CHAPTER 3

3.1 MATERIAL AND METHODS

The experimental study was conducted at the Agricultural Research Council in Pretoria (25° 55′ S; 28° 12′ E), South Africa. The area is situated in a typical highveld climate at an altitude of 1525 m above sea level. The weather condition ranges from hot days and cool nights in summer to moderate winter days with cold nights. Two breeds of male goats were used in the experiment, namely Gorno Altai and indigenous goats which were randomly selected. The Gorno Altai bucks were 4 years of age and the indigenous bucks 5 years of age and weighed between 45 to 65 kg. The experimantal animals were housed in enclosed pens with covered roofing and an open-air area. Every morning, the bucks were fed lucerne and hay, while water and mineral licks were provided *ad libitum*. The trial commenced in January 2000 and was completed at the end of December 2000.
Monthly air temperatures were obtained from the Weather Bureau Services of South Africa in Pretoria. To determine the effects of environmental temperatures on semen production, the average daily temperatures were calculated according to the following formula (Loubser *et al,* 1983):

Average daily temperatures (°C) = Maximum temperatures +Minimum temperatures /2 and converted to a monthly average (Loubser *et al*, 1983).

3.2 PREPARATION OF BUCKS FOR ELECTRO-EJACULATION

Semen was collected during the second week of each month. Before semen was collected, the prepuce of the penis was washed with a sterile gauze swab in order to remove dirt and excess urine from the sheath. Preputial hairs were clipped and the preputial orifice was cleansed thoroughly.

Semen collection is similar to harvesting any crop in a season. The effective harvesting of semen involves obtaining the maximum number of sperm of the highest possible quality from superior males. This involves proper semen collection procedures with males sexually stimulated and prepared.

3.3 SEXUAL STIMULATION

Sexual stimulation prior to semen collection was induced by parading the teaser doe for several minutes amongst the males. Sexual stimulation was done in order to increase the number of spermatozoa per ejaculate, for the highest possible quality ejaculate. False mounts without ejaculation were allowed in order to enhance the degree of sexual excitement. Sexual stimulation was conducted according to the method of Hale and Almquist (1960) and Collins *et al* (1951) in order to increase semen production as sexual stimulation and preparation increases the semen volume and sperm concentration of the ejaculates in males. Ejaculates with a larger volume, high concentration, and higher motility are generally considered to have a higher fertility rate.

3.4 PREPARATION OF THE ELECTRO-EJACULATOR

Semen was collected by means of an electro-ejaculator. The apparatus consisted of a bipolar electrode and a variable source of alternating electrical current. The voltage ranged from 0 to 30 volts with a low amperage (0.5 to 1.0). The electrode had negative and positive conductors running longitudinally along the electrode. Prior to the rectal insertion of the rectal probe, the electrode was lubricated with KY-jelly ease insertion. Lubrication was also done in order to avoid injuries and causing stress to the male animal. The electrode was placed in the rectum (12 cm) immediately above the accessory sex glands in order to stimulate reproductive system. Three levels of voltage were applied with the buck responding at a peak of 8 volts (Maxwell and Evan, 1987; Bearden and Fuquay, 1980). The process of electro-ejaculation was accomplished with the buck on its side.

3.5 SEMEN COLLECTION USING ELECTRO-EJACULATOR

Semen was collected in a graduated glass collection tube. Semen was collected from each buck once a month over a period of a year. Immediately following collection, the semen sample was placed in a water bath at 35°c. Each ejaculate was examined for volume, percentage progressive motile sperm and sperm concentration.

The semen volume was measured in a collection tube enclosed in a holding swab and the colour evaluated. Semen was also taken to the laboratory as soon as possible. A microscope was mounted with a hot stage in order to maintain the body temperature of the ejaculate.

The slide was allowed to warm up to 38°C. The intensity of the wave motion (WM) in an uncovered drop was estimated microscopically on a warm stage and rated between 0 to 5 (very turbulent). Motility was observed under a light microscope at a magnification of 100 X. Several fields were examined in order to estimate the percentage of sperms that were progressively motile. Measurements that were observed were mainly motility or wave motion.

3.6 ASSESSMENT OF SPERM CONCENTRATION WITH THE AID OF A HAEMOCYTOMETER

Semen concentration was determined with the use of a haemocytometer. A sample of 0.01 ml semen was diluted with 4 ml of physiological saline solution. A cover slip was placed on the counting grid of the haemocytometer. The edge of the counting chamber was touched with the tip of the pipette to allow a drop of diluted semen to run under the cover glass.

Spermatozoa were allowed to settle for 5 to 6 minutes before the haemocytometer was placed on the warm stage of the microscope. The haemocytometer was placed on the microscope under direct lighting with a magnification 400X to cover a large square on the grid. At least five large squares were counted and the total number of spermatozoa recorded.

3.7 STAINING OF THE GLASS SLIDES WITH SPERM

A staining agent was obtained from the Onderstepoort Faculty of Veterinary Medicine, University of Pretoria. The agent was composed of 1 % eosin and (Nigrosin 5 %) compounds. Both eosin and the background stains were dissolved in 2.9% sodium citrate dihydrate buffer. The slides with sperm were dried quickly on a warmed plate (55°C-60°C) with a small electric fan directed across the plate. This was done to overcome early death of spermatozoa and the penetration of the staining agent before the slide was completely dry. Several fields at random were examined and sperm counted under a microscope.

3.8 ASSESSMENT OF PERCENTAGE DEAD AND ABNORMAL SPERMATOZOA.

A clean slide was place a on the warm stage of the microscope. A drop of eosin-negrosin stain was placed on the slide and a drop of pure semen was mixed with the staining agent for ten seconds. The mixture was left to stand for approximately 50 seconds. The mixture was spread and drawn and a thin film was made on the microscope slide. The preparation was stored away from humidity for later microscopic analysis.

3.9 Semen evaluation

3.9.1 Visual appearance of semen

When semen is visually examined in a collection tube, semen samples should be a homogenously milky or creamy fluid and free of pus, urine, blood and dust. A watery semen sample contains few sperm and is almost certainly without a fertilizing capacity. Whey-like specimens have low sperm counts and are usually associated with infertility. Cryptochoid males and those with bilateral epididymitis yield a sperm-free fluid, while cases of marked hypoplasia or degeneration of the testes show low sperm density. Purulent samples usually come from cases of seminal vesiculitis or epididymitis. In bulls,

semen with a yellow tinge is obtained, but the colour is due to vesicular secretions and it is a familial trait of no consequence (Arthur *et al*, 1982).

3.9.2 Evaluation of sperm motility

Sperm motility is markedly influenced by changes in temperature. So for example, in winter semen placed on a slide immediately after collection cools down very rapidly and motility soon approaches zero, whereas in summer, air temperatures are sometimes sufficiently high for good motility to be maintained throughout the whole period of evaluation. Motility should be assessed as quickly as possible, either on the farm or immediately on return to the laboratory.

In order to make accurate comparisons, it is necessary to warm the specimens to body temperature immediately before the motility estimation. In the laboratory, the tube of semen and the glass slides are placed in the incubator for several minutes at 37°C. The microscope is then prepared with respect to focus and illumination. A drop of semen is quickly transferred from the tube to the slide, a warm cover slip applied and the specimen evaluated under the two-thirds objective.

A constant elevated temperature for more prolonged evaluations may be obtained by employing a thermostatic slide carrier or a hot stage. Walton (1939) postulated that, in respect to the warming of the slides, it is important to understand that temperatures above 60°C are lethal to sperm. At body temperature, under low magnification, normal semen exhibits mass sperm movement in the form of recurrent swirling waves. Under high magnification, sperm make an active, progressive motion that can only be followed momentarily. Poor semen samples indicate only rotatory or oscillatory activity of a minority of the sperm, while the occasional specimen is completely devoid of motility. Absence of motility is found in cases of orchitis and epididymitis, while various grades of poor motility are observed in instances of testicular hypoplasia and degeneration (Arthur *et al*, 1982).

3.9.3 Determination of sperm concentration

The number of spermatozoa per millilitre of semen may be determined by means of the Thoma/ Neubauer haemocytometer and an appropriate diluting pipette-in the same method used for doing erythrocytes counts. The sperm head will be included only if spermatozoa are lying across the boundary lines of the large square.

Cleaning of the haemocytometer is followed by the second reloading and a second count made. The mean of the two sperm counts is taken. As a simple alternative to haemocytometer counts, sperm density may be estimated with sufficient accuracy by employing the comparison with a standard opacity tube. Low sperm density is a feature of testicular hypoplasia and degeneration (Arthur *et al*, 1982).

3.9.4 Evaluation of the percentage dead and live sperm

Hancock (1952) reported that immediately after the motility estimation, a drop of semen can be mixed on a warm slide with two drops of warm negrosin-eosin stain and a thin smear of semen made with a cover slip, in a same manner used for making blood smears. Maxwell and Evans (1987) found that dead sperm take up the eosin stain whereas live sperm do not, and this difference may be assessed against the dark background imparted by the negrosin-eosin and a percentage count made of the dead sperm.

Good samples of semen have an average of 25 % dead sperm. A high percentage of dead sperm usually indicate severe infection of the testicles (Arthur *et al*, 1982).

3.10 MORPHOLOGICAL EVALUATION OF SPERMATOZOA

3.10.1 Tailless sperms and sperms with looped tails

The most common sperm abnormalities are the detachment of the sperm head (tailless) and bending of the middle piece and a tail around and over the sperm head (looped tail). Looping of tails is sometimes caused by sudden temperature changes. Tailless and bending of the tails over the head also arises from rough manipulation of the semen, but these are indications of sperm weaknesses under standard conditions. Both types of sperm abnormalities are seen most frequently in the ejaculate of sterile males. In normal bulls it has been found that both tailless sperm and sperm with looped tails are present at an average percentage of 40%. In a specific hereditary sterile condition of Guernsey bulls, there are nearly 100% of tailless sperm. Hammond (1940) deduced that sperm might survive storage in the tail of the epididymis for approximately 60 days. It was further stated that it is reasonable to assume that changes occur in older sperms and that such changes will render them more fragile. This was based on the findings of a high percentage of tailless spermatozoa in the ejaculate of fertile bulls after long sexual rest, for example, 23 %, after a sexual rest of 72 days. That such tailless sperms are not necessarily dead is shown by the motility of the headless tails.

Tailless sperm may reach the oviduct but they will be incapable of penetrating the ovum. It has also been found that semen samples with numerous tailless sperms often showing a relatively high percentage of the looped or coiled tails is intrinsic sperm weakness including aging (Arthur *et al*, 1982).

3.10.2 Sperm with coiled tails

The coiling of sperm tails come in two forms. The first involves the coiling of the tail at the extremity of the tail only, and the second involves the coiling of the whole of the tail and sometimes the middle piece as well.

In the coiling of the tail and the middle piece, it may appear as a tight coil adjacent or surrounding the sperm head. In the coiling of the extremities of the tail, motility can be seen, whereas motility cannot be seen in the coiling of the complete tail. Coiling of the middle piece with the head where there is no motility is regarded as a serious abnormality, and occurs mainly in the semen of infertile bulls (Zemjanis, 1962; Arthur *et al*, 1982).

3.10.3 Immature or unripe sperm

Immature sperm are characterized by the presence of the droplet of protoplasm (cytoplasmic droplet) at the junction of the sperm head with the middle piece (the neck). The presence of such droplets is physiological and they are discarded along the way as they progress to the epididymis. Immature sperm with or attached to cytoplasmic droplets usually exhibit a low motility. A large number of immature sperm have been recorded in the semen of many infertile bulls, including young animals with hypoplasia and older bulls with a degeneration of the testes. It is generally considered that the appearance of these sperm in the ejaculate is an indication of the dysfunction of the testis and epididymis (Arthur *et al*, 1982).

3.10.4 Abnormal acrosomes

The importance of the abnormality of the sperm head must be recognized, as this is where the chromosomes genetic material is contained. Sperm shapes and sizes vary depending on whether the sperm has been obtained from a fertile or an infertile bull. Some authors

have classified head defects, in contrast to those of the middle piece and the tail as the primary sperm abnormalities (Perez and Mateos, 1996; Arthur *et al*, 1982).

Very small and unduly large heads, short, narrow and pear-shaped heads, detachment, loosening or distortion of the galela capitis (acrosome cap), shrunken, misshapen or abnormally staining heads are all observed to a large extent in those sperm that have defects in middle piece (Hancock, 1949). It was further stated that sperm with double heads and others with an abaxial attachment of the middle piece, could also be seen. The integrity of the acrosome is an important prerequisite for the fertilizing ability, as it contains enzymes, which assist penetration of the ovum. One such enzyme which occurs only in an intact acrosome is acrosin.

Other varieties of defective sperm include an unduly thick middle piece. In other occasions its proximal portion appears as a filiform structure. The sperm tail sometimes shows an angular junction with the middle piece. Red blood cells are rarely seen but leucocytes are present in cases of seminal vesiculinitis, prostates, epididymitis and orchitis.

3.11 The following data was recorded on a monthly basis

Buck body weight (W)

Semen volume (V) in ml

Wave motion of the spermatozoa (WM) (on a scale of 1-5)

Concentration of the spermatozoa (Cont)

Number of live spermatozoa (L)

Number of dead spermatozoa (D)

Number of normal spermatozoa (N)

Total number of spermatozoa (N)

Semen colour (Consistency)

Scrotal circumference in centimetres (SC)

3.12 Measurement of sperm morphological abnormalities

Number of tailless spermatozoa (TLS)

Number of spermatozoa with enlarged heads (EH)

Number of spermatozoa with small heads (SH)

Number spermatozoa with coiled tails (CT)

Number of spermatozoa with cytoplasmic droplets (CD)

Number of spermatozoa with tapped heads (TH)

Number of spermatozoa broken at neck (BN)

Number of spermatozoa broken at mid-piece (BM)

Number of spermatozoa with abnormal acrosomes (AA)

3.13 STATISTICAL ANALYSIS

The results were analysed using the SAS package [SAS Institute Incorporation; User Guide Version 8.Cary.N.C:NC SAS Institute. IN. 1999]