on post-thaw sperm quality and fertility rates after artificial insemination. Theriogenology. 2007;68:168-177. DOI: 10.1016/j.theriogenology.2007.04.048

[67] Khalifa TAA, EI-Saidy BE. Testing usability of butylated hydroxytoluene in conservation of goat semen. American-Eurasian Journal of Agricultural and Environmental Sciences 2006;1(3):288-293. DOI: 10.1111/j.1439-0531.2007.00947.x

[68] Hidalgo M, Rodriguez I, Dorado J. Influence of staining and sampling procedures on goat sperm morphometry using the sperm class analyzer. Theriogenology. 2006;66:996-1003. DOI: 10.1016/j.theriogenology.2006.02.039

[69] Okab OB. Semen characteristics and plasma testosterone of new-Zealand male rabbits as affected by environmental temperatures. Slovak Journal of Animal Science. 2007;40(4):161-167.

[70] Jimenez F, Puchades S, Gadea J, Vicente JS, Viudes-De-Castro MP. Effect of semen collection method on pre- and post-thaw Guirra ram spermatozoa. Theriogenology. 2005;64:1756-1765. DOI: 10.1016/j.theriogenology.2005.04.006

[71] Nur Z, Dogan I, Gunay U, Soylu MK. Relationships between sperm membrane integrity and other semen quality characteristics of the semen of Saanen goat bucks. Bulletin of the Veterinary Institute of Pulawy. 2005;49:183-187.

[72] Molinia FC, Evans G, Maxwell W.C. In-vitro evaluation of zwitterions buffers in diluents for freezing ram spermatozoa. Reproduction Nutrition Development. 1994;34:491-500. DOI: 10.1051/rnd:19940510

[73] Pezzanite L, Bridges A, Neary M, Hutchens T. Breeding Soundness Examinations of Rams and Bucks [Internet]. 2015. Available from: https://www.extension.purdue.edu/extmedia/as/as-599-w.pdf. [Accessed: 2015-08-02]

[74] Mellado M, Herrera CAM, Arevalo JR, Garcia JE, Veliz FG. Effect of dietary energy intake and somatotropin administration after

weaning on growth rate and semen characteristics of Granadina goat bucks. Turkish Journal of Veterinary and Animal Sciences. 2012;36(4):338-345. DOI: 10.3906/vet-1101-721

[75] Ajani OS, Oyeyemi MO, Moyinoluwa OJ. Correlation between age, weight, scrotal circumference and the testicular and epididymal parameters of red Sokoto bucks. Journal of Veterinary Medicine and Animal Health. 2015;7(5):159-163. DOI: 10.5897/JVMAH2014.0303

[76] Food and Agriculture Organization. Commission on genetic resources for food and agriculture. Draft guidelines on phenotypic characterisation of animal genetic resources. Thirteenth Regular Session; 18-22 July 2011; Rome; 2011. p. 1-78

[77] African Union Interafrican Bureau for Animal Resources. Improving the utilization of Animal Genetic Resources characterization [Internet]. 2014. Available from: http://au-ibar.org/ [Accessed: 2015-06-02]

[78] Turner WC, Versfeld WD, Kilian JW, Getz WM. Synergistic effects of seasonal rainfall, parasites and demography on fluctuations in springbok body condition. Journal of Animal Ecology. 2012;81(1):58-69. DOI: 10.1111/j.1365-2656.2011.01892.x

[79] Gaias G. Body Condition Score and Body Composition of Sarda Dairy Ewes [Thesis]. Italy: Universits Degli Studi di Sassari; 2012.

[80] Kridli RT, Tabbaa MJ, Barakeh FS. Seasonal variation in scrotal circumference and semen characteristics of Black Bedouin and Black Bedouin-Damascus crossbred bucks. Asian-Australasian Journal of Animal Sciences. 2007;20(3):359-364. DOI: org/10.5713/ajas.2007

[81] Bozkurt Y, Ogretmen F, Secer FS, Ugur E. Relationship between seminal plasma composition and spermatological parameters in Scaly Carp (*Cyprinus carpio*). Journal of Animal and Veterinary Advances. 2009;8(12):2745-2749. DOI: javaa.2009.2745.2749

[82] Novak S, Ruiz-Sanchez A, Dixon WT, Foxcroft GR, Dyck, M.K. Seminal plasma proteins as potential markers of relative fertility in boars. Journal of Andrology. 2010;31(2):188-200. DOI: 10.2164/jandrol.109.007583

[83] Bozkurt Y, Ogretmen F, Kokcu O, Ugur E. Relationships between seminal plasma composition and sperm quality parameters of the *Salmo trutta* macrostigma (Dumeril, 1858) semen: with emphasis on sperm motility. Czech Journal of Animal Science. 2011;56(8):355-364. DOI: 10.17221/2394-CJAS

[84] Purdy PH. A review on goat sperm cryopreservation. Small Ruminant Research. 2006;63:215-225. DOI: 10.1016/j.smallrumres.2005.02.015

[85] Dyck MK, Foxcroft GR, Novak S, Ruiz-Sanchez A, Patterson J, Dixon WT. Biological markers of boar fertility. Reproduction in Domestic Animals. 2011;46(2):55-58. DOI: 10.1111/j.1439-0531.2011.01837.x

[86] Souza CEA, Moura AA, Lima-Souza AC, Killian GJ. Binding patterns of seminal plasma proteins on bovine epididymal and ejaculated sperm membrane. Brazilian Journal of Veterinary and Animal Sciences. 2011;63(3):535-543. DOI: 10.1590/S0102-09352011000300001

[87] Killian G. High-fertility proteins enhance reproduction rates in dairy cattle. National Research Initiative Competitive Grants Program, The Pennsylvania State University [Internet]. 1999. Available from: www.csrees.usda.gov/nea/animals [Accessed: 2015-06-14]

[88] Farshad A, Yousefi A, Moghaddam A, Khalili B. Seasonal changes in serum testosterone, LDH concentration and semen characteristics in Markhoz goats. Asian-Australian Journal of Animal Science. 2012;25(2): 189-193. DOI: 10.5713/ajas.2011.11179

[89] Ramukhithi FV. Prediction of Male Fertility in South African Indigenous Goats Using Semen and Phenotypic Parameters [Thesis]. Pretoria: Tshwane University of Technology; 2016.

[90] Leone MG, Haq HA, Saso L. Lipocalin type prostaglandin D-synthase: Which role in male fertility? Contraception. 2002;65:293-295. DOI: 10.1016/s0010-7824(02)00280-9

[91] Nynca J, Dietrich MA, Bilinska B, Kotula-Balak M, Kiełbasa T, Karol H, Ciereszko A. Isolation of lipocalin-type protein from rainbow trout seminal plasma and its localisation in the reproductive system. Reproduction, Fertility and Development. 2011;23:381-389. DOI: 10.1071/RD10118

[92] Gerena RL, Irikura D, Urade Y, Eguchi N, Chapman DA, Killian GJ. Identification of a fertility-associated protein in bull seminal plasma as lipocalin-type prostaglandin D synthase. Biology of Reproduction. 1998;58:826-833. DOI: 10.1095/biolreprod58.3.826

[93] Ax RL, Bellin ME, Zhang HM, Hawkins HE. New approach to estimate bull fertility. In: Proceedings of The Range Beef Cow Symposium XVI. 14-16 December 1999; Greeley.

[94] Dalton JC, Deragon L, Vasconcelos JLM, Lopes CN, Peres RFG, Ahmadzadeh A. Fertility-associated antigen on Nelore bull sperm and reproductive outcomes following first-service fixed-time AI of Nelore cows and heifers. Theriogenology. 2012;77:389-394. DOI: 10.1016/j.theriogenology.2011.08.011

[95] Asadpour R. Relationship between mineral composition of seminal plasma and semen quality in various ram breeds. Acta Scientiae Veterinariae. 2012;40(2):1-8.

[96] Market CL. Biochemistry and function of lactate dehydrogenase. Cell Biochemistry and Function. 1984;2:131-133. DOI: 10.1002/cbf.290020302

[97] Colagar AH, Marzouni ET, Chaichi MJ. Zinc levels in seminal plasma are associated with sperm quality in fertile and infertile men. Nutrition Research. 2009;29:82-88. DOI: 10.1016/j.nutres.2008.11.007

[98] Lotfi R, Ragab N, Ghazala W. Evaluation of seminal plasma magnesium in premature ejaculation. Scientific reports. 2012;1(2):156. DOI: 10.4172/.156 page.

[99] Nikoobakht M, Aloosh M, Hasani M. Seminal plasma magnesium and premature ejaculation: a case-control study. Urology Journal. 2005;2(2):102-105.

[100] Aguiar GV, van Tilburg MF, Catunda AGV, Celes CKS, Lima ICS, Campos ACN, Moura AAA, Araujo AA. Sperm parameters and biochemical components of goat seminal plasma in the rainy and dry seasons in the Brazilian northeast: The season's influence on the cooling of semen. Brazilian Journal of Veterinary and Animal Sciences. 2013;65(1):6-12. DOI: 10.1590/S0102-09352013000100002

[101] Cortada CNM, Lucci C, Gonzalez RAF, Valentin R, Mattos CB. Plasma urea levels on reproductive parameters of wool-less rams (Ovis Aries, LINNAEUS, 1758). Brazilian Journal of Veterinary Research and Animal Science. 2000;37(6):87-91. 10.1590/S1413-95962000000600007

[102] Cross NL. Role of cholesterol in sperm capacitation. Biology of

Reproduction. 1998;59:7-11. DOI: 10.1095/biolreprod59.1.7

[103] Sorensen MB, Bergdahl IA, Hjollund NHI, Bonde JPE, Stoltenberg M, Ernst E. Zinc, magnesium and calcium in human seminal fluid: Relations to other semen parameters and fertility. Molecular Human Reproduction. 1999;5(4):331-337. DOI: 10.1093/molehr/5.4.331

[104] MandaL R, Badyakar D, Chakrabarty, J. Role of membrane lipids fatty acids in sperm cryopreservation. Advances in Andrology. 2014;1-9. DOI: 10.1155/2014/190542

[105] Gundouan M. Some reproductive parameters and seminal plasma constituents in relation to season in Akkaraman and Awassi rams. Turkish Journal of Veterinary and Animal. 2006;30:95-100.