PHENOTYPIC AND REPRODUCTIVE CHARACTERISATION OF KOLBROEK PIGS

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by

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Dissertation submitted in fulfilments of the requirements for the degree

MAGISTER TECHOLOGIAE: AGRICULTURE

in the

Faculty of Health and Environmental Sciences

Department of Agriculture

at the

Central University of Technology, Free State

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September 2018

ACKNOWLEDGEMENT OF FINANCIAL SUPPORT

I wish to express my heartfelt gratitude and appreciation to the Central University of Technology, Free State (CUT, FS). The Agricultural Research Council (ARC) Professional Development Program (PDP) at Irene, Gauteng. Department of Agriculture Forestry and Fisheries (DAFF), National Research Foundation (NRF) and Southern African Science Service Centre for Climate Change and Adaptive Land Management – Council for Scientific and Industrial Research (SASSCAL) for their financial contribution to the successful completion of this study.

DECLARATION AND COPYRIGHT

I, Lemohang Gladys Makhanya, student number , declare that this dissertation: **Phenotypic and Reproductive Characterisation of Kolbroek pigs**, submitted to the Central University of Technology, Free State for the degree MAGISTER TECHNOLOGIAE is my own independent work and that all sources used and quoted have been duly acknowledged by means of complete references; and complies with the codes of academic integrity as well as other relevant policies, procedures, rules and regulations of the Central University of Technology; and has not been submitted before to any other institution by myself or any other person in fulfilment or (partial fulfilment) of the requirements for attainment of any qualification. I also disclaim this dissertation in the favour of Central University of Technology, Free State.

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Lemohang Gladys Makhanya Date

DEDICATION

This dissertation is dedicated to my family, my friends, my son and the staff at Germplasm Conservation and Reproductive Biotechnologies of the Agricultural Research Council (ARC), Irene, who contributed in so many ways in making this study experience incredible, gaining of knowledge and remarkable personal growth.

ACKNOWLEDGEMENTS

My sincere gratitude goes to the management personnel of the Pig Nutrition Experimental Unit, Animal Production Institute (API) of the Agricultural Research Council (ARC), for allowing me to use the animals for the study. I would also like to extend my sincere gratitude and appreciation to the following people:

- Professor D.O. Umesiobi, my main project supervisor and the former Head of Department of the Department of Agriculture at the Central University of Technology, Free State, for giving me an opportunity to work with you. I am so grateful for your support, teaching, understanding, patience and encouragement.
- Professor T.L. Nedambale, my project co-supervisor and Research Professor at the Tshwane University of Technology, for his mentorship, support and valuable scientific guidance in this research project.
- Mrs M.B Matabane, my project co-supervisor and the former Acting Research Team Manager at the Germplasm Conservation and Reproductive Biotechnologies, ARC for her guidance, constructive criticism and willingness to listen, assist and constant feedback throughout the duration of this study.
- Dr Arnold Kanengoni for his ever-ready support and willingness to offer assistance to the successful conclusion of this study.
- Dr Liberty Sibanda and the staff at ÄRC-OVI-Residue laboratory for their assistance and patience in helping me with analysis of samples during this study.
- Mr Philemon Tlhako, Mr Thomas Madungaone, Mr Philemon “Bra Phil” Molokomme, Mr Thabo Bohlolo, Mr Vincent Khoza and Mr Albert Mphuloane

for helping me in data collection and also contributing tremendously to the successful collection of data during this study.

- To Ntombizodwa Bovula for travelling this journey with me from the beginning to the end.
- To my colleagues at GCRB for their support and assistance.
- To Dieketseng “keke” Lesako and Dr Nontete Nhlapo my dearest friends thank you for the support and encouragement you gave me throughout this period it meant a lot to me.
- To my best friend Johannes ”Mokolobetsi” Molieleng, thank you for caring, listening for the laughter and support.
- To my dearest mother Malehlohonolo Makhanya, my late grandparents Tamolo and Matsibela Qhala, my sisters Motshidisi and Mpolokeng Makhanya, my brother and his wife Lehlohonolo and Makatleho Makhanya and their two girls Katleho and Neo Makhanya, and lastly my lovely son Kabelo, for their love, belief in me and never ending support, prayers and for taking care of my son during my time of absence from home. I love you very much and thank you from the bottom of my heart.
- Above all, I thank God Almighty for his grace, for giving me strength, courage, wisdom and the ability to successfully complete this study.

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LIST OF ACRONYMS AND ABBREVIATIONS

ABBREVIATION	DESCRIPTION
n	Number of animals
CASA	Computer Aided Sperm Analysis
VIAB	Viability
TM	Total Motility
RAP	Percentage of rapidly moving sperm
PM	Progressive Motility
VCL	Velocity on the curve line
VSL	Velocity Straight Line
VAP	Velocity Average Pathway
LIN	Linearity
STR	Straightness
WOB	Wobble
BW	Body Weight
FSH	Follicle Stimulating Hormone
LH	Luteinizing Hormone
E_2	Estradiol

GENERAL ABSTRACT OF THE STUDY

Indigenous pigs are considered as a valuable component of rural livelihood farming systems in South Africa. However, the population of rural pigs such as Kolbroek are considered stagnated due to the absence of a comprehensive improvement and conservation strategy. Therefore, in domestic pigs (*Sus Scrofa domestica*), phenotypic and reproductive characterisation are some of the most important characteristics for improved pig production. The objectives of the study were to evaluate the phenotypic characteristics of Kolbroek males and female pigs, to correlate the testicular size with semen parameters and to detect the onset of puberty by hormonal assays in Kolbroek boars and sows then evaluate the reproductive characteristics of the sows following natural mating. A total of 57 Kolbroek pigs (34 sows and 23 boars) were used for phenotypic characterisation. Data for various phenotypic characteristics were evaluated by visual appraisal. Morphometric characteristics such as body weight, body length, chest girth, head length, width of head, ear length, hair length, tail length and number of teats were recorded using a measuring tape. The scrotal measurements were done using a vanier calliper. A total of fifteen ejaculates from three boars were collected using a gloved hand technique method. Semen parameters were evaluated macroscopically and microscopically. Blood was collected randomly from thirteen Kolbroek pigs from the jugular vein every two weeks for 5 months using a 10ml syringe. Blood was then centrifuged at 2400rpm for 14 minutes and the serum was stored at -20^{0}C. The serum profile of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and Estradiol (E_2) of the gilts were determined using Elisa kits. Data were analysed using the Statistical Analysis System (SAS). The predominant coat colour pattern of the pigs was a patchy black and white colour type. All the pigs had straight long and dense hair and tusks were present only in boars. In all the descriptive

characteristics, majority of the sows had a rough skin and ears which projected forward (57.9%). Moreover, majority of boars had erect ears (69.7%). All the pigs had concave heads with a short and cylindrical snout, a straight tail and sows had an average of 10 teats. There were no significant differences ($P>0.05$) for morphometric characteristics, irrespective of the gender. However, sows (123kg) had a significantly higher ($P<0.05$) body weight compared to boars (114kg).

There was a significantly positive correlation ($P<0.05$) between bodyweight and testicular size ($r = 0.90$). There was also a highly positive correlation ($P < 0.01$) between testicular size and sperm concentration ($r = 0.47$) as well as total motility ($r = 0.30$). Moreover, there was a highly positive correlation between body weight and semen pH ($r = 0.91$). A significant positive correlation was observed between body weight and semen volume ($r = 0.80$) as well as sperm concentration ($r = 0.90$). A highly significant positive correlation was also observed in testicular size and semen volume ($r = 0.90$). Weak positive correlations ($P>0.05$) were found between semen pH and sperm concentration ($r= 0.18$) and semen volume ($r= 0.18$), although not significant. Furthermore, a weak positive correlation was observed between body weight and sperm motility ($r= 0.30$). There was strong positive correlation ($P<0.05$) between sperm motility and semen volume ($r= 0.66$), pH (0.78) and sperm concentration ($r= 0.60$). There were no significant differences ($P>0.05$) for hormonal serum concentrations in Kolbroek sows. There was a significant increase (0.22-0.35ng/ml) in serum concentrations of LH, FSH and E_2 in all sows from 3 to 7 months of age. In conclusion, Kolbroek pigs have a patchy black and white coat colour. The head was concave in form and the ears were erect and projected forward. It was also found that the effect of gender was not significant on all body weights and measurements in Kolbroek pigs, except for body length. It was indicated that the measurements of

testicular size and bodyweight of Kolbroek boars was positively correlated with semen volume. The age of attainment of puberty of Kolbroek sows was found to be 3 months. The study showed that phenotypic and reproductive characteristics are useful in selection, identification and conservation of South African Kolbroek pigs.

Keywords Kolbroek pigs, puberty, semen quality

CHAPTER 1

CHAPTER 1: BACKGROUND AND OVERVIEW OF THE STUDY

1. 1 GENERAL INTRODUCTION

Indigenous pigs are considered as a valuable component of rural livelihood farming systems in South Africa. South Africa has a diversity of indigenous pig breeds that play a major role in the social, farming and economic sectors of the country. Kolbroek is one of South Africa's indigenous pig breeds (Swart *et al*, 2010). Kolbroek pigs have specific characteristics that contribute to their adaptability which is hardiness, tolerance, harsh environmental conditions, and the ability to survive on little or no input and adjust to fluctuations in feed availability (Masenya *et al.*, 2011). Due to these characteristics, the production potential of indigenous Kolbroek pig breeds should not be entirely criticised like other indigenous livestock species. However, the population of indigenous pigs is considered stagnated due to the absence of a comprehensive improvement and conservation strategy. Therefore, phenotypic and reproductive characterisation in domestic pigs (*Sus scrofa domestica*) is one of the most important economic characteristics for improved pig production.

According to Rodero & Herrera. (2000), studies are necessary to characterize, identify and differentiate pig populations. Furthermore, Carnerio *et al.* (2010) emphasized that identifying the origin and history of breeds as well as phenotypic description and morphological characteristics are of utmost importance. Indigenous pigs manifest a great deal of variation which is due to genetic and environmental factors. Phenotypic characterisation provides useful information on the suitability of animals for selection. It includes description of the animal's physical characteristics, productive parameters, status of the breed and mating systems allowing distinction between breeds (ANGR, 2006). Indigenous pigs which are basically non-descriptive types vary widely in body shape, body length, coat colour and other phenotypic characteristics. Most of the research has been focusing on the imported genotypes which cannot be sustained under small holder conditions (Ncube *et al*., 2003). Furthermore, local research to evaluate the phenotypic characteristics of indigenous pigs has been erratic and inadequate. Hence there is a need to evaluate the reproductive potential of the South African indigenous pigs in comparison with imported pigs.

The process of reproduction is extremely complicated and generally involves highly specific biological functions (Thompson, 2015). However, external environmental factors such as nutrition, housing, social surroundings, temperature and disease, has a far greater effect on reproductive performance than on any other biological process (Okere & Nelson, 2002). In pig production, growth and reproductive performance characteristics are of utmost importance (Matta *et al.*, 2005). Furthermore, it depends on complex physiological pathways which determine male and female age at sexual maturity, gamete production, libido, fertilization, foetal and piglet survival (Okere & Nelson, 2002). Female reproductive characteristics are determined by conception rate, farrowing rate, litter size, number of piglets born alive, litter birth weight, and number of piglets weaned, age at puberty, weaning to oestrus interval and farrowing interval (Phiri, 2006). Furthermore, male reproductive characteristics are determined by a positive correlation between testis size and sperm concentration, as well as a superior mating ability (Janyk, 2004). It is important in improving reproductive efficiency which in turn will increase the overall efficiency of pig production (Phiri, 2006). It is well recognised that reproductive function is of utmost importance in commercial piggeries (Barb *et al.*, 2008; Kolesarova *et al.*, 2010).

Puberty is a fundamental developmental process experienced by all reproductively competent adults, yet the specific factors regulating age at puberty remain elusive in pigs (Yang *et al.*, 2008). Sexual maturity is recognised by first oestrus, which is determined by the presence and physiological state of ovarian follicles that occurs at different ages and body weights in diverse pig genotypes due to genetic variances in mature size and growth rate. Although molecular techniques have aided in the identification and characterization of individuals and breeds, phenotypic and reproductive characterisation is also crucial. Due to the risk of extinction, Kolbroek pigs require in depth studies and conservation strategies through characterisation and preserving their germplasm.

1.2 MOTIVATION OF THE STUDY

The phenotypic characteristics and reproductive potential of indigenous Kolbroek pigs have not been fully exploited in South Africa as compared to their exotic counterparts (Masenya *et al.*, 2011). According to Umesiobi (2009), the reproductive performance of most indigenous pig breeds found in South Africa is declining mostly because of over dependence on the imported pig breeds. Consequently, little attention is given to the improvement and utilization of indigenous pig breeds. Moreover, indigenous pig breeds and strains are particularly vulnerable as selection for improvement and uncontrolled mating strategies (inbreeding) may lead to genetic dilution and loss of genetic variation within breeds and eventually leading to their extinction (Shresta, 2005; Scherf *et al.,* 2006; Koster *et al.,* 2008). The lack of sufficient characterisation of the local genotypes makes it difficult to use them in pig improvement schemes (Ncube *et al*., 2003). Furthermore, diverse roles Kolbroek pigs play are not simply quantifiable, meanwhile the information that would be derived from this study would assist in finding mechanisms for preserving the breed. Therefore, it is of utmost importance to characterise the Kolbroek pigs, to conserve them and use them in future breeding programs.

1.3 PROBLEM STATEMENT

There is limited available data on the phenotypic and reproductive characterisation of Kolbroek pigs in South Africa. Moreover, there is limited information pertaining to the age of attainment of puberty and reproductive performance in Kolbroek sows. A recent survey indicated an appalling downfall in South Africa's indigenous pigs germplasm. Thus it is of utmost importance to characterize and conserve their genetic materials as they possess a unique gene pool with characteristics of economic importance. Therefore, it is important that their phenotypic and reproductive characteristics are recorded and studied for conservation or future breeding programme purposes.

1.4 PRIMARY OBJECTIVE

To characterise the phenotypic and reproductive parameters of Kolbroek pigs.

1.5 SPECIFIC OBJECTIVES

1) To evaluate the phenotypic characteristics of Kolbroek boars and sows.
2) To correlate the testicular size with semen parameters of Kolbroek boars.
3) To detect the onset of puberty in Kolbroek pigs using hormonal assays and evaluate reproductive performance of Kolbroek sows following natural mating.

1.6 HYPOTHESIS

1) There are no differences in phenotypic characteristics between males and female Kolbroek pigs.
2) There is a correlation between testicular size and semen parameters of Kolbroek boars.
3) The onset of puberty in Kolbroek pigs is detectable using hormonal assays natural mating has no effect on reproductive performance of Kolbroek sows.

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CHAPTER 2

CHAPTER 2: LITERATURE REVIEW

2.1 HISTORY AND ORIGIN OF KOLBROEK PIGS

South African pigs comprise of a mixture of pigs introduced through diverse pathways. Kolbroek is a South African indigenous pig breed. The name "Kolbroek" originates from the name of the ship called Coalbrook which was wrecked on the Eastern Cape coast close to Betty's bay in 1778 (Hoffman *et al*., 2005). Another theory is that this breed was introduced to South Africa by the earliest traders from the Far East (Blench & MacDonald, 2000). These pigs are characterised by their "spotted" colour pattern (Figure 2.1), while it is shorter than most other "modern" pig breeds. It is fat, it has a short snout, sturdy legs, strong feet, extremely hardy and survives under harsh conditions and all these features makes Kolbroek ideally suited for free range or small holder systems. They are farmed in a way that enables them to free range and free farrow. Therefore the use of Kolbroek pigs may possibly contribute to crossbreeding programmes for improving adaptability characteristics of other pig breeds (Mapeka *et al.*, 2009). This breed is dark in colour being either black or brown and some are often striped at birth, these stripes may have been the origin of the name Kolbroek (breech markings).

Figure 2.1 Kolbroek sow with a piglet

Figure 2.2 Kolbroek boar

The Kolbroek has been reported to be an early maturing breed and grows slower than other modern pig breeds (Masenya *et al*., 2011). The advantages of Kolbroek pig is that it's extremely hardy, good forager and can survive even if they are not well fed and managed properly. The hardiness of these breeds and their good mothering ability ensures a high survival rate particularly after the first farrowing, litter size is between 8 and 10 piglets (Halimani *et al*., 2012).

2.2 OVERVIEW OF PORK PRODUCTION IN SOUTH AFRICA

The word pork refers to all domesticated pigs which are reared for the production of meat, ham, bacon, spreads, meat rolls and sausages for human consumption as well as economic benefit. The terminology used to describe pigs is unclear as they are referred to as "indigenous", "native", and/or "local". According to the Oxford Dictionary (1990) these terms are defined as:

- Indigenous: living naturally in an area; not introduced
- Native : belonging by birth to a specific area or country
- Local: native inhabitant

Hence for the purpose of this study it was decided to use the word "indigenous" for characterisation. Based on the FAO data (presented under World Pork Market), South Africa produced 338 000 metric tons of pork during 2013, representing 0.33% of the world's pork production. To date this number has slowly declined due to farmers also to keeping the smaller herd sizes in order to adequately meet the animals' nutritional requirements (Thomas *et al*., 2010), leading to small populations that are vulnerable to inbreeding and natural disasters (Halimani *et al*., 2012). What is the scope of the pork industry in South Africa?

The South African pork industry produces less than 0,5% of the world's total pork output, and slaughters approximately 2,8 million pigs per annum. There are only nine stud breeders. It is estimated that around half of all South African pork is utilised by the meat processing industry to manufacture bacon, sausages, hams and other meat products. This pork is produced by 400 commercial producers in the industry.

2.3 THE PRESENT STATUS OF SOUTH AFRICAN INDIGENOUS ANIMALS

South Africa harbours a full spectrum of indigenous livestock breeds from highly productive modern breeds of cattle, sheep, and pigs which are well adapted to the demands of modern production practices. However, recent developments have shown that South African indigenous breeds have the potential to produce competitive qualities of beef, mutton and pork meat per unit area (Smital, 2004). The advantage of farming with indigenous pigs is that they are not raised and selected in highly intensive piggeries. They are owned by economically vulnerable people in South Africa. In areas where they are utilized, they are a valuable source of food, income and expenditure (Halimani *et al.*, 2012). Moreover, these pigs have been sustained by poor communities for many years and have added values that are often not exploited but these Indigenous animals are underutilized in the mainstream economies of South Africa. According to FAO (2014), the level of extinction of indigenous pigs species that are held in each country is reaching a serious downfall. Out of the 7,600 unique breeds listed by FAO, 20% are designated as endangered, which includes pigs (18%) and chicken (33%) breeds.

The present rate of extinction, at two pig breeds per week, is expected to accelerate FAO (2014). Moreover, in developing countries, the situation is worse due to a lack of information on the existing indigenous genetic resources (Ayalew *et al*., 2003). In order to conserve the genetic diversity of livestock, it is vital to establish a genetic resource conservation system with collaboration between genetic resource conservation centres, academia, breed associations, breed supply companies and farms (DAFF, 1999). To achieve this, efforts are required from a variety of institutions, but the construction of a database with accurate information on indigenous farm animal genetic resource should head such a system. Through the construction of a detailed information system of genetic resources including information on the population size, distribution and phenotype, it is possible to grasp the status of the endangered native species and take measures to prevent extinction of the species (Dessie *et al*., 2009).

2.4 CHARACTERISATION AND CONSERVATION OF PIG GENETIC RESOURCES

The Food and Agriculture Organization (FAO) of the United Nations has proposed an integrated programme for the global management of genetic resources (Project MoDAD, on an international level (Scherf, 1995; Gandini & Olddenbroek, 1999). In addition, a communication and information system called the Domestic Animal Diversity Information System (DAD-IS) has been developed by FAO, with the main objective of assisting countries by providing extensive searchable database and guidelines for better characterisation, utilization and conservation of animal genetic resources. Such programmes are important because the Animal Genetic Resources (AnGR) has been faced with genetic dilution due to foreign or exotic germplasm use, changes in production systems, market preferences, environment changes, natural catastrophes, unstable policies from public and private sectors and the availability of very limited funds for conservation activities (Rege and Gibson, 2003).

2.5 METHODS OF CONSERVING INDIGENOUS ANIMALS GENETIC RESOURCES

Conventional methods for conserving indigenous animal breeds include *in-situ* and *ex-situ* conservation (Köhler-Rollefson, 2000). *In situ* conservation is whereby breeding population of live animals are kept in their adaptive environment along with appropriate management practices (Brem *et al.*, 1989; Henson, 1992). *Ex situ* conservation can either be *in vivo* or *in vitro*. The latter is when living are conserved outside their productive environment or where the breed is normally found, whereas the former is when genetic material is conserved in an artificial environment outside the living organism. *In vitro* is usually conservation of animal genetic material in the form of living ova, embryos or semen stored cryogenically in liquid nitrogen (Brem *et al*., 1989; Köhler-Rollefson, 2000). In view of a number of breeds that are considered endangered and the financial implications that comes with the programme to conserve Animal Genetic Diversity, it is imperative that strict selection pertaining to AnGR to be conserved be made (FAO, 2014). Selection criteria should include the following:

- Degree of endangerment
- Adaptation characteristics
- Characteristics of economic importance
- Unique characteristics

2.6 ECONOMICALLY VALUABLE CHARACTERISTICS OF KOLBROEK PIGS

The reasons for keeping Kolbroek pigs are diverse besides the fact that they require minimal care, they are hardy and resistant to diseases which is beneficial as this will save on stock medicaments (NDA, 1999). According to a survey done in Botswana on indigenous pigs in communal areas, after slaughtering for home consumption, the by-products such as fat are used to make soap and also making a lubricant in traditional leather rope manufacturing (Nsoso *et al*., 2001). In turn these ropes are used in harnessing draft animals in crop production by observation. One local commercial farmer used both indigenous and commercial pigs and combining the traditional fat with meat from commercial breeds to make sausages (Nsoso *et al*., 2001). Furthermore, the fat is also used as traditional medicine and seed preservative. These kinds of usages need to be explored to identify market related incentives (niche market) to promote the rearing of indigenous pigs.

2.7 PHENOTYPIC CHARACTERISTICS OF PIGS

Ogah *(*2011) defines phenotypic characters as the observable variation in a population that includes both genetic and environmental components. Phenotype is defined as the observable physical characteristics of an organism as determined by both genetic makeup and environmental influences. The biggest numbers of domestic livestock breeds originate in the developing countries of the world (Notter, 1999). A breed is defined as a group of animals with an even, heritable appearance (Ellis *et al.,* 1997). The main characteristics that distinguish breeds are features such as coat colour (hair and skin colour in pigs), morphological properties such as ear size and shape and body conformation (Ellis *et al.,* 1997). Livestock breeds in developing countries have been less thoroughly characterised (Notter, 1999).

2.7.1 Coat Colour characteristics

The colour of a pig depends on the breed. The chemistry of coat colour genetics has been described by (Prota & Sarle 1978; Ollivier & Sellier, 1982; Hur *et al*., 2013). One of the first phenotypic characteristics that has been modified during the domestication process in livestock (Fontanesi & Russo, 2013). A survey was done by Olivier & Sellier (1982) to evaluate the range of phenotypic variation and to make an initial evaluation of the phenotypic frequencies of pig coat colour patterns in the villages and concluded that a variant of spotted was seen in which the black patches are replaced by brown ones. The overall coat colour of indigenous pigs varies from black in Papua, New Guinea; with white patches to black and white/ginger (Chinese indigenous pigs). Prominent white spots (which should be round) are normally found on the flanks and hindquarters of the animal. In pigs, domestication and selection have produced a large variety of coat colours and patterns that are characteristics of different breeds and populations (Porter, 1993; Legault, 1998). Coat colour in *Sus scrofa* has been the matter of pioneering genetics studies carried out at the beginning of the last century, just after the re-discovery of the Mendel's laws (Spillman, 1906; Spillman, 1907).

2.7.2 Head and Ear characteristics

Indigenous pigs have concave heads (Giuffra *et al.*, 1999). The snout of wild pigs are a bit longer than of domestic pigs. There are two pairs of tusks protruding from the head. The whole body is covered with coarse, bristly hair. The ear set varies between breeds: (i) erect, (ii) semi-erect or (iii) droopy, with a tendency to drop to the front. Domestic pig males have tusks, but never as big as wild boars (Giuffra *et al*., 1999). Furthermore, the latter have two pairs of them, one in the upper jaw and one in the lower jaw. These tusks are big that they don't fit into the mouth and protrude outside, wrapping the upper lip.

2.7.3 Body and Tail characteristics

The body frame of indigenous pigs is small and short of length compared to exotic pig breeds (Lauvergne, 1982). Some indigenous pigs have a potbelly, which is a characteristic of the breed. Furthermore, the fat belly almost reaches the ground and contains an accumulation of lard/fat (Lauvergne, 1982). The legs are short, generally down on the pasterns with fine bone, none of which seem to hinder their gait or movement which makes them extremely good walkers. The hooves are dark in colour and can grow out substantially due to lack of exercise**.** There are a few pigs with long hair. Logically it is not usual for pigs to have long hair because they are not raised for their hair but their meat. The Kune Kune is a hairy small domestic pig breed that originates from New Zealand it is characterized by pirpiri or wattles on its lower jaw whereas Kolbroek pigs have straight, long and dense hair (Drickamer *et al.,* 1999). It also comes in different colours such as black and white, cream, and ginger, black and brown skin. The size and placement of the tail in pigs can vary between breeds and individuals within a breed. In most cases indigenous pigs are found to have short tails that are often curly. A pig which is sound tends to have a flatter top, a more level rump and higher tail setting.

2.7.4 Morphometric characteristics

Morphological characters have been used to detect genetic relationships between breeds in different domestic species (Jordana *et al.*, 1993; Herrera *et al.*, 1996). Traditionally, pigs are simply selected for breeding purposes if they have adequate number of teats, with the threshold often set to 14 functional teats (Rydhmer, 2000). However, in South Africa the threshold for number of teats is set at twelve for indigenous pigs meaning that each sow must have atleast six well positioned functional teats on each side (Drickamer *et al.,* 1999). The number of teats signify the main phenotypic characteristics for reproduction and mothering ability of the sow.

2.8 REPRODUCTIVE CHARACTERISTICS OF THE SOW

The economy of pig production are based on the ability of the sow to reproduce and nurture her piglets efficiently at the estimated cost and in good time (Hernandez *et al*., 2014). The pig is widely recognized as a non-seasonal polyestrous species, meaning that they can reproduce at any time of the year (Dunkin & Taverner, 1996). The reproductive system of a mature sow comprises of a pair of ovaries, joining to the ovaries is the uterus which consists of two horns and a 5cm body (Dunkin & Taverner, 1996; Phiri, 2006). The ovaries (female gonads) are considered the primary reproductive organs for the female because they produce gametes (ovum) and the female sex hormones (oestrogen and progestins). The cow, mare and ewe are considered monotocous because they normally give birth to one offspring per each gestation period, therefore one ovum is produced in each oestrous cycle. The sow on the other hand is polytocous, producing 10 to 15 ova in each oestrus cycle and giving birth to numerous offpring (Bearden & Fuquay, 2000). The physiological and behavioural changes associated with the oestrous cycle are controlled by hormones produced by endocrine glands. Gonadotropin releasing hormone (GnRH) is released from a specific area of the brain called the hypothalamus, travels through blood vessels to the pituitary gland, and there stimulates secretion of Follicle Stimulating Hormone (FSH) and Luteinizing hormone (LH). During the two to three-day period just prior to oestrus, increasing blood levels of FSH and LH cause follicles to rapidly grow on each of the two ovaries (Cassar, 2009). These follicles secrete increased levels of the hormone oestrogen into circulation, which in turn causes the behavioural and physiological changes associated with oestrus (e.g., reddening and swelling of the vulva, lordosis or the "standing response" in the presence of a boar, etc. Rising concentrations of oestrogen eventually triggers increased secretion of GnRH, resulting in a massive release of LH during oestrous (Cassar, 2009). This "LH surge" stimulates the process of ovulation. Multiple ova, or eggs, are released from the follicles on the ovaries during the process of ovulation, which occurs approximately 40 hours after the onset of oestrous (Cassar, 2009). Each ovulation site on the ovaries subsequently forms a structure called a corpus luteum (CL) which secretes another hormone, progesterone, into the circulation.

2.8.1 Oestrous cycle

Following attainment of puberty, gilts display spontaneous ovarian and oestrous cycles of about 21 days throughout their lives, interrupted only by pregnancy or lactation (Hughes & Varley, 1980). According to Hughes & Varley. (1980), the oestrous cycle is characterised by periods of reproductive inactivity, lasting 18 to 20 days followed by short periods of sexual receptivity having a mean duration of 53 hours with a range of 12 – 72 hours.

The porcine oestrous cycle can be divided into 3 different phases, namely:

(i) Luteal phase (Di-oestrous)

The luteal phase is characterised by the secretion of progesterone from of the corpus luteum. The corpus luteum is derived from the luteinization of the cells of the follicle after ovulation. In pigs this phase lasts about 16 days, if there is no pregnancy, the uterus secretes prostaglandin PGF2alfa that induces luteolysis and therefore a drop in progesterone secretion. In absence of progesterone a new cycle begins and a new set of follicles can ovulate.

(ii) Follicular phase (Pro-oestrous)

The phase immediately preceding oestrus. It lasts to 5 days, depending on the species. Marked increase in reproductive activity. Endocrine transition from progesterone to oestrogen dominance under the influence of gonadotrophins LH and FSH. Follicular growth and regression of the corpus luteum of the previous cycle (in polyoestrous species). Uterus enlarges slightly Endometrium becomes congested and oedematous, glands show secretory activity. Vaginal mucous becomes hyperaemic. Increase in cell numbers in the vaginal epithelium. Superficial layers become cornified.

(iii) The oestrous period which is the period of sexual activity

The period where the female will accept the male for copulation. The onset and the end of oestrus are the only stages of the oestrous cycle that can be accurately measured and used to determine the cycle length. Female seeks out the male and 'stands' to be mounted. Uterine, cervical and vaginal glands secrete increased amounts of mucus. Vaginal epithelium and uterine endometrium becomes hyperaemic and congested. Cervix is relaxed Ovulation occurs during this phase in all domestic species except the cow. The cow ovulates ~12 hours after the end of oestrus.

Ovulation is spontaneous in all domestic species except the cat, rabbit and camelids, which is induced by the act of copulation. During pro-oestrous and oestrus there is follicular growth with no functional corpora lutea present on the ovary (follicular phase). The main ovarian hormone production is Oestrogen.

2.8.2 Oestrous synchronization and heat detection in sows

One of the most critical components of a successful piggery is to accurately detect oestrus. The duration of oestrus is variable, but gilts average 38 hours and sows average 53 hours. In response to high concentrations of Estradiol in the blood, all or some of the following signs may be exhibited by a sow or gilt approaching or in oestrus:

1. Red, swollen vulva and enlarged clitoris,
2. Mucous discharge from the vulva,
3. Nervous, restless behaviour,
4. Moving back and forth along pen partitions,
5. Frequent urination,
6. Increased vocalization,
7. Decreased appetite,
8. Mounting other females and/or standing to be mounted by other females, and
9. Elevation of ears (pinning ears), locking knees and elevating the back (immobilization or lordosis response).

The immobilization response is the best indicator that sows are in oestrus and ready to be mated. Sows and gilts in oestrus exhibit the immobilization response as a reaction to a combination of visual (sight), auditory (sound), olfactory (smell) and tactile (touch) stimuli originating from the boar (Britt *et al.*,1999). Obviously, the best effective oestrus detection system is one that employs all of these stimuli. Thus, wherever possible, producers should place a mature (at least 12 months of age) oestrus detection or "heat-check" boar in physical contact with the sows or gilts being checked for oestrus.

The mature ova are normally released from the ovary during the second half of the oestrous period normally within the range of 38 to 42 hours after the onset of oestrous. Ovulation in the pig occurs 38-42 hours after the onset of oestrous (Soede *et al*., 2011). Soon after ovulation, the walls of the raptured follicle collapse. The granulosa cells of the raptured follicle then undergo changes known as luteinisation. Hypertrophy of these luteal cells results in rapid increase in the size of the collapsed follicle and then it develops in to a functional *corpus luteum*. Assuming that no fertilization took place there is a rapid decline of the *corpus luteum* (Hughes & Varley, 1980). However, a sow cannot conceive if mating does not take place during the appropriate period of the oestrus cycle. Morphological changes in the ovary are responsible for hormonal changes in the outlying circulation during the estrous cycle (Dunkin & Taverner, 1996).

2.9 REPRODUCTIVE CHARACTERISTICS OF THE BOAR

The male reproductive characteristic consists of the scrotum, spermatic chords, testes, accessory glands, penis and the prepuce. The testes are the primary organ of reproduction in the male (male gonads) just like the ovaries in females. According to Bearden & Fuquay (2000) the testis is the primary organs of reproduction because they produce male gametes (spermatozoa) and sex hormones (androgens). For a mature boar the testis is a large organ about the same size as that of a bull and weighs as much as 300 to 500g. It is about 10 – 13 cm long and 5 – 6.5cm wide (Bearden & Fuquay 2000).

2.9.1 Collection of boar semen

There are different methods applied in the collection of boar semen. The first one is artificial vagina (Strzežek, 1995). This method is only appropriate if the female is on standing heat and it requires regular check-ups of visible signs for heat in the mornings and late in the afternoon and lastly the size of the female should match that of the boar. The second one is the dummy sow and because not all boars are capable of mounting the dummy sow they should be trained in order for the boar to learn to associate the dummy sow with ejaculation and pleasure (Strzežek, 1995). Young boars are easily trained to mount the dummy sow but boars which are used for natural mating are more difficult (Shipley, 1999). The dummy must be set at the right height, must be stable and have no sharp projections. The smell of another boar's semen, saliva and sow urine on the dummy helps stimulate the trainee boar to mount and thrust.

Figure 2.3 Semen collections from Kolbroek boar using a customized gloved hand technique method at ARC

2.9.2 Semen analysis

Semen analysis is an essential tool in assessing the fertility of boars (Masenya *et al.*, 2011). Determining the initial quality of a boar ejaculate is the first step in semen processing and should ensure that prior to further processing, a high quality artificial insemination dose of semen will be produced. Effective screening methods for ejaculates prior to processing are necessary for improving on farm reproductive performances. Ideally, ejaculates that are thoroughly evaluated prior to processing help identify poor quality semen. Daily evaluations of gross motility and morphology of stored semen sample will ensure that due to some unforeseen reason, deteriorated semen doses are not used at the farm level (Rozeboom, 2001).

2.9.3 Semen volume

The semen volume is measured in a warm, dry calibrated flask. The volume of semen varies between 50 - 500mL (Sutkeviciene *et al*., 2009). Main boar ejaculate volume is 250 ml, with variation between 100 and 1200 ml (Holt *et al*., 2007). Large volume of semen does not necessarily mean the total sperm content is greater than that from smaller ejaculates. The colour of semen is creamy white but a good ejaculate is very milky. The ejaculate becomes watery if the boar is overused. In boars, ejaculate volume varies in relation with age, individual, frequency of ejaculation, feeding, health status, method of collection, collection time and stress. Younger boars have a smaller ejaculate volume, arriving to maximum at sexual maturity and the level remains increased until 7 - 8 years (Aparicio *et al*., 2005; Holt *et al*., 2007).

2.9.4 Semen pH

Semen pH is an important indicator of seminal quality (Frunză *et al*., 2008). According to King and MacPherson (2005), the pH of boar semen fraction should be 7.69. Strzezek *et al*.(1995) had determined boar semen pH from four different breeds and obtained the following results: Landrace 7.1 Yorkshire 7.1 Duroc 7.1; Chester 6.9 a high sperm pH denotes a low quality sperm or the presence of an infectious process in the genital tract or in accessory sex glands (Strzezek *et al*.,1995). Within the species and certainly in the same male, sperm pH can differ from one ejaculate to another (Newth, 1999). The explanation is that inside the epididymis pH mean is 6.10 and can oscillate between 5.57 and 6.9 Paulez *et al*. (2000). Accessory sex gland contribution with lower or higher secretion levels to the ejaculate, will determine an alkaline or more acid reaction. Semen pH changes negatively influence sperm viability and motility (Estienne & Harper, 2004; Kamp *et al*., 2003; King & Macpherson, 2005).

2.9.5 Sperm concentration

Determining sperm concentration or total number of sperm cells is not a component of semen quality but more so as a means to monitor the health and productive output for improving the genetic potential of the boar (Rozeboom, 2001). Sperm concentration is an important indicator of sperm quality (Frunză *et al*., 2008). Furthermore, the normal ejaculate of a boar is 100-500mL with a total sperm concentration of 10-100 billion sperm cells per ejaculate (Frunză *et al*., 2008). Consequently, boar sperm is 5-10 times less concentrated than bull sperm and approximately equal in concentration with stallion sperm (Cheon, 2002). According to Hafez (1993), the mean sperm concentration of boars is 0.1 - 0.2x10^8 spermatozoa/L.

In a study done by Strzezek *et al*. (1995), ejaculates from four different breeds of boars obtained the following values for sperm concentration: Landrace 286x 10^6 sperm/ml; York 211x 10^6 sperm/ml; Duroc 315x 10^6 sperm/mL; Chester 363 ± 50.16 x 10^6 sperm/mL. Accurate assessment of sperm numbers is not the only factor for increasing semen doses per ejaculate and boar stud efficiency in terms of semen output. In recent years, artificial insemination (AI) has been widely implemented in the production of pigs. The quality and quantity of semen used for artificial insemination is determined by the main parameters; sperm volume, sperm concentration, number of live sperm and number of insemination doses obtained from one ejaculate after dilution (Smital *et al.,* 2004). Therefore, the choice of boars is becoming increasingly important and urgent to the pig industry (Ren *et al.,* 2009). Understanding the basic genetic differences between the male reproductive characteristics of different breeds of boars is necessary to increase the efficiency of sperm production in insemination stations (Safranski, 2008).

2.9.6 Sperm motility

Previously, subjective assessment for sperm motility was used however due to these biases, emphasis has been placed on the use of objective methods such as Computer Aided Sperm Analysis (CASA). Sperm motility depends on how much of each fraction is collected if the collection is mainly sperm rich fraction there is higher motility (Cheon, 2002). According to Flowers (1997) sperm motility is then estimated to the nearest 5% by viewing groups of sperm in at least four different fields on the slide at 200 or 400X; these readings are then averaged. Only ejaculates with at least 70% gross motility should be used for further processing. This is especially important because sperm motility and viability normally decrease during storage. If ejaculates are used shortly after collection, samples with a minimum of 60% motility can be used for AI. Dilution rate of raw semen can be established based on various parameters. CASA is used for evaluation of younger and adult boar semen, in relation to subsequent fertility for emphasizing special sperm features such as hyperactivation or presence of sperm subpopulations (Mircu *et al*., 2008). In order to achieve all these goals, each ejaculate has to be well managed, in order to generate good fertility parameters, and the crucial problem is represented by the dilution rate (Mircu *et al*., 2008).

2.9.7 Sperm morphology

Sperm morphology and acrosome integrity are effective tools in estimating sperm viability and provides more information regarding the ejaculate in terms of quality, since sperm abnormalities have been linked to poor fertilization (Knecht *et al*., 2014). However, having higher amounts of abnormally shaped sperm has been associated with infertility in some studies. Usually, higher numbers of abnormally shaped sperm are associated with other irregularities of the semen such as low sperm count or motility (Graham *et al*., 1990). Therefore, to conduct sperm morphology evaluation and accentuate the outline of the sperm, stains are used under a light microscope. Many different staining techniques have been devised for examining sperm morphology. A nigrosin-eosin stain is commonly used because it is effective, simple and allows sperm to be readily visualized (Garner *et al*., 1994). However, much emphasis has been directed toward using combinations of stains to determine sperm viability. One of the first attempts to assess sperm viability Rhodamine 123 (R123) was utilized (Evenson *et al.,* 1992). Other combinations that have been used to examine the functional capacity of sperm are carboxyfluorescein diacetate (CFDA) and Propidium Iodide (PI) (Thomas & Garner, 1994). SYBR-14, in combination with PI, is used in estimating the proportions of living and dead sperm in both fresh and cryopreserved porcine semen samples (Garner, 1996).

Table 2.1 Overview criteria to use for pigs semen in artificial insemination. Adapted from (Masenya *et al*., 2011).

Semen parameters	**Requirements**			
	Kuster & Althouse, (1999)	**Martin-Rillo *et al*, (1996*)**	**Shipley, (1999)**	**Britt *et al*, (1999)**
Abnormal morphology	<20		<20	
Normal acrosome		70-100		
Cytoplasms droplets	<15		<15	
Proximal droplets		0-20		
Coiled tails		0-30		
Primary abnormalities		0-4		<10
Secondary abnormalities				<20

* Recommendation for 2 x 10^9 sperm per dose.

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CHAPTER 3

Phenotypic Characterisation of Kolbroek pigs

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3.1 Abstract

In domestic pigs (*Sus Scrofa domestica*), phenotypic characterisation is one of the most important economic traits for improved pig production. The objective of the study was to evaluate the phenotypic characteristics of Kolbroek pigs. A total of 57 Kolbroek pigs (34 sows and 23 boars) were used in this study. Data for various phenotypic characteristics were collected by visual appraisal. Body weight, body length, chest girth, head length, width of head, ear length, hair length, tail length were collected using a measuring tape and the number of teats were counted. Data were analysed using the Statistical Analysis System (Chimonyo *et al.*, 2005). The predominant coat colour pattern of the pigs was a patchy black and white colour type. All the pigs had

straight long and dense hair and tusks were present only in boars. In all the descriptive traits, majority of the sows had a rough skin and ears which projected forward (57.89%). Moreover, majority of boars had erect ears (69.67%). All boars and sows had concave heads with a short and cylindrical snout, a straight tail with sows having an average of 10 teats. The morphometric results revealed that there was no significant (P>0.05) difference between pigs for morphometric characteristics, irrespective of the gender. However, a significant (P<0.05) difference was observed for body weight between boars (114kg) and sows (123kg). There was also a significant (P<0.05) difference for body length between boars (95cm) and sows (105cm). This study showed significant differences between Kolbroek pigs in diversity exemplified by phenotypic and morphological characteristics; consequently, useful in selection, identification and conservation of South African indigenous pigs.

Keywords: kolbroek pigs, body weight, descriptive characteristics, morphometric characteristics, Conservation

3.2 INTRODUCTION

Kolbroek is a South African indigenous pig breed (Masenya *et al.*, 2013). It is an extremely fatty pig breed, fairly uniform with phenotype of unknown origin. Although slow in growth performance, these pigs play an important role in human nutrition, food security, alleviation of poverty, improved livelihoods and in the creation of employment for rural communities (Antwi & Seahlodi, 2011; Dietze, 2011; Mergenthaler *et al.*, 2009). According to the Department of Agriculture Forestry and Fisheries (DAFF, 2013), the pork industry in South Africa contributes about 2.2% to the primary Agricultural sector. This low contribution of pork entail that there is a need for utilization of indigenous pigs to support sustainable agricultural development. Indigenous pigs are underutilized in the conventional economies of South Africa (Halimani *et al.,* 2012). Numerous factors contributing to this such as are traditional biases in the meat and carcass grading system, prejudice against indigenous pigs and lack of markets and market orientation (Halimani *et al.*, 2010; Halimani *et al.,* 2012).

A recent survey indicated a significant decrease in the population of South Africa's indigenous germplasm (FAO, 2007). This downfall was attributed to unplanned breeding, cross breeding and the introduction of imported exotic genes (Scholtz, 2005; Masenya *et al.*, 2013). The erosion of the gene pool of indigenous livestock breeds in South Africa is a cause for concern as they signify a valuable contribution to the rich biodiversity of the land (Nedambale *et al.*, 2008). Furthermore, Kolbroek pigs are particularly vulnerable as inbreeding may lead to genetic dilution and even loss of genetic differences within breeds and ultimately lead to extinction (Berthouly *et al*., 2012). Therefore, phenotypic characterisation is critical as it provides useful information on the suitability of animals for selection and definition of unique characteristics. It also includes the description of the animal's physical characteristics, productive parameters, status of the breed and mating systems thus allows distinction between breeds (ANGR, 2006). Hence the objective of the study was to phenotypically characterise the South African Kolbroek pigs.

3.3 MATERIALS AND METHODS

3.3.1 Study area

The study was conducted at Agricultural Research Council (ARC), Germplasm Conservation and Reproductive Biotechnologies (GCRB) at Irene Pretoria, South

Africa. The ARC is situated at 25^0 55' South; 28^0 12'East. The institute is located in Highveld region of South Africa and situated at an altitude of 1525m above sea level.

3.3.2 Experimental animals

A total of 57 indigenous Kolbroek pigs were used in the study comprising of 34 sows and 23 boars due to the limited number available of these Kolbroek pigs in conservation programme. The Kolbroek pigs were sexually matured (>2 years) and were fed boar and sow feed which was formulated to meet the nutritional requirements of the pigs (National Research Council (NRC), 1998. Water was provided *ad-libitum* throughout the duration of the study. Experimental pigs were cared for according to the Agricultural Research Council, Animal Production Institute Ethics Committee (Ref: APIEC14/032).

3.3.3 Data collection

The study was conducted in summer from 31 October to 30 November 2014. Prior to data collection, a data sheet was prepared in accordance with the objective of the study and the standards of Commission on Genetic Resources for Food and Agriculture (CGRFA, 2011). The descriptive characteristics were taken by visual appraisal and the morphometric characteristics were taken using a measuring tape. All measurements were carried out by the same person to avoid differences among individuals **(Table 3.1)**. Descriptive characteristics such as hair, tusks, snout, coat colour pattern, coat colour type, head profile, ear type, ear orientation, skin and tail type were recorded in both sows and boars based on subjective visual appraisal. The body weight was taken before measuring using a weighing scale.

Table 3.1: Phenotypic morphometric characteristics

Character	Description
Body length	Length from the base of the tail to the middle of the shoulder blade along the back on the midline
Head length	External occipital protrusion occipital to the tip of the nasal bone
Tail length	From attachment of the tail to the tip of the tail
Ear length	Distance between the tip of the ear and the base,
Chest girth	Total distance around the animals circumference measured directly behind the animals forelegs height midpoint of the shoulder blade to the floor in the perpendicular plane
Number of teats	Recorded by counting teats individually

3.4 DATA ANALYSIS

Data analysis was performed with Statiscal Analysis System version 9.3 (SAS, 1999). The data was subjected to an appropriate analysis of variance (ANOVA). The Shapiro-Wilk's test was performed on the standardised residuals to test for deviations from normality (Shapiro and Wilk, 1965). Student's t-LSD (Least significant difference) was calculated at a 5% significance level to compare means of significant source effects (Snedecor and Cochran, 1980).

3.5 RESULTS

Descriptive characteristics of Kolbroek pigs are presented and summarised in Table 3.2. Approximately (95.0%) of Kolbroek pigs had straight long and dense hair, short and cylindrical snout (100%) a patchy black and white coat colour type (73.9%) with tusks present in boars only. Furthermore, the majority of boars (69.7%) had erect ears as well as sows (55.8%). The head profile in Kolbroek pigs was concave, the skin was rough with straight tails and ears projecting forward.

Table 3.2: Descriptive characteristics of Kolbroek pigs

Descriptive Traits	Sows (34)	Boars (23)
Hair		
Straight long and dense	95.0%	95.0%
Short	4.9%	4.9%
Tusks		
Present	0%	100%
Absent	100%	0%
Snout		
Short and cylindrical	100%	100%
Long	0%	0%
Coat colour pattern		
Patchy	100%	100%
Coat colour type		

Black and white	73.9%	73.9%
Black and Fawn	26.1%	26.1%
Head profile		
Concave	100%	100%
Convex	0%	0%
Ear type		
Droopy	41.2%	30.3%
Erect	55.8%	69.7%
Ear orientation		
Project forward	98.5%	69.7%
Project upward	1.5%	30.3%
Skin		
Smooth	42.1%	42.1%
Rough	57.8%	57.8%

Tail		
Straight	100%	100%
Curly	0%	0%

3.6 DISCUSSION

Phenotypic Descriptive Characterisation

3.6.1 Coat Colour Characteristics

The colour combination of the Kolbroek boars and sows were black and white (73.9%) and black and fawn (26.1%). In contrast, the coat colour for indigenous pigs from Southern Africa had been previously recorded as predominantly black (Chimonyo *et al.*, 2005), black with white stripes along the body length (Ncube *et al.*, 2003; Dandapart, 2012), light brown coat colour in wild pigs and a black coat colour in village pigs (Subalini *et al.*, 2011).

Figure 3.1 Coat colour characteristics of Kolbroek pigs

3.6.2 Head and Ear Characteristics

The shape of the head in all the Kolbroek pigs were concave (100%). The snout was short and cylindrical in shape and the tusks were only present in boars. In sows, the ear type was erect (55.8%) and projected forward (98.5%). Majority of the Kolbroek pigs had erect ears (55.8%) that projected forward (98.5%). Similarly, it was reported that the Moo Nonghad local pigs in Lao Peoples Democratic Republic are characterised by erect ears which project forward (Saukanh *et al.*, 2011). In contrast, native pigs at Bangladesh had erect ears that projected backwards (Ritchil *et al*., 2014).

3.6.3 Body and Tail Characteristics

In the present study it was found that Kolbroek pigs have straight tails (100%). Ritchil *et al*. (2014) reported that straight tails are common in indigenous pigs at Bangladesh. Furthermore, majority of the Kolbroek pigs had a rough skin (57.9%). The results also revealed that (96%) of Kolbroek pigs have straight long and dense hair.

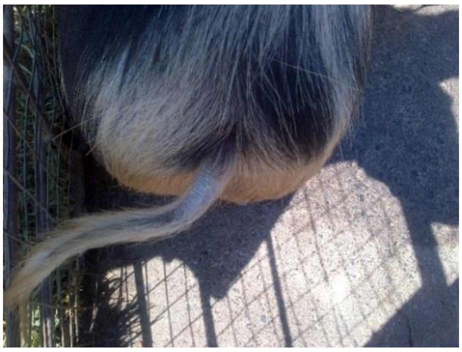

Figure 3.2: Body and tail characteristics

3.7 Morphometric Characterisation

Morphometric characteristics of Kolbroek pigs were presented and summarized in Table 3. The mean live body weight of sows (123.4kg) and boars (113.6kg) recorded show that the Kolbroek pigs are generally smaller than commercial pig breeds. Similarly, Holness (1991), reported that indigenous pigs are smaller in size than commercial breeds and this small size may yield a greater ability to survive under harsh conditions than greater sized breeds as an evolutionary adaptation to conditions of low input production (Lukele & Kyvsgaard, 2003;Borkotoky, 2014). Boars tend to have bigger body conformation than the females (Subalini *et al.*, 2010), however in the present study the Kolbroek sows had a higher bodyweight than the boars. Similar reports were observed in Moo Nonghad and Moo Hmong indigenous pigs in Lao PDR (Keonouchanh *et al.*, 2011) and the local Mali pigs in Tripura India (Dandapat *et al.*, 2010).

Table 3.3: Morphometric Characteristics of Kolbroek pigs (mean ± SD)

	Sows (n=34)	**Boars (n=23**
Morphometric characters	**Mean ± SD**	**Mean ± SD**
Body weight (kg)	123.40 ^{a} ±24.97	113.58 ^{b} ±18.78
Body length (cm)	105.24 ^{a} ±25.36	95.11 ^{b} ±16.09
Head length (cm)	20.04 ^{a} ±4.35	21.26 ^{a} ±2.88
Tail length (cm)	19.68 ^{a} ±3.26	20.94 ^{a} ±2.75
Ear length (cm)	14.12 ^{a} ±2.69	15.72 ^{a} ±2.15
Chest girth (cm)	128.99 ^{a} ±17.10	130.95 ^{a} ±12.62
Body height (cm)	67.51 ^{a} ±9.41	71.85 ^{a} ±5.21
Teat number (count)	±10	

^{ab}Means within a row with the different superscripts are significantly different ($P < 0.05$). n= number of animals

Kolbroek pigs had a body length of 95-105cm, girth circumference of 129-130cm and height of 68-72cm. Similar results were observed for body length and chest girth in Lao PDR pigs (Keonouchanh *et al*., 2011). In contrast, the average body length and chest girth of Sri Lanka and Bangladesh village pigs were relatively smaller than

Kolbroek pigs (Subalini *et al.*, 2010; Ritchil *et al.*, 2014). The head length of the sows (20.04cm) and boars (21.26cm). The mean ear length in sows (14.12cm) and boars (15.72cm). The wider head, the erect ears projecting forward clearly indicates that the Kolbroek pig represents a largely unselected group of pigs (Sonaiya, 1986). The mean tail length of Kolbroek sows (19.68cm) and boars (20.94cm) with sows having an average of 10 teats. Similar results were also observed in tail length in village pigs in Sri Lanka. According to Yaetsu *et al*. (1987) and Ritchil *et al.* (2014), indigenous pigs from different regions of Bangladesh had four to six teats. A pattern of five pairs was most frequent in all the populations examined which is similar to the results of the present study.

3.8 CONCLUSIONS

Kolbroek pigs have a patchy black and white coat colour. Kolbroek pigs have a distinguishing coat colour pattern, it is unique and differentiates it from other indigenous pig breeds which are predominantly black (e.g. Mukota in Zimbabwe, Windsnyer in South Africa, Mali pigs in Tripura India). The hair was straight long and dense and the tusks were present in boars only. The head is concave in form and the ears are erect and projected forward. It was also found that the effect of gender was not significant on all body weights and measurements in Kolbroek pigs, except for body length. Kolbroek pigs have the potential to be developed in order to contribute significantly to the indigenous pig industry. Kolbroek pigs should be conserved to prevent from being extinct because they are hardy and adaptable to harsh environmental conditions. These fatty pig breeds reveal both national value and tremendous economic potential. Thus their preservation and propagation needs comprehensive collaboration of commercial, government sectors and researchers. Descriptive and Morphometric analysis are useful in breed comparisons it is thus recommended that advanced phenotypic characterisation studies on all South African Kolbroek pigs should be undertaken with more number of Kolbroek pigs. Detailed characterisation of Kolbroek pigs including DNA profiling, estimates of genetic distance are needed to establish the distinctiveness of the breed.

3.9 ACKNOWLEDGEMENTS

The authors wish to acknowledge National Research Foundation (NRF), Southern African Science Service Centre for Climate Change and Adaptive Land Management – Council for Scientific and Industrial Research (SASSCAL), Department of Agriculture Forestry and Fisheries (DAFF) and Agricultural Research Council (ARC) Professional Development Program (PDP) for funding the project. The Germplasm Conservation and Reproductive Biotechnologies (GCRB) staff for the support and assistance.

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CHAPTER 4

Relationship between testicular size and semen parameters of Kolbroek boars

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4.1 Abstract

Measurement of the testicular size is an important part of evaluation of the breeding soundness of breeding boars. The objective of the study was to correlate scrotum size and semen parameters of Kolbroek boars. A total of three Kolbroek boars were selected and used for this study. The testicular length was taken using a Vanier calliper. A total of 15 ejaculates were collected using a gloved hand technique method twice weekly from February to March. During collection the semen was filtered through a gauze inside a pre warmed (37^{o}C) insulated thermos flask and only sperm rich fractions were collected. Semen samples collected were transported to the laboratory within 30 minutes. Upon arrival, semen volume was measured using graduated falcon tube, pH was measured using litmus paper and sperm motility parameters were evaluated using CASA. Analysis of variance (ANOVA) was used to test the differences between the boars. The average bodyweight of the Kolbroek boars was 65.9kg. There was no significant difference in the length of the right (103.3 cm) and left (110cm) testicles between the three boars. Furthermore, there was a high positive correlation between semen volume and testicular size (r=0.90). There was a highly positive correlation between bodyweight and testicular size (r = 0.90) of Kolbroek boars. There was also a highly positive correlation between body weight and semen pH (r = 0.91) and testicular size (r = 0.66). Furthermore, a highly positive correlation was observed in body weight, semen volume (r = 0.80) and testicular size (r = 0.90). Likewise, a highly positive correlation was also observed in body weight and sperm concentration (r = 0.90). Lastly, there was a highly positive correlation between total motility, testicular size (r = 0.78) and semen pH (r = 0.78). No significant differences were observed in sperm concentration (5.0±1.3 x10^6sperm/ml), pH (7.0), total motility (94.8%) and morphology (73.9%) between the three boars. In conclusion, testicular size and bodyweight of Kolbroek boars was positively correlated with ejaculated semen volume. The results indicated that measurements of testis size, body weight and semen parameters can accurately guide the assessment of the reproductive performance of boars.

Keywords: Kolbroek boars, semen quality, testis size

4.2 INTRODUCTION

Measurement of testicular size in young boars is one of the basic selection criteria in young boars. The growth and development of the testes may be an indicator of the reproductive performance of the boars (Webb *et al*., 2006) and develop the testicular size at a young age (Mccoard *et al*., 2003). In boars, testis size can only be determined by the assessment of the testis volume using the formula described by (Esbenshade, 1999). The breeding value of a boar depends on the quality and quantity of semen produced (Kondracki *et al*., 2012). Testis size has been correlated with the sperm and testicular morphology (Umesiobi, 2006). Ejaculate variation is influenced by many factors like season of the year (Wysokinska & Kondraki, 2014), age of the boar (Deka *et al*., 2002) and utilization intensity (Savić *et al*., 2013). There are also differences in fertilization efficiency, fertility and overall semen evaluation (Kondracki *et al*., 2012). A high frequency of sperms with morphological abnormalities is an important indicator of declining boar fertility. Boars can differ with respect to the frequency of sperms with morphological abnormalities (Wysokińska & Kondracki, 2009). Assessment of sperm motility, morphology and viability is considered as a major component of the spermiogramme or semen parameters as it relates to fertility of the ejaculate (Saravia *et al*., 2007). Visual motility assessment is subjective by nature which is used to determine the quality of boar ejaculates (Tejerina *et al*., 2008). Subsequently, for more objective assessment using conventional image analysis or particle counting, Computer-Assisted Sperm Analysis (CASA), with different software designs has been developed (Tejerina *et al*., 2008). The aim of the study was to correlate testicular size with semen parameters in Kolbroek boars.

4.3 MATERIALS AND METHODS

4.3.1 Study area

The study was conducted at Agricultural Research Council (ARC), Germplasm Conservation and Reproductive Biotechnologies (GCRB) at Irene Pretoria, South Africa. The Agricultural Research Council is situated at 25^0 55’ South; 28^0 12’East. The institute is located in Highveld region of South Africa and situated at an altitude of 1525m above sea level.

4.3.2 Experimental animals

A total of three indigenous Kolbroek boars from ARC conservation program were used in the study due to scarcity of indgenous Kolbroek boars. The boars were housed in individual pens at pig testing centre throughput the duration of the study. The Kolbroek boars were 8 months of age and were fed boar and sow ration which was formulated to meet the nutritional requirements of the pigs (National Research Council (NRC), 1998. Water was provided *ad libitum* throughout the duration of the study. Experimental pigs were cared for according to the Agricultural Research Council, Animal Production Institute Ethics Committee (Ref: APIEC14/032).

4.3.3 Semen collection and processing

A total of fifteen ejaculates from Kolbroek boar were collected using a gloved hand technique method twice weekly from February to March. During collection the semen was filtered through a gauze inside a pre warmed (37^{o}C) insulated thermos flask and only sperm rich fractions were collected. Semen samples were collected and transported to the laboratory within 30 minutes. Upon arrival, semen volume was measured using graduated falcon tube, pH was measured using litmus paper. Sperm concentration was measured using the spectrophotometer (Jenway 6310 spectrophotometer, Bibby Scientific, England) using the formula for pigs (Dilution factor) *[21.39*(Absorbance) – 1.09] and was recorded in billion (x 10^9 sperm/mL).

Semen was firstly evaluated undiluted on the Computer Aided Sperm Analysis (CASA). Sperm motility and velocity characteristics were evaluated using CASA. Semen dilution took place immediately after the assessment using a short-term boar semen extender, Beltsville Thawing Solution (BTS) Version 13525/0100 Antibiotic free, Minitube International, Verona, USA.

4.3.4 Assessment of plasma membrane integrity

Plasma membrane integrity was assessed through SYBR-14/Propidium Iodide (PI). In the first case, the LIVE/DEAD Sperm Viability Kit (Molecular Probes) was used according to the protocol described by Garner & Johnson (1995). Briefly, sperm samples were incubated at 38ºC for 10min with SYBR-14 at a final concentration of 1 µl, and then with PI at a final concentration of 5µl for10min at the same temperature. 5µl of the sample was placed on a microscope slide for evaluation. After this assessment, three sperm populations were identified:

i. Viable green-stained spermatozoa (SYBR);
ii. Non-viable red-stained spermatozoa (PI), and
iii. Non-viable spermatozoa that were stained both green and red (SYBR-14/PI).

4.3.4 Testicular Measurements

The testis size was measured using a Vanier calliper. The length of the testes was measured by placing a fixed arm of the calliper at the proximal end and at the sliding arm at the distal end of the testes width of the left and right testes.

4.4 Data analysis

Data analysis was performed with SAS version 9.3 statistical software (SAS, 1999). The data was subjected to an appropriate analysis of variance (ANOVA). Pearson correlations coefficients was used among scrotal measurements. Student's t-LSD (Least significant difference) was calculated at a 5% and 1% significance level to compare means of significant source effects (Snedecor & Cochran, 1980).

4.5 RESULTS

The results on macroscopic evaluation of Kolbroek boar sperm characteristics are summarised in Table 4.1. The body weight of Kolbroek 3 (72.8±8.2) was significantly higher ($P<0.05$) compared to Kolbroek 1 (66.8±8.3) and Kolbroek 2 (58.2±8.5). No significant differences ($P>0.05$) were observed in Kolbroek boar's semen volume (79.9±12.1mL), semen pH (7.0) and sperm concentration (5.0±1.3x 10^9 sperm/ml). Furthermore, no individual variation was observed for semen volume, semen pH and sperm concentration.

Table 4.1: Macroscopic evaluation of Kolbroek boar semen (Mean±SEM)

Boars	Body weight (kg)	Semen Volume (ml)	Semen pH	Semen Concentration m/l ($x10^9$ sperm/ml)
Kolbroek 1	66.8±8.3[b]	88.8±13.3[a]	7.1±0.5[a]	5.8±1.3[a]
Kolbroek 2	58.2±8.5[a]	76.8±12.4[a]	7.0±0.3[a]	4.8±1.2[a]
Kolbroek 3	72.8±8.2[b]	74.3±10.5[a]	7.0±0.1[a]	4.4±1.3[a]
Averages	**65.9±8.2**	**79.9±12.1**	**7.0±0.3**	**5.0±1.3**

[ab] Means with the same letter within a column are not significantly different (P>0.05)

Table 4.2 represents the results for testicular measurements of Kolbroek boars. There was a significant difference ($P<0.05$) between testicular size of the left and right testicles. Kolbroek 2 (120.2±6.4) had a significantly higher left testicular length than Kolbroek 1 (110.8±7.9) and Kolbroek 3 (100.5±3.1). Likewise, in all the boars, the left testicle width (50±4.7) was higher than the right testicle width (43.3±6.2).

Table 4.2: Testicular measurements (cm) of Kolbroek boars (Mean±SEM)

Boars	Right Testicle	Left Testicle	Right Testicle	Left Testicle
	Length	Length	Width	Width
Kolbroek 1	105.2±6.3[a]	110.8±7.9[b]	40.4±7.1[a]	50±6.4[b]
Kolbroek 2	105.8±7.3[a]	120.2±6.4[b]	44.3±5.0[a]	50±4.3[b]
Kolbroek 3	100.8±6.4[a]	100.5±3.1[a]	45.2±6.5[a]	50±3.9[b]
Averages	**103.3±6.6**	**110±5.8**	**43.3±6.2**	**50±4.7**

[ab] Means within a column with the same letter within each row are not significantly different (P>0.05)

Table 4.3 represents the results for sperm motility and velocity rates for Kolbroek boars. The average sperm mortility of Kolbroek pigs was 94.8±2.5%. However, Kolbroek 1 had a significantly higher rapid motility (66.8±8.3) as compared to Kolbroek 2 (31.2±7.3) and Kolbroek 3 (44.5±9.3). Moreover, Kolbroek 1 had a significantly higher ($P<0.05$) progressive motility (74.7±7.1) as compared to Kolbroek 2 (41.2±6.1) and Kolbroek 3 (57.5±8.1). Kolbroek 2 showed a significant difference ($P<0.05$) in lower VSL (15.0±6.9) and LIN (24.1±4.1) compared to Kolbroek 1 (15.0±6.9) and Kolbroek 3 (34.0±6.9). Moreover, in Kolbroek 1 there was also a significant difference ($P<0.05$) in STR (70.8±5.7) compared to Kolbroek 2 (50.2±5.3) and Kolbroek 3 (54.3±5.6).

Table 4.3: Sperm motility and sperm velocity rates for Kolbroek boars (Mean±SEM)

Boars	Sperm motility			Sperm velocity					
	TM (%)	RAP (%)	PM (%)	VCL (µm/sec)	VSL (µm/sec)	VAP (µm/sec)	LIN (%)	STR (%)	WOB (%)
Kolbroek 1	89.3[a]±2.5	66.8[a] ±8.3	74.7 [a]±7.1	50.8 [a] ±12.9	27.9 [a]±6.9	40.4 [a]±8.2	55.7[a]±9.1	70.8[a]±5.7	78.9 [a]±4.4
Kolbroek 2	87.4[a]±5.2	31.2[b]±7.3	41.2[b]±6.1	62.8[a]±13.9	15.0[a]±6.9	31.6[a]±8.2	24.1[b]±7.1	50.2[b]±5.3	47.9[b]±4.5
Kolbroek 3	96.8[a]±7.5	44.5[b]±9.3	57.5[b]±8.1	67.1[a]±14.9	34.0[a]±6.9	38.8[a]±8.2	41.5±8.4[a]	54.3[b]±5.6	68.3[a]±5.5
Averages	**94.8±4.9**	**47.5±8.3**	**57.8±7.1**	**60.2±13.9**	**25.6±6.9**	**36.9±8.2**	**40.4±8.2**	**58.4±5.5**	**65.0±4.8**

[ab] Values with different superscripts within a column differ significantly at $P < 0.05$. TM (total motility); RAP (rapid); PM (progressive motility); VCL (velocity on the curve line); VSL (velocity on the straight line); VAP (velocity on the average path); LIN (linearity); STR (straightness); WOB (wobble).

Data in Table 4.4 indicates the sperm morphology and viability of Kolbroek boars. The average percentage of live sperm Kolbroek boars' live sperm was 73.9%±5.7. Kolbroek 3 (80%±7.5) had a higher live sperm percentage compared to Kolbroek 1 (74%±3.1) and Kolbroek 2 (67%±6.5). Furthermore, Kolbroek 1 (4.2%±1.4) had a significantly higher percentage of bent midpiece abnormality compared to Kolbroek 2 (2.6±1.2) and Kolbroek 3 (2.8%±1.4). Additionally, Kolbroek 2 (2.6%±1.8) had a higher percentage of lose head abnormalities compared to Kolbroek 1 (4.2%±1.2) and Kolbroek 3 (4.2%±2.2). No significant difference (P>0.05) was found for proximal percentage and coiled tail percentage.

Table 4.4: Sperm morphology and sperm viability of Kolbroek boars (Mean±SEM)

Boars	Live (%)	Dead (%)	Abnormalities (%)			
			Proximal	Distal bent midpiece	Abnormal lose head	Coiled tail
Kolbroek 1	$74^{a}\pm3.1$	$8.1^{a}\pm7.5$	$4.4^{a}\pm1.2$	$4.2^{a}\pm1.4$	$4.2^{a}\pm1.2$	$4.6^{a}\pm1.7$
Kolbroek 2	$67^{a}\pm6.5$	$61.2^{b}\pm6.6$	$3.1^{a}\pm1.2$	$2.6^{b}\pm1.2$	$2.6^{b}\pm1.8$	$3.4^{a}\pm1.8$
Kolbroek 3	$80^{b}\pm7.5$	20 ± 3.1^{c}	$3.6^{a}\pm2.2$	$2.8^{b}\pm1.4$	$4.2^{a}\pm2.2$	$3.4^{a}\pm1.8$
Averages	**73.9±5.7**	**33.1±5.7**	**3.6±1.5**	**3.4±1.3**	**3.6±1.7**	**3.8±1.7**

$^{a-c}$ Values with different superscripts within a column differ significantly at $P<0.05$

Pearson correlations coefficients of Kolbroek boars body weight, testicular size, semen pH, semen volume, sperm concentration and total motility are summarized in Table 4.5. There is a significantly positive correlation ($P<0.05$) between bodyweight and testicular size ($r = 0.90$). There was also a highly positive correlation ($P < 0.01$) between testicular size and sperm concentration ($r = 0.47$) as well as total motility ($r = 0.30$). Moreover, there was a highly positive correlation between body weight and semen pH ($r = 0.91$). A positive correlation ($P<0.05$) was observed between body weight and semen volume ($r = 0.80$) as well as sperm concentration ($r = 0.90$). A positive correlation ($P<0.05$) was also observed in testicular size and semen volume ($r = 0.90$). A non-significant positive correlations ($P>0.05$) were found between semen pH and sperm concentration ($r= 0.18$) and semen volume ($r= 0.18$). Furthermore, a weak positive correlation ($P<0.05$) was observed between body weight and sperm motility ($r= 0.30$). There was strong positive correlation ($P<0.05$) between sperm motility and semen volume ($r= 0.66$), pH (0.78) and sperm concentration ($r= 0.60$).

Table 4.5: Pearson correlations coefficient of Kolbroek body weight, testicular size, semen pH, semen volume, sperm concentration and total motility

	Body weight	**Testicular size**	**Semen pH**	**Semen volume**	**Sperm concentration**	**Total Motility**
Body weight	1.00					
Testicular size	0.90*	1.00				
Semen pH	0.91*	0.66*	1.00			
Semen volume	0.80*	0.90*	0.18	1.00		
Sperm concentration	0.90*	0.47**	0.18	0.47	1.00	
Total Motility	0.30**	0.78	0.78*	0.60*	0.60*	1.00

* Values are significantly correlated P<0.05

**Values are highly correlated P<0.01

4.6 DISCUSSIONS

The study demonstrated that there was no significant difference in body weight, testicular size, semen pH, sperm volume and sperm concentration among the three Kolbroek boars. Moreover, macroscopic characteristics of Kolbroek boars were similar. However, Kolbroek boar semen volume, sperm concentration and motility rate were positively correlated to bodyweight. The length of the left testicle was larger than the length of the right testicle and the width of the left testicle was larger than the width of the right testicles. Macroscopic evaluation of Kolbroek boar sperm characteristics showed that the bodyweight of Kolbroek boars is low compared to Mangalica boars in Hungary, Ugandan village pigs in Kenya (Egerszegi *et al.*, 2008). Furthermore, Chimonyo *et al*. (2005) and Masenya *et al*. (2012) stated that indigenous pigs in Southern Africa are smaller in size compared to exotic pig breeds.

According to (Smital *et al*., 2009) and Leidinger *et al*. (1998) high sperm concentration and semen volume is an important semen parameter of indigenous pigs which was evident in this study. Freking *et al*. (2013) previously reported that a breed does not have a significant effect on boar sperm characteristics. In contrast (Egerszegi *et al*., 2008) found that semen volume of 178 ml is common in Hungarian indigenous Mangalica boars. Frunză *et al*. (2008) reported that the pH of raw boar semen varies between 7.0 and 7.5. Semen pH of higher than 8 denotes a low quality sperm or the presence of an infectious process in the genital tract or in accessory sex glands (Frunză *et al*., 2008). Within the species and in the same male, sperm pH can differ from one ejaculate to another Purdy *et al*. (2010). pH changes negatively, influence sperm viability and motility (Kamp *et al*., 2003; Estienne & Harper, 2004; King & Macpherson, 2005).

The current results of this study indicated that the length of the left testicle was larger than the length of the right testicle. The average values for length and width of the testicles within this study are similar to values already recorded for young boars of various breeds in other studies (Ugwu, *et al*., 2009; Oberlender *et al*. 2012). Moreover, previous reports by Harder *et al*. (1995) stated that heavier boars which possessed larger testes may produce more sperm than boars with a smaller testis size. Furthermore, Borg *et al*. (1993) reported that testicular measurements provide reliable estimates of sperm-producing capacity in boars. The results, therefore suggest that testicular size is a good indicator of reproductive efficiency in boars and that semen volume increases with age due to an increase in body weight and testes size.

The average sperm motility of the Kolbroek boars was greater than 90% which is similar to that reported in other pig breeds (Smital, 2004; Tejerina *et al*., 2008). The three major parameters in the evaluation of boar sperm quality are concentration, motility, and morphology (Frunză *et al*., 2008). For instance, if a given sperm sample contains more than 30% abnormal sperm, the fertility will be reduced (Waberski *et al*., 1994; Flowers, 1997; Frunză *et al*., 2008). In this study, the quantity of abnormal sperm was 20% indicating that the fertility of Kolbroek boar sperm was acceptable. The Kolbroek sperm motility are comparable to the sperm motility of other pig breeds such as the Landrace 86%, Duroc 89% and Large White 87% (Tejerina *et al*., 2008; Yeste *et al*., 2010). The percentage of sperm with normal morphology was above 80% for Kolbroek boars. Such percentages of normal morphology are correlated with fertility (Sanchez *et al*., 1998; Xu *et al*., 1998; Alm *et al*., 2006). The results from this study indicated that there were no individual variations between the three boars.

Morphological abnormalities of sperm can have a detrimental impact upon fertilization and embryonic development (Wolf & Smital, 2009; Wysokińska *et al*, 2009). According to Borg *et al*. (1993), sperm morphology do not differ among different breeds Duroc, Meishan, Fengjing and Minzhu. The new membrane permeant nuclear stain (SYBR 14) which brightly fluoresces the nuclei of living cells, has been used in combination with PI to determine the proportion of living sperm in semen from several different mammals and has been proven effective for assessing sperm viability (Garner & Johnson, 1995; Wolf, 2009). Live sperm, which stained green with SYBR-14, and the dead sperm, which stained red with PI, were examined through use of fluorescence microscopy. Non motile sperm, apparently dead, fluorescence red when stained with PI alone, and moribund sperm that fluoresesced both green and red were evident in the results. The present results of this study are in agreement with previous reports by (Siqueira *et al*., 2011; Wysokińska & Kondracki, 2014) stating that preparations for analysing cell membrane integrity using SYBR 14/PI make it possible to successfully assess the integrity of the plasma membrane of domestic pig sperm cells.

The average values for semen quality characteristics evaluated indicated that the ejaculates of the various indigenous Kolbroek boars were positively correlated with bodyweight and testicular size, semen pH, semen volume, sperm concentration. A positive and significant correlation of testicular size with the percentage of total motility and sperm concentration was noted in men (Sakamoto *et al*., 2008; Condorelli *et al.,* 2013). The correlations of testes volume with sperm concentration and total number of sperm recorded during the current study are consistent with previous reports (Huang & Johnson 1996; Owsianny *et al*., 1998; Ugwu *et al*., 2009; Ytournel *et al.* 2014). Consideration should be given to including testicular size in the selection objective in sire lines when boars are used predominantly in artificial insemination programs. Therefore, it would make economic sense and add extra value in producing boars with larger testes for artificial insemination. These results indicate that boars with larger testes produce ejaculates with higher numbers of sperm compared to boars with smaller testes. In contrast, Huang & Johnson (1996) found that the selection for larger testes in boars had no effect ($P>0.05$) on the percentage of motile sperm. An important fact observed in this study was the highest estimated correlation coefficients between the length and width of the left testicle and sperm concentration, total motility in semen indicates that testicular measurements can be used to estimate the reproductive performance of boar's sperm.

4.7 CONCLUSIONS

In conclusion there was no significant difference in body weight, testicular size, semen pH, sperm volume and sperm concentration between individual Kolbroek boars. Moreover, macroscopic characteristics of Kolbroek boars were similar. Furthermore, Kolbroek boar semen volume, sperm concentration and motility rate were positively correlated to bodyweight. In Kolbroek boars the length of the left testicle was larger than the length of the right testicle and the width of the left testicle was larger than the width of the right testicles. Testicular size is a useful indicator of breeding soundness and should be used as an important criterion for selection of young boars for breeding purposes. Testicular size and bodyweight of Kolbroek boars was positively correlated with semen volume. Selection for increased size of testes in boars is an effective way to increase semen volume, sperm concentration. The emphasis to give testis size in a selection program depends on current boar fertility relative to other economic traits. Moreover, fluorescent staining can be used for the assessments of fresh boar semen and it is recommended that further studies should be conducted with more number of boars to validate the sperm motility characteristics information. Since body weight is also highly correlated with semen volume in all three Kolbroek boars, these characteristics can jointly be employed in estimating the reproductive performance of live boars of different genotypes particularly in their sperm production capacity. Furthermore, future seminal parameters that will be correlated with testicular parameters are necessary to confirm the present findings. Also the result of the present study show that simple scrotal measurements can be used to estimate the reproductive performance of boars. This simple technique will save pig breeders time and labour of having to euthanize or castrate a boar for sole purpose of evaluating reproductive performance. Therefore, current swine industry selection practices would be expected to result in reduced male fertility. Additional work is needed to understand the relative economic importance of sperm characteristics in the development of breeding objectives.

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4.8 ACKNOWLEDGEMENTS

The authors wish to acknowledge National Research Foundation (NRF), Southern African Science Service Centre for Climate Change and Adaptive Land Management – Council for Scientific and Industrial Research (SASSCAL), Department of Agriculture Forestry and Fisheries (DAFF) and Agricultural Research Council (ARC) Professional

Development Program (PDP) for funding the project. The Germplasm Conservation and Reproductive Biotechnologies (GCRB) staff for the support and assistance.

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CHAPTER 5

Detection of the onset of puberty in Kolbroek pigs using hormonal assays method and evaluating reproductive performance in Kolbroek sows following natural mating

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5.1 Abstract

The onset of puberty is one of the basic reproductive events in the life cycle of domestic animals but factors that control these events are poorly understood. The objectives of the study were to determine the onset of puberty in Kolbroek using hormonal assays and evaluating reproductive performance of sows following natural mating. A total of thirteen selected indigenous Kolbroek gilts were used in the study. Blood were collected from the jugular vein using a 10ml syringe. Thereafter blood serum was centrifuged at 2400rpm for 14 minutes and stored at -20^{0}C. The serum profile of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and Estradiol

(E_2) of the gilts were determined using ELISA kits. Data was analysed using SAS. The results revealed that there was no difference (P>0.05) between Kolbroek gilts for hormonal concentration in serum. However, there was a significant increase (0.22-0.35ng/ml) in mean serum concentration for all the hormones from 3-7 months of age with a mean body mass of ±19.10kg with a gradual decline from the 7th month. In conclusion the age of attainment of puberty in Kolbroek gilts was found to start from 3 months. The average age at first farrowing was ±7months with a bodyweight of ±25.45kg and an average litter size of 12. The hormonal assays method was found to be are clear indicators of the exact onset of puberty in Koelbroek sows.

Keywords: Kolbroek sows, puberty, hormone assay, LH, FSH, E_2, Oestrus detection

5.2 INTRODUCTION

The onset of puberty is one of the basic reproductive events in the life cycle of domestic animals. Guthrie (2005) reported that puberty is the time when the hypothalamic-hypophyseal unit first becomes responsive to increasing concentrations of circulating oestrogens resulting in a surge of Luteinizing Hormone (LH) and Follicle Stimulating Hormone. The Follicle Stimulating Hormone is a gonadotropin hormone synthesized and secreted by the gonadotropic cells of the anterior pituitary gland and regulates the development, growth, pubertal maturation and reproductive processes of the body (Noakes *et al*., 2003). Hormones work synergy with the reproductive system. Factors that control reproductive events are poorly understood in gilts (Noakes *et al*., 2003). The occurrence of delayed puberty in gilts has become apparent and is recognized as an important component of economic loss (Martinant-Botte *et al*., 2011). This is due to lack of understanding of the basic endocrinology associated with the onset of puberty in swine. To effectively detect oestrus (heat) and successfully employ methods of oestrus synchronization, the producer must have a basic understanding of the anatomy and reproductive physiology of sows and gilts (Ranjit, 2015). Oestrus begins with the pituitary gland, which is located just below the brain and secretes several hormones into the blood stream, including *luteinizing hormone* (LH) and *follicle-stimulating hormone* (FSH) known as gonadotropins. According to Sterning *et al*. (1998), in prepubertal gilts, gonadotropin secretion is low but intensely increases prior to puberty. During the two to three-day period just prior to oestrus, increasing blood levels of LH and FSH causes the follicles on each of the two ovaries to grow rapidly. These follicles in turn secrete increased levels of the hormone estradiol into the blood, causing the behavioral and physiological changes associated with oestrus (Estienne, 2009) Rising concentrations of estradiol in the blood reach a threshold which triggers a massive release of LH from the pituitary gland around the onset of oestrus (Estienne, 2009). This LH surge stimulates ovulation, the release of multiple ova from the follicles into the oviducts. Though the timing of ovulation is extremely variable, it is recorded that on average it occurs 40 hours after the onset of oestrus (Peltoniemi *et al*., 2005). Reproductive performance of sows in the herd is the major factor controlling the efficiency of swine production (Rekwot *et al*., 2001) and is considered to be the most important economic parameter in the swine industry (Ranjit *et al*., 2015). It is measured primarily by the number of live piglets at birth. The goal of reproductive performance is to have 2.0 litters per year of 8 piglets/litter at weaning, that is, a total of 16 pigs weaned per year for each female maintained in the herd (Ranjit, 2015). To date limited scientific data on the age of attainment of puberty and reproductive performance in Kolbroek sows contrary to commercial pig breeds is not available. The objective of the study was to detect the onset of puberty using hormonal assays and the common reproductive characteristics in Kolbroek sows following natural mating.

5.3 MATERIALS AND METHODS

5.3.1 Study area

The study was conducted at Agricultural Research Council (ARC), Germplasm Conservation and Reproductive Biotechnologies (GCRB) at Irene Pretoria, South Africa. The Agricultural Research Council is situated at 25^{0} 55' South; 28^{0} 12'East. The institute is located in Highveld region of South Africa and situated at an altitude of 1525m above sea level.

5.3.2 Experimental animals

A total of 13 indigenous Kolbroek pigs (7 sows and 6 boars) were used in the study. The pigs were housed individually at the ARC Pig Testing Centre, Irene during blood collection and were quarantined for two weeks before commencement of the study. The Kolbroek pigs were gilts of the same age (3months) and were fed boar and sow feed which was formulated to meet the nutritional requirements of the pigs (National Research Council (NRC), 1998. Water was provided *ad libitum* throughout the duration of the study. Experimental pigs were cared for according to the Agricultural Research Council, Animal Production Institute Ethics Committee (Ref: APIEC14/032).

5.3.3 Blood collection

Blood were collected from the jugular vein every 2 weeks for 5 months using a 10ml syringe and collected in to heparinized glass tubes and stored in to Styrofoam box containing crushed ice. Immediately after collection blood was transported to the laboratory. Thereafter blood serum was separated from the whole blood by centrifugation at 2400rpm for 14 minutes, serum samples were then stored in Nalgene tubes at -20^0C until the day of analysis.

5.3.4 Hormonal assays

The serum profile of luteinizing hormone (LH), follicle stimulating hormone (FSH) and estradiol (E_2) of the gilts were determined using ELISA kits designed for quantitative determination of hormonal concentration in serum. The kits were LH-0802-1, LH-080-1 and ES-0702-1 respectively (biorex diagnostic limited). Procedures for the determination of individual hormones were carried out following manufacturer's instructions and assays were done in duplicate.

5.3.5 Synchronizing Oestrus and natural mating in Sows

In order to detect oestrus a boar was slowly moved in front of the sows, pressure was then applied to the back of the sow to detect oestrus, and if not on heat the sow will move in an attempt to escape. An oestrus sow that was in standing heat was then introduced to the boar to let them mate naturally and then separated immediately after the boar dismounts. This was done early in the morning and late in the afternoon.

5.4 Data analysis

The data were entered into the Excel datasheet and descriptive statistics were performed. All mean values for age at attainment of puberty, concentration of luteinizing hormone (LH), follicle stimulating hormone (FSH) and estradiol (E_2) and litter size were reported as mean ± standard error of the mean (SEM).

5.5 RESULTS

The mean age at attainment of puberty, weight at puberty and number of piglets at first farrowing are presented in **Table 5.1**. The mean age at attainment of puberty of indigenous Kolbroek sows was 254.5 ± 34.4 days. The minimum age at puberty was 3 months and the maximum age at puberty of Kolbroek sows was seven months.

Table 5.1: Reproductive characteristics of Kolbroek sows

Parameters	**Mean ±SEM**
Age of attainment of Puberty (months)	3±0.7
Weight at puberty (kg)	19.10±1.1
Oestrus cycle length (days)	21.2±1.2
Gestation length (days)	114.3±2.9
Number of piglets born in first farrowing (n)	12±1.7

Mean serum concentrations of LH in samples collected are illustrated in **Fig 5.1**

Figure 5.1: Serum LH concentration in Kolbroek sows

All sows showed changes in the pattern of LH secretion from the age of 3 months and an overall LH concentrations increased significantly ($P<0.001$) from (0.22 -0.35ng/ml). Low levels of LH (0.22±0.2 ng/ml; Mean±SEM) were seen in all gilts from the age of 3 months. An ovulatory LH surge followed the onset of standing oestrus (mean ± SEM). Mean serum concentrations of LH reached a peak of 0.45 ng/ml.

Figure 5.2: Mean bodyweight of Kolbroek sows

A low body weight of 16.63kg from the age of 3 months was observed in Kolbroek gilts which gradually increased every month by ±4kg. The average bodyweight achieved at 7 months of Kolbroek sows was ±25kg.

Mean serum concentrations of FSH in samples collected are illustrated in **Fig 5. 3**

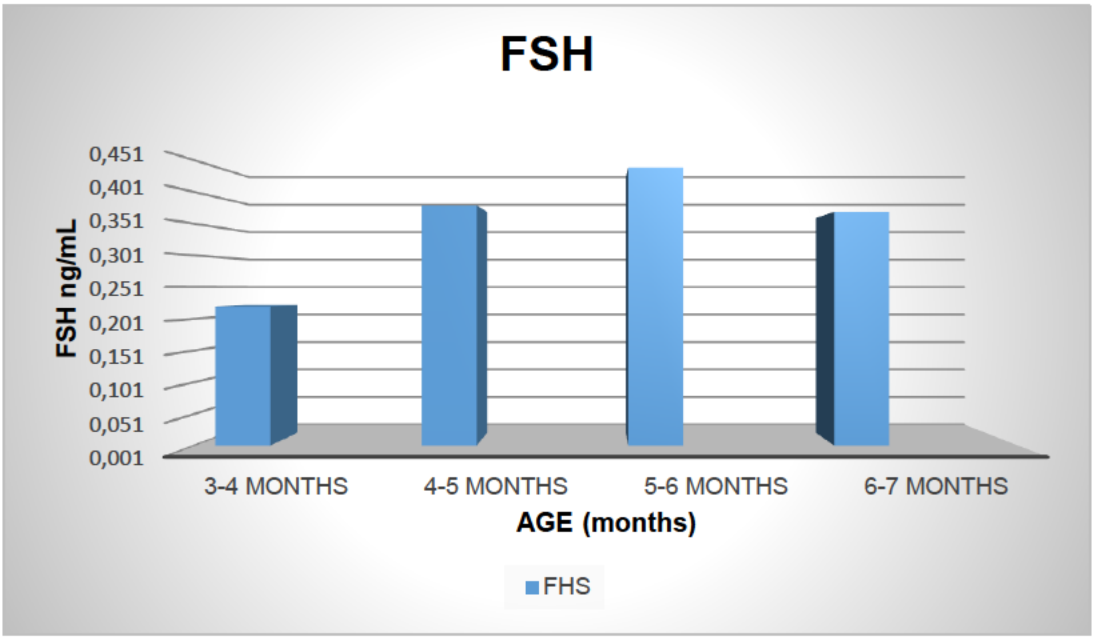

Figure 5.3: Serum FSH concentrations of in Kolbroek sows

Low levels of FSH (0.22±0.2 ng/ml; Mean±SEM) were seen in all gilts from the age of 3 months. The mean maximal level achieved was 0.35ng/ml. FSH peaked at 5-6 months and gradually declined from 6-7 months. There was a gradual increase of serum FSH from the age of 3 months. The concentrations of FSH increased significantly ($P<0.001$) from (0.20 - 0.35ng/mL).

Mean serum concentrations of E_2 in samples collected are illustrated in **Fig 5.4**

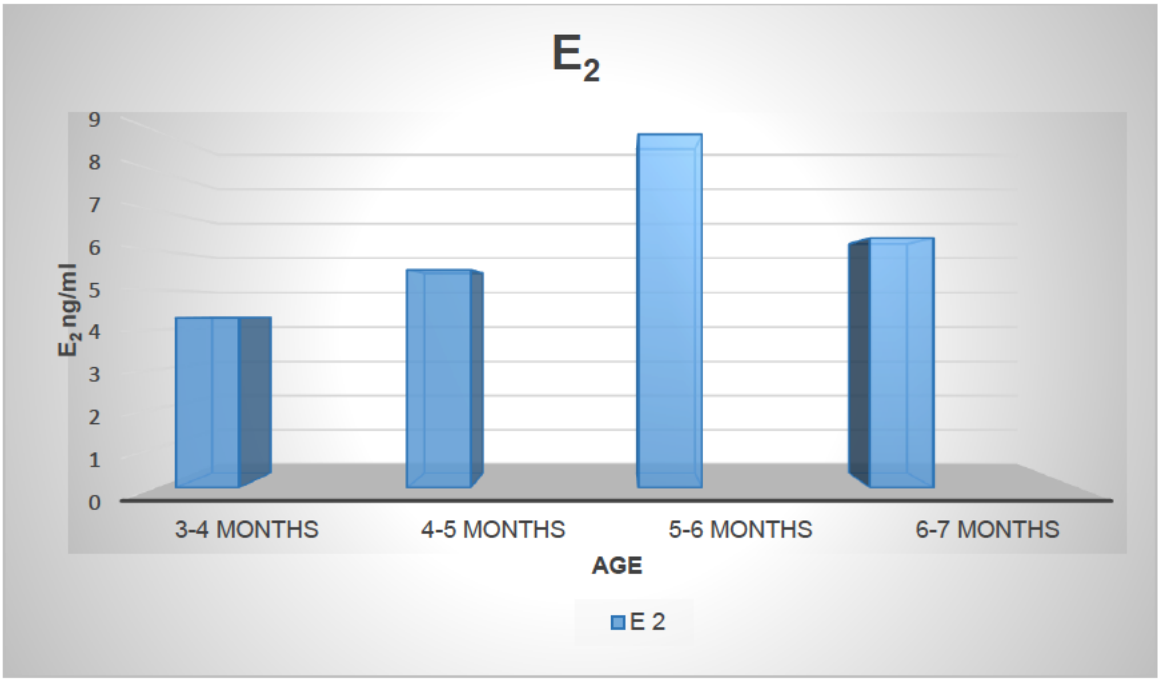

Figure 5.4: Serum E_2 concentration of in Kolbroek sows

Plasma concentration of Estradiol (E_2) did not vary significantly with age even though large fluctuations were observed (**Figure 5.4**). E_2 concentration (4.3-8.9ng/mL) peaked at 5-6 months.

5.6 DISCUSSIONS

This study reveals that the age of attainment of puberty of South African Kolbroek pigs is at 3 months. Moreover, age and weight are related to puberty but within the 3-7 months window for expression of oestrus these two factors do not provide much selection criteria since the weight of gilts ranges and smaller bodyweight delays puberty in gilts. However, concentration of LH, FSH and E_2 did not appear to be depressed ($P<0.05$) in the present study from the age of trhree months. The findings are in agreement with Thompson (2007) that LH and FSH tends to peak from the age of three months in indigenous pigs. The present study broadly supports the view that endocrine changes occurring after weaning are similar to those at oestrus in cycling sows (Stewart *et al*., 2010). Overall, the absolute levels of the hormones in the gilts from that early age is responsible for the increased number in sows which shows oestrus early. There was no difference ($P<0.05$) in the gonadotrophin profiles between the ages of 3 and 6 months gilts which means that ovulation rate in the gilts studied suggests that corpus luteum formation was normal.

Kolbroek pigs reached puberty early with sows showing signs of heat as early as three months of age. Kolbroek gilts the present study reached puberty early compared to previous studies where the age of attainment at puberty of indigenous pigs was 204.1±28.0 days (Tummaruk *et al*., 2003). Similarly, occurrence of puberty at the age of 3 months has been reported in Chinese Meishan breed (Evans & O'Doherty. 2001). The occurrence of puberty between 5 and 8 months of age has been documented in exotic breeds (Evans & O'Doherty. 2001). Moreover, Ritchil *et al*. (2014) reported that the mean age at puberty of native gilts in Bangladesh was 206 days. Furthermore, Stewart *et al*. (2010) found that ages at puberty at different photoperiods were 193.4, 175.6 and 177.1 days, respectively. The variations at the age of puberty among different studies may be due to variations in breeds of swine, feeding practices and agro-climatic conditions among studies. In the present study, the mean gestation length was 114.3 ± 2.9 days. Likewise, average gestation length of 114±2.0 was reported by (Pitcher & Springer, 1997). Irfan (2011) also reported a gestation length of 114 days in domestic sows and 119 days in wild sows. The mean number of piglet born alive production was12.2 which were similar to the previous findings by Zaleski *et al*. (1993) where he obtained 12.2 piglets per litter after evaluating 98 farrowings. Ranjit (2015) also reported 12 piglets per litter in domestic sows and 5 piglets in wild sows. The number of piglets born alive between the present study and that of the mentioned authors are the same. Llitter size was high with the first parity. These results are consistent with the earlier studies where litter size usually increased from first to second litter and again from second to third litter, but then plateaus until the seventh or eighth litter (Hughes & Varley, 1980; Irfan, 2011).

The pregnancy rate of Kolbroek gilts at their first service was 98%. Similarly, to the findings of this study, during the present study a higher pregnancy rate (98.9%) has been documented in swine which received natural service in Illinois, USA (Pitcher & Springer, 1997. Low levels of reproductive performance may not only result in low profit per sow, but will also limit attempts to improve the herd genetically (Rekwot *et al*., 2001). Some authors suggest that age is the most important aspect determining puberty attainment (Prunier *et al*., 1987; Newton & Mahan, 1992). However, it is generally perceived that unstimulated gilts may reach puberty from as early as 170 days to as late as 260 days of age (Kauffold & Althouse, 2007). Part of the variability of age at puberty seems to be related to the genetic background of the gilts and to other aspects of the environment in which they are kept. Therefore, age is not considered to be an accurate predictor of puberty in gilts (Rozeboom *et al*., 1995). Knowing about reproduction in the sow is important for the best system of breeding or the secret of success in any and all conditions. However, the improvement of reproductive performance is difficult and time-consuming. Unfortunately, there are no quick and easy solutions to improving reproductive performance. It is necessary to follow sound management practices as well as a rigorous genetic selection program to upgrade reproductive parameters of swine farms

5.7 CONCLUSIONS

The average age of attainment of puberty of South African Kolbroek pigs was found to be 3 months. Moreover, age and weight are related to puberty but within the 3-7 window of expression of oestrus these two factors do not provide much selection criteria since the weight of gilts ranges and smaller bodyweight delays puberty in gilts. In particular, the characteristics of LH, FSH and E_2 are considered to be important in follicular development. The number of piglet born per sow was 12 in the first litter. The average gestation length of Kolbroek pigs was 114 days. The overall reproductive performance of the sows in this experiment, that were *ad libitum* fed to breeding, bred naturally at a relatively young age. Under improved conditions of management, the fertility of indigenous Kolbroek pigs are on par with those of commercial breeds. For sows, true productivity must combine litter size, piglet survival and farrowing therefore an understanding of the sow's reproductive system is essential for a successful management program.

5.8 ACKNOWLEDGEMENTS

The authors wish to acknowledge National Research Foundation (NRF), Southern African Science Service Centre for Climate Change and Adaptive Land Management – Council for Scientific and Industrial Research (SASSCAL), Department of Agriculture Forestry and Fisheries (DAFF) and Agricultural Research Council (ARC) Professional Development Program (PDP) for funding the project. The Germplasm Conservation and Reproductive Biotechnologies (GCRB) staff for the support and assistance.

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CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 GENERAL CONCLUSIONS

Results from the Kolbroek pigs studied indicate that Kolbroek pigs, such phenotypic traits as body weight and scrotal measurements can jointly be employed in estimating the reproductive performance of live boars of different genotypes particularly in their sperm production capacity. Furthermore the result of the present study show that simple scrotal measurements can be used to estimate the reproductive performance of boars. This simple technique will save pig breeders the time and labour of having to kill or castrate a boar for sole purpose of evaluating reproductive performance.

Current results indicate that in South African indigenous boars such as Kolbroek pigs, the testicular measurement is a useful indicator of breeding soundness and should be used as an important criterion for selection of young boars for breeding purposes. However, estimates of changes in testicular development can be considerably improved by following the same boars at different ages to avoid confounding between boars and ages.

The present results of this study reveal that the average age of attainment of puberty for South African Kolbroek pigs, is at 3 months. Under improved conditions of management, the fertility of indigenous Kolbroek pigs is on par with those of commercial breeds. In sows, true productivity must combine litter size, piglet survival and farrowing therefore an understanding of the sow's reproductive system is essential for a successful management program.

6.2 RECOMMENDATIONS

- It is recommended that advanced phenotypic characterisation studies on all South African Kolbroek pigs should be undertaken with more number of Kolbroek pigs. Detailed characterisation of Kolbroek pigs including DNA profiling, and estimates of genetic distance are needed to establish the distinctiveness of the breed.
- Further studies on the suitability of semen ejaculates of Kolbroek pigs for conservation and preservation at different temperatures should be carried for dissemination of quality germplasm to different parts of the country. Seminal parameters should be correlated with testicular parameters are necessary to confirm the present findings.
- Further studies in hormone LH, FSH and E_2 secretions of pigs at 8 months of age are essential. Litter size at first farrowing is only one component in sow productivity, thus it is recommended that litter size should be checked at different parities and ages of indigenous Kolbroek pigs. The breeder should keep the time to keep records, monitor the feed and breeding, keep facilities in good conditions, and monitor the health status of the entire pig herd.