RELATIONSHIPS BETWEEN COCK SEMEN VIABILITY AND THE FERTILITY OF ARTIFICIALLY INSEMINATED SOUTH AFRICAN INDIGENOUS CHICKEN BREEDS

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NOVEMBER 2007

RELATIONSHIPS BETWEEN COCK SEMEN VIABILITY AND THE FERTILITY OF ARTIFICIALLY INSEMINATED SOUTH AFRICAN INDIGENOUS LAYER BREEDS

by

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Dissertation submitted in partial fulfilment of the requirements for the degree

MAGISTER TECHNOLOGIAE: AGRICULTURE: ANIMAL PRODUCTION

in the

Faculty of Health and Environmental Science
School of Agriculture and Environmental Science

at the

Central University of Technology, Free State

Supervisor: Dr D. O. Umesiobi (PhD., MA HES)

NOVEMBER 2007

DECLARATION OF INDEPENDENT WORK

I, Julian Thabo Molekwa, identity number [redacted] student number 205069070, declare that this dissertation*: Relationships between cock semen viability and the fertility of artificially inseminated South African indigenous layers* submitted to the Central University of Technology, Free State for the degree MAGISTER TECHNILOGIAE: AGRICULTURE is my own independent work and that all the sources used and quoted have been duly acknowledged by means of complete references; and complies with the Code 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 institution by myself or any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification. I also disclaim the copyright of this dissertation in favour of the Central University of Technology, Free State.

MOLEKWA JULIAN THABO DATE

ACKNOWLEDGEMENTS

First and foremost, I would like to express my heartfelt thanks to my supervisor Dr D. O. Umesiobi. I greatly appreciate his meticulous guidance in planning and implementation of this study, his patience, encouragement, leadership, financial support and the conducive environment that he created for me to complete my study. I am extremely thankful to my former manager, Mr H. J. Fourie, for his valuable support, encouragement and technical guidance during the course of the study.

I would like to express my gratitude to the Agricultural Research Council (ARC) at Glen for the donation of chickens and to my colleagues Silas, Hendrik, Bernard, John and Ernst for the great job they did through all the ups and downs during the research project. Sincere thanks to Country Bird Holdings for donating broilers.

I wish to extend my gratitude to members of my family, my beloved mother Iponeng, my late loving girlfriend, my son and my sisters for their moral support, prayers and encouragement.

Above all, I thank the Almighty God, for giving me the inner strength and making it possible for the accomplishment of this study.

DEDICATION

I dedicate this work to my late loving and caring girlfriend Modiehi Joyce Makhosi Mangoejane, our lovely and cute son Hlompho, my beloved mother Iponeng and my two beautiful sisters Sekgopi and Caroline who were at my side to add courage to my study.

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LIST OF ACRONYMS AND ABBREVIATIONS

Abbreviation	Description
µl	Microliter
μm^3	Micro meter qube
®	Trademark
AI	Artificial Insemination
ARC	Agricultural Research Council
bn	Billion
DNA	Deoxyribonucleic acid
DOA	Department of Agriculture
FAO	Food and Agricultural Organisation
HPC	High performing cock
LPC	Low performing cocks
ME	Metabolizable Energy
NN	Naked neck
OVB	Ovambo
P	Probability
PK	Potchefstroom Koekoek
RH	Relative humidity
SAS	Statistical Analysis Software
±s.e	least square mean
USDA	United States Department of Agriculture
VD	Venda

ABSTRACT

***Key words**: Cocks, semen viability, fertility, hatchability, South African indigenous chicken breeds*

Four different South African indigenous (Naked Neck (NN), Ovambo (OVB), Venda (VD) and Potchefstroom Koekoek (PK) chicken breeds were used in this study. From each of the four breeds of chicken, 40 hens and 8 cocks were selected randomly. Two groups each of sixteen cocks were subsequently formed: high performing (HP) and low performing (LP) groups to determine the relationships between cock semen viability and the fertility of artificially inseminated South African indigenous layer breeds. Semen was collected following five minutes of sexual massage (5SM) and evaluated for semen volume (ml), sperm motility (%), live sperm (%) and total sperm ($x10^9$/ml). Semen from each cock was then used to inseminate five hens per breed, in each treatment. Each hen was inseminated twice a week throughout the duration of the trial. During the experimental period, each hen was inseminated with 0.05 ml diluted semen. The artificially inseminated hens were examined for average egg weight (g), fertility (%), hatchability of set eggs (%), live chicks (%), normal chicks (%) and chick weight (g). A total of 1600 eggs, i.e. 400 eggs from each breed were collected in three batches following artificial insemination from individually caged hens and were hatched to compare hatching parameters among breeds. The hatchability traits of hens of the four breeds (NN, OVB, PK and VD) were

compared. Hatching egg weight had significant ($P < 0.05$) difference among the four breeds.

The results of this study indicate that semen viability exemplified by ejaculate volume, sperm motility; live sperm and total sperm per ejaculate were significantly ($P < 0.01$) superior in the HP cocks compared to the LP cocks. Hens inseminated with semen from the HP cocks in each experimental group resulted in higher egg weight (g), fertility (%), hatchability of set eggs (%), live chicks (%), normal chicks (%) and chick weight (g). Significant positive relationships existed between semen volume and sperm motility ($P < 0.05$), semen volume and live sperm cells ($P < 0.01$), semen volume and total sperm ($P < 0.01$) in NN, OVB and VD, with negative correlations in PK. Some positive correlations were found between sperm motility and live spermatozoa ($P < 0.01$), sperm motility and total sperm ($P < 0.01$), live sperm and total sperm ($P< 0.01$) in NN, OVB, PK and VND.

Fertility was the highest in the HP group. Fertility was also the highest in PK, intermediate and similar in OVB and NN and lowest in VD ($P<0.05$). Breed had a significant effect on hatchability of fertile eggs ($P<0.05$). Hatchability of total eggs set was highest in PK and NN, intermediate in OVB and lowest in VD ($P<0.05$). Breed had a significant effect on live, normal chicks and chick weight ($P<0.05$). Live chick was the highest in NN, whereas at day-old, normal chick and chick weight at hatching were the highest (23.50 ± 0.11) ($P<0.05$) in PK (98.14 ± 0.67

vs. 37.90 ± 0.28 g), intermediate and similar in NN (87.90 ± 0.63 vs. 23.50 ± 0.11) and OVB (87.75 ± 0.45 vs. 32.81 ± 0.49 g) and the lowest but with an acceptable value in VD (76.85 ± 0.46 vs. 26.90 ± 0.36 g). There were some correlations among different hatchability traits depending on breed. The correlations were more profound among PK. It was clear that chick weight as percent of egg weight was not just a function of egg weight, and that genotype also played an important role favouring the heavier breeds.

The results obtained in this study on the relationships between cock semen viability and the fertility of artificially inseminated South African indigenous layer breeds elucidate that the use of high performing (HP) cocks following five minutes of sexual massage, prior to semen collection and artificial insemination of layers is a practical method for optimising sperm viability and subsequent fertility of hens. The results of this study suggest that the Potchefstroom Koekoek (PK) cocks and hens are superior to the Naked Necks (NN), Ovambo (OVB) and Venda (VD). The Ovambo and Naked Neck cocks ranked second in donating quality semen as well as in improving the fertility and hatchability traits of the indigenous chicken breeds.

Thus selection of high performing cocks through five minutes sexual massage prior to semen collection and use is recommended for poultry AI breeding programmes.

Chapter 1

Orientation

1.1 INTRODUCTION

Poultry production, and especially broiler production, is becoming big business in South Africa. The South African poultry industry currently accounts for an estimated 16% of the total gross value of primary agriculture. The broiler industry in South Africa currently produces on average 13.8 million broilers per week, growing steadily from 1990 when only 7.6 million broilers per week were produced (South Africa Online, 2007). Domestic demand for poultry meat is growing at the rate of approximately 7% per annum, which outstrips the performance of any other protein on the market (Republic of South Africa, Department of Agriculture (RSA DoA), 2007). According to the United States Department of Agriculture (USDA) (2007), South Africa's poultry market is estimated to have grown by 11% to R13.5bn (US$2.1bn) for the financial year ending 31 March 2007, contributing approximately 16% of the total gross value of primary agriculture in South Africa. Expansion plans flow from a realisation amongst producers that there is a long-term structural change occurring in chicken consumption patterns, mostly due to quick returns, poverty alleviation, black empowerment and income generation (South Africa Online, 2007).

However, while South Africa's poultry industry continues to expand through increasing production of exotic chicken breeds, the future remains uncertain for

indigenous South African chicken breeds. The genetic diversity within indigenous chicken breeds is shrinking due to their replacement by high-producing exotic commercial hybrids. Indigenous breeds of chickens are a product of their environment and have survived under the harsh conditions of many generations (Fourie *et al*., 2005).

As a result of the high cost of meat, rural dwellers are constantly looking for cheaper sources of protein. Poultry are kept all over the world for various reasons. They are one of the cheapest sources of meat and can be kept by anyone, even in backyards. In South Africa poultry are kept by smaller farmers, as well as by households in backyards (RSA DoA, 2007). The indigenous breeds of chickens provide meat and eggs which are a valuable yet affordable source of high quality protein and vitamins required for improved nutrition and health.

The limited production of South African indigenous chicken breeds in large numbers may be attributed to their slow growth rates, poor egg production (Sonaiya and Odubote, 1997), high rearing mortalities and susceptibility to diseases (Alders *et al*., 1997; Moreki *et al*., 1997). Most of South Africa's population lives in rural areas where the indigenous chicken breeds are best adapted to the harsh living conditions. It is speculated that with minimal technical and institutional support, the indigenous chicken breeds could contribute significantly to the rural economy and could curtail the vicious cycle of unemployment and poor human nutrition. The reproductive potentials of the

South African indigenous chicken breeds have not been exploited in South Africa, as much as has been done in other African countries. The purpose of this study therefore, was to evaluate relationships between cock semen viability and the fertility of artificially inseminated South African indigenous layer breeds.

Rapid human population growth and low protein intake are two of the major problems facing developing countries like South Africa. Poultry offers an avenue for rapid transformation in animal protein production. This is because poultry ranks first in population among domesticated animals species (FAO, 1995). There is almost no household in the rural and peri-urban areas that does not keep some form of poultry. There is overwhelming evidence that indigenous poultry production plays a significant role in the socio-cultural, economic and nutritional aspects of the lives of rural households in Africa. Poultry also has a shorter generation interval than most farm animals, thus genetic improvement could be attained at a faster rate. Artificial insemination (AI) is one of the animal production technologies that augments production and returns from livestock and poultry at a faster rate and enhances cross-breeding programmes. The benefits of this technology are however derived only when it is available to the farmers and when it is effectively utilized by them.

Profitable poultry farming depends mainly upon the fertility of the chickens. To fulfil the increasing demand, production of quality chicks from South African indigenous breeds should be encouraged at a reasonable price in commercial

hatcheries using artificial insemination techniques. AI is a vital component of commercial poultry production. In AI, semen collected from one cock is used to inseminate 20-30 breeder hens, as opposed to a ratio of 1 cock to 8-10 hens in natural mating (Bayyari *et al*., 1990). As a result the cost of producing day-old chicks is also reduced and farmers can get quality sound chicks at a minimum cost. The degree of multiplication of any breeding stock is an essential factor in determining rate of success of poultry operations (Hammerstedt, 1996). Also, Salahuddin *et al*. (1990) recorded 80.8% egg fertility and 71.7% hatchability of fertile eggs for Deshi chickens with 72% sperm motility of cock semen. However, Demmining and Middelkoop (1999) observed that hatchability in both types of chickens was affected by rate of infertility rather than early embryonic mortality.

To maximise hatch of total eggs set after AI, the efficient use of cock semen of the highest quality is essential (Hammerstedt, 1996). Unfortunately, genetic selection for growth and carcass traits reduces reproductive performance (Donoghue *et al*., 1999). Several tests to evaluate semen quality have been described (Bayyari *et al*., 1990; Bakst *et al*., 1991; Umesiobi, 2000, 2004; Umesiobi *et al*., 2004; Umesiobi, 2006a, b), but they are rarely applied in on-farm settings. Rather, the industry has relied on evaluation of semen for colour and volume (Donoghue, 1997), which give estimates of sperm quantity; no information on quality of the sperm from on-farm experiments is available, however.

The assessment of the semen quality characteristics of poultry species provides an excellent indicator of their reproductive potential, and is a *sine qua non* to effective artificial insemination programmes. There is, however, a paucity of information on this aspect of the South African indigenous species of poultry in particular. There are no studies currently on reproduction and artificial insemination of South African indigenous chicken breeds. It is therefore imperative to understand and improve the reproduction potentials of these birds in order to improve the basis for rapid breeding programmes. It is necessary that the programme should always include local poultry since they possess some innate resistance to certain local disease in addition to adaptability to prevailing climatic conditions.

1.2 PROBLEM IDENTIFICATION

The establishment of a strong breeding programme to combat constraints related to commercial production of the South African indigenous chicken breeds is essential, for which a wider genetic base of germplasm is a prerequisite. However, the lack of genetic resources in the indigenous chicken breeds is becoming acute due to the high rate of genetic erosion caused by diseases (Newcastle) and neglect. Furthermore, the massive distribution of exotic chicken breeds via fertile eggs, day-old chicks and three-month-old pullets and cockerels by both governmental and non-governmental organisations has resulted in the dilution of the indigenous genetic stock. If this trend continues at the current

pace, the gene pool of the indigenous chickens could be lost in the near future – even before they are described and studied. In South Africa, studies to determine the effects of cock semen viability on the fertility and hatchability of artificially inseminated South African layer breeds have never been done.

Moreover, to date, little or no information is available in terms of artificial insemination and fertility studies in South African indigenous chicken breeds. More importantly, lack of methods of evaluation of sperm quality, especially objective tests, apparently obscures the accurate identification of indigenous males that are likely to be sub-fertile. Recent studies in exotic chicken breeds found correlations between measure of sperm motility and fertility (Froman and McLean, 1996; McDaniel *et al*., 1998). However, many seminal attributes such as sperm concentrations, percentage live, morphologically normal spermatozoa and percentage sperm motility are essential for spermatozoa to achieve full fertilizing potential, and only semen containing a high percentage of sperm with all essential functional attributes was noted to be of high fertility (Amann and Hammerstedt, 1993; Hammerstedt, 1996). Therefore, this investigation was initiated to evaluate relationships between cock semen viability and the fertility of artificially inseminated South African indigenous layer breeds.

1.3 PROJECT RATIONALE

Artificial insemination (AI) is a vital tool for rapid improvement of infertility in chickens by allowing maximum use of the best cocks on numerous hens, ensuring disease prevention and high fertility rates, as well as for economic reasons. AI is also carried out when natural mating is impossible in genetically improved birds due to body size (Alkan *et al*., 2002). AI requires the regular collection of semen. Little has been done, however, to evaluate the semen of South African cock breeds. The poultry industry could benefit greatly from a semen assessment. For example, roosters with substandard fertility could be eliminated if identified early in production, which would save the producer the cost of keeping this bird throughout its breeding life.

In addition, most commercial farms pool the semen of many males for the insemination of hens. A recent study indicated that only a few cocks produced the majority of offspring after pooled inseminations (Wishart *et al*., 1992; Donoghue *et al*., 1999). In order to lower costs and maximise profits, an effective method of determining semen quality on-farm is therefore imperative.

1.4 OBJECTIVES OF THE STUDY

The objectives of the study are to determine:

- relationships between cock semen viability and the fertility of artificially inseminated South African indigenous layer breeds;
- the effects of cock semen viability on egg laying performance;
- the effects of cock semen viability on egg quality and hatchability; and
- the effects of semen viability on survivability of day-old chicks.

1.5 PROJECT HYPOTHESES

The project hypotheses include:

- Poor cock semen quality is detrimental to egg laying performance of South African indigenous layer breeds.
- The detrimental effects on laying performance have a link to egg quality, hatchability and survivability of day-old chicks.
- Artificial insemination (AI) in the South African indigenous layer breeds will improve egg production, egg quality and survivability of day-old chicks.

1.6 CONCLUSION

Throughout the continent of Africa, and especially in South Africa, the keeping of indigenous chicken breeds by village communities has been practised for many generations. These birds, which are generally kept on a free range system, currently make up substantial income for the South Africa's local poultry farmers and rural dwellers. Although requiring minimal resource input and considered

secondary to other agricultural activities by farmers, this type of production plays an important role in supplying local populations with additional income and high quality protein. However, high mortality, especially in growers, constitutes the greatest constraint to development. Growth and egg production of the indigenous birds are low and their limits of performance are rapidly improved when feeding and management are improved. However, the meat and eggs are much preferred by the consumers and fetch premium prices compared with commercial birds. The genetic potential of the indigenous stocks could be improved through artificial insemination with cocks selected for their optimum semen viability potential.

Chapter 2

Literature review

2.1 INTRODUCTION

The chicken (*Gallus gallus*) is a type of domesticated fowl, believed to be descended from the wild Indian and south-east Asian Red Jungle fowl. With a population of more than 24 billion in 2003 (Havenstein *et al*., 2003; RSA DoA, 2007; USDA, 2007), there are more chickens in the world than any other bird. They are used to produce two sources of food: meat and eggs.

In South Africa and most parts of the world, chickens were raised primarily on family farms until roughly 1960. Originally, the primary value in poultry keeping was in eggs, and meat was considered a by-product of egg production (McDaniel *et al*., 1998). Supply was lower than the demand, and poultry was expensive. Excepting in hot weather, eggs can be shipped and stored without refrigeration for some time before going bad; this was important in the days before widespread refrigeration.

According to Guèye (1998), local chicken breeds tended to be small because the hens largely fed themselves through foraging, with some supplementation of grain, scraps, and waste products from other farm ventures. Such foodstuffs were in limited supply, especially in the winter, and this tended to regulate the size of the farm flocks. Soon after poultry keeping gained the attention of agricultural researchers, improvements in nutrition and management made

poultry keeping more profitable and businesslike (Froman and McLean, 1996; Umesiobi, 2000).

In Africa, prior to the nineteenth century, chicken was served primarily on special occasions or Sunday dinner. Poultry was shipped live or killed, plucked, and packed on ice (but not eviscerated). The "whole, ready-to-cook broiler" wasn't popular until the Fifties, when end-to-end refrigeration and sanitary practices gave consumers more confidence. Before this, poultry were often cleaned by the neighbourhood butcher, though cleaning poultry at home was a commonplace kitchen skill (Mushi *et al*., 2000).

Two kinds of poultry were generally used: broilers, which were young male chickens and a by-product of the egg industry, which were sold when still young and tender, and "old layers," also a by-product of the egg industry, which were old hens past their prime for laying (Sonaiya and Odubote, 1997)

The major milestone in 20th century poultry production was the discovery of vitamin D, which made it possible to keep chickens in confinement all year round. Before this, chickens did not thrive during the winter (due to lack of sunlight), and egg production, incubation, and meat production in the off-season were all very difficult, making poultry a seasonal and expensive proposition (Havenstein *et al*., 2003).

The vertical integration of the egg and poultry industries was a late development, occurring after all the major technological changes had been in place for years (including the development of modern broiler rearing techniques, the adoption of the Cornish Cross broiler, the use of laying cages, etc.) (Umesiobi, 2000; Havenstein *et al*., 2003).

By the late Fifties, poultry production had changed dramatically. Large farms and packing plants could grow birds by the tens of thousands. Chickens could be sent to slaughterhouses for butchering and processing into prepackaged commercial products to be frozen or shipped fresh to markets or wholesalers. Meat-type chickens currently grow to market weight in six to seven weeks whereas only fifty years ago it took three times as long (Havenstein *et al*., 2003). This is due to genetic selection and nutritional modifications (and not the use of growth hormones, which are illegal for use in poultry in the US and many other countries). Once a meat consumed only occasionally, the availability and lower cost has made chicken a common meat product in developed nations. Growing concerns over the cholesterol content of red meat in the 1980s and 1990s further resulted in increased consumption of chicken.

Today, eggs are produced on large egg ranches on which environmental parameters are controlled. Chickens are exposed to artificial light cycles to stimulate egg production year-round. In addition, it is a common practice to

induce moulting through manipulation of light and the amount of food they receive in order to further increase egg size and production (Gueye, 2001).

On average, a chicken lays one egg a day for a number of days (a "clutch"), then does not lay for one or more days, then lays another clutch. Originally, the hen presumably laid one clutch, became broody, and incubated the eggs. Selective breeding over the centuries has produced hens that lay more eggs than they can hatch. Some of this progress was ancient, but most occurred after 1900. In 1900, average egg production was 83 eggs per hen per year. In 2000, it was well over 300 (Moreki *et al*., 1997; Havenstein *et al*., 2003).

In the United States, laying hens are butchered after their second egg-laying season (Havenstein *et al*., 2003). In Europe, they are generally butchered after a single season (Alders *et al*., 1997). The laying period begins when the hen is about 18-20 weeks old (depending on breed and season). Males of the egg-type breeds have little commercial value at any age, and all those not used for breeding (roughly fifty percent of all egg-type chickens) are killed soon after hatching. The old hens also have little commercial value. Thus, the main sources of poultry meat 100 years ago (spring chickens and stewing hens) have both been entirely supplanted by meat-type broiler chickens (Alders *et al*., 1997; Havenstein *et al*., 2003).

In South Africa, as in other parts of African continent, chicken production was distributed across the entire agricultural sector. During the twentieth century, it gradually moved closer to major cities to take advantage of lower transportation costs. This had the undesirable side effect of turning the chicken manure from a valuable fertilizer that could be used profitably on local farms to an unwanted by-product. This trend may be reversing itself due to higher disposal costs on the one hand and higher fertilizer prices on the other, making farm regions attractive once more (Guèye *et al*., 2000).

In Southern Africa, from the farmer's point of view, eggs used to be practically the same as currency, with general stores buying eggs for a stated price per dozen. Egg production peaks in the early spring, when farm expenses are high and income is low. On many farms, the flock was the most important source of income, though this was often not appreciated by the farmers, since the money arrived in many small payments. Eggs were a farm operation where even small children could make a valuable contribution (Alders *et al.*, 1997; Moreki *et al*., 1997). In terms of poultry distribution, the Food and Agriculture Organisation (FAO) (2004) reports that China was the top chicken market in 2004, followed by the USA.

Under natural conditions most birds lay only until a clutch is complete, and they will then incubate all the eggs. Many domestic hens will also do this – and are then said to go broody. The broody hen will stop laying and instead will focus on

the incubation of the eggs (a full clutch is usually about 12 eggs). Hens will sit or set fast on the nest, protesting or pecking in defence if disturbed or removed, and will rarely leave the nest to eat, drink, or dust-bathe. While brooding, the hen maintains the nest at a constant temperature and humidity, while also turning the eggs regularly during the first part of the incubation (Wishart *et al*., 2001). To stimulate broodiness, an owner may place many artificial eggs in the nest, and to stop it they may place the hen in an elevated cage with an open wire floor.

At the end of the incubation period (about 21 days), the eggs, if fertile, will hatch. Development of the egg starts only when incubation begins, so they all hatch within a day or two of each other, despite perhaps being laid over a period of two weeks or so. Before hatching, the hen can hear the chicks peeping inside the eggs, and will gently cluck to stimulate them to break out of their shells. The chick begins by pipping – pecking a breathing hole with its egg tooth towards the blunt end of the egg, usually on the upper side. It will then rest for some hours, absorbing the remaining egg-yolk and withdrawing the blood supply from the membrane beneath the shell (used earlier for breathing through the shell). It then enlarges the hole, gradually turning round as it goes, and eventually severing the blunt end of the shell completely to make a lid. It crawls out of the remaining shell and its wet down dries out in the warmth of the nest (Havenstein *et al*., 2003).

The hen will usually stay on the nest for about two days after the first egg hatches, and during this time the newly-hatched chicks live off the egg yolk they

absorb just before hatching. Any eggs not fertilized by a rooster will not hatch, and the hen eventually loses interest in these and leaves the nest. After hatching, the hen fiercely guards the chicks, and will brood them when necessary to keep them warm, at first often returning to the nest at night. The hen leads them to food and water – the hen will call them to edible items, but rarely feeds them directly. She continues to care for them until they are several weeks old, when she will gradually lose interest and eventually start to lay again.

Modern egg-laying breeds rarely go broody, and those that do often stop part-way through the incubation. However, some "utility" (general purpose) breeds, such as the Cornish, do regularly go broody, and they make excellent mothers, not only for chicken eggs but also for those of other species – even those with much smaller or larger eggs and different incubation periods, such as quail, pheasants, turkeys or geese (Graham and Wishart, 1994). Chicken eggs can also be hatched under a broody duck, with varied success (Umesiobi, 2000).

Chicken egg incubation can successfully occur artificially as well. Nearly all fertilized chicken eggs will hatch after 21 days of good conditions (37.5℃) and around 55% relative humidity. Many commercial incubators are industrial-sized with shelves holding tens of thousands of eggs at a time, with rotation of the eggs a fully automated process (Graham and Wishart, 1994).

Home incubators are usually large boxes (lead incubators are popular) holding a few to 75 eggs. Eggs must be turned three to eight times each week, rotating at least 180 degrees. If eggs aren't turned, the embryo inside will stick to the shell and may hatch with physical defects. This process is natural; hens will stand up three to five times a day and shift the eggs around with their beak. However, eggs should not be turned during the last week of incubation, or the chick may have difficulty settling in the correct hatching position (Demmining and Middelkoop, 1999; Rodriguez and Lok, 2000).

2.2 CHARACTERISTICS OF SOUTH AFRICAN INDIGENOUS AND EXOTIC CHICKEN BREEDS

The chicken breeds of South Africa are diverse and can be divided into four main breed types: indigenous (local breeds), exotic (commercial breeds), crossbred strains of indigenous and exotic breeds, and hybrids developed from exotic breeds. Several indigenous breeds have been selected for many years (Bayley and Phororo., 1992; Aganga *et al*., 2000, 2003).

The most abundant indigenous chicken breeds in South Africa are the Naked Neck, Venda, Ovambo and Potchefstroom Koekoeks. Records of Naked Neck chickens have been found in areas as far apart as central Europe and Malaysia. The Venda, Ovambo, and Naked Neck are regarded as native to South Africa and are adapted to the prevailing harsh conditions in rural areas. The

Potchefstroom Koekoek, Rhode Island Red and New Hampshire were bred to be adaptive and to survive under harsh, low input conditions with basic requirements of shelter, feed, water and hygiene (Joubert, 1996; Gunaratne, 1999).

2.2.1 The Naked Necks

The origin of the strange looking Naked Neck chickens is disputed. According to archaeologists, the Naked Neck breed originated in Malaysia; from there it spread all over the world. It is therefore possible that the Dutch East India Company introduced the Naked Neck to South Africa in the 17th century (Merat, 1996; Ramsey *et al*., 2000). They are now found mainly in the rural areas around the huts of the local population. These chickens have a variety of colour patterns. There are two types of Naked Necks, one of which is purebred and has a completely naked neck and the other, which is not purebred, has a tassel on the front part of the neck (Aganga *et al*., 2003).

Figure 2.1 Naked Neck chickens (Courtesy: ARC Poultry Unit at Glen)

In most parts of the world, the naked neck factor is used to advantage in commercial production for three reasons. Firstly, a considerable amount of dietary protein is used in the growing of feathers. The Naked Necked chickens have 30% less feathers than fully feathered birds and can therefore produce the same body weight with less food. Secondly, there are fewer feathers to remove in the slaughter line and therefore they pass through much faster and, lastly, they are more heat tolerant.

The naked neck is characterised as a dual-purpose breed adaptive to hot climates. They are very colourful – white, red and black feather combinations are found (Joubert, 1996). They reach sexual maturity at 155 days with an average weight of 1.95 kg for males and 1.4 kg for females at 20 weeks of age. These chickens carry the major gene Na- for naked neck, which has autosomal inheritance with incomplete dominance and was mapped on the chromosome of the chicken genome (Pitel *et al*., 2000). Chickens that are homozygous have a little tuft of feathers on the neck area (Merat, 1996; ARC, 2006) while the heterozygous chickens have a little tuft of feathers on the lower portion of the neck. The Na- gene is associated with significantly less plumage cover than chickens not carrying the gene.

2.2.2 The Venda chickens

In 1979 a veterinarian, Dr Naas Coetzee, noticed a distinctive new breed in Venda and named it after the region in which he found it. Similar chickens were later seen in the southern Cape and in Qwa-Qwa (Joubert, 1996). Vendas are multi-coloured with white, black and red as the predominant colours. Rose-coloured combs and five-toed feet are not uncommon. In contrast to other indigenous breeds, the Venda is fairly large and lays tinted eggs of a generous size (Joubert, 1996). The hens are broody and are very good mothers. Little is known about this breed which is presently being collected and evaluated.

Figure 2.2 Venda chickens (Courtesy: ARC Poultry Unit at Glen)

The Venda is characterised by lower egg production, instinct to broodiness and adaptability for household production. These chickens reach sexual maturity at

the age of 143 days with an average body weight of 2.1 kg in males and 1.4 kg in females at 20 weeks old. The colour of the eggs is cream and sometimes tinted. The average egg weight is 52.7g. These chickens have white and black or white and brown plumage with shades of dark green on the feather tips (Joubert, 1996; Kitalyi, 1999).

2.2.3 The Ovambo chickens

The Ovambo chickens originated in the northern part of Namibia and Ovamboland. Unlike the Venda chicken where white feathers occur, the Ovambo is dark coloured. It is also smaller in size and it is these two differences which help to camouflage the bird and protect it from raptors. The Ovambo is very aggressive and agile. It has been known to catch and eat mice and young rats. This chicken can fly and roosts in the top of trees to avoid predators (Joubert, 1996; Kitalyi, 1999; ARC, 2006).

Figure 2.3 Ovambo chickens (Courtesy: ARC Poultry Unit at Glen)

Their broodiness ensures their propagation and survival. These chickens are characterised as layers and survive under harsh conditions. Their average weight at 20 weeks is 2.16 kg for males and 1.54 kg for females. They reach sexual maturity at 143 days and the average egg weight is 52.5g (Joubert, 1996).

2.2.4 Potchefstroom Koekoeks

The Potchefstroom Koekoek chicken has been popular for a long time. What is not generally known is that the term Koekoek describes the colour pattern rather than the breed (Joubert, 1996; Lebajoa, 2001). The Koekoek colouring is recognised as a variety and is present in as many as nine different breeds.

The first chickens with the Koekoek colouring in South Africa were the North Dutch Blue breed, brought into this country by Jan van Riebeeck. Later, the Barred Plymouth Rock chicken was imported from the United States of America - this was also known as the Koekoek. This breed was very popular at that time because it laid large numbers of dark brown eggs. When slaughtered at the end of its productive life, this hen had very attractive deep yellow meat (Mosoeunyane and Nkebenyane, 2001).

The Koekoek feather colouring is sex-linked which makes it very useful in breeding programmes. If a black or red cock is crossed with a Koekoek hen, the

sexes of the offspring can be separated when the chicks are only a day old. Sexes can be identified as the females are completely black whilst the males have a white spot on the head (ARC, 2006).

Figure 2.4 Potchefstroom Koekoeks (Courtesy: ARC Poultry Unit at Glen)

The Potchefstroom Koekoek was bred from crosses between the Black Australorp and the White Leghorn and is recognised as a breed developed in South African. It also resembles the barred Plymouth Rock. It is characterised by relatively high egg production and adaptability for household production. The Koekoek is classified as a heavy breed, with an average adult body weight varying from 3-4 kg for cocks and 2.5 - 3.5 kg for hens (Joubert, 1996). The average egg weight is 55.7g and the colour of the eggs is brown (Ramsey *et al*., 2000). These birds reach sexual maturity at 130 days. They have a characteristic black and white speckled colour pattern, also described as barred, which is present in as many as nine different poultry breeds. The male inherited the bar

gene which is sex-linked, and they are easily distinguished, having light grey bars on the feathers. The females are darker (Van Marle-Köster and Nel, 2000).

2.2.5 White Leghorns

The White Leghorn is an egg-type chicken. Leghorns figured in the development of most of our modern egg-type strains. The Leghorn chickens take their name from the city of Leghorn in Italy, where they are considered to have originated (Umesiobi, 2000; Dumpala *et al*., 2006).

Leghorns are excellent layers of white eggs (around 300 per year), but they can be noisy, flighty, and easily excited. Leghorns mature quickly, but are generally not considered to be large birds, averaging from 1.5 to 2.5 kg. Due to their prolific egg-laying, they are preferred by laboratories for embryonic and avian biological research. They are also the number one breed used for large-scale commercial egg production in most parts of the globe.

Figure 2.5 White Leghorns (Courtesy ARC Poultry Unit at Glen)

They are good foragers and can often glean much of their diet from ranging over fields and barnyards. Leghorns are capable of considerable flight and often roost in trees if given the opportunity. Leghorns and their descendants is the most numerous breed in America today. The Leghorn has relatively large head furnishings (comb and wattles) and is noted for egg production. Leghorns rarely go broody.

2.3 Cock semen

The sperm cells of the domestic birds are long, cylindrical, and tapered at both ends. As in other animals, the spermatozoa contain an acrozome, a head, a mid-piece and a tail. The sperm are about 0.5 µm at their widest point and approximately 100µm in length, and their volume is about 10 μm^3. As the cells are transformed from spermatogonia, which measure about 6 µm in diameter and contain about 75 μm^3, into spermatozoa, they are greatly elongated and their volume is substantially reduced (Etches, 1996; Umesiobi, 2000)

Sire selection, which is based on performance tests, and which correlates semen viability and fertilizing potential, is extremely beneficial to the poultry industry. The ability to differentiate cocks with high semen viability provides a rational, objective reason to cull cocks with low fertility from a flock. Technologies such as semen viability tests provide valuable information as to how sperm function in a pooled sample and this information could alter the way cocks are currently managed. If

males with substandard fertility do not contribute to the production of offspring they can be evaluated early in production and removed from the breeding flock. The semen viability tests have been shown to predict fertility and show promise as management tools for sire selection (Umesiobi and Iloeje, 1999; Umesiobi, 2000, 2004; Dumpala *et al*., 2006).

Sperm viability is determined by simple microscopic examination combined with nigrosin-eosin stain. Live viable sperm do not take up the pink-coloured eosin stain, remaining white on the blue (nigrosin) background. Dead sperm take up the eosin, and appear pink. When viewed under the microscope at 80-100x magnification, a field of view is measured for live vs dead sperm (Umesiobi and Iloeje, 1999). At the same time, live abnormal sperm can be counted because these will also probably be incapable of fertilization. The sperm of normal avian species are “worm-like” in appearance with a thin symmetrical-shaped body culminating in a short (15-20% length) thin tail. These normal sperm are gently curved. Most abnormal sperm are characterised by severe bending in the head, mid or tail region (Holsberger *et al*., 1998; Umesiobi and Iloeje, 1999; Hazary *et al*., 2000).

Semen viability is affected by various factors, the most important of which are live weight and semen collection technique. There is a significant positive correlation between body weight and seminal volume, pH, and abnormal spermatozoa rate, whereas there is a negative correlation between body weight and motility,

spermatozoon concentration and live spermatozoon rate in poultry (Donoghue, 1999; Alkan *et al*., 2002). It is reported that a semen collector can affect semen quality while harvesting the semen and it is also advised that every breeding male should be stimulated prior to semen collection according to its nature by applying appropriate pressure to the genitalia so as to avoid contamination with faeces, urine or blood (Dumpala *et al*., 2006). It is very important to know the proportion of defective (abnormal) spermatozoa in a semen sample to determine fertility. Zahraddeen *et al*. (2005), who studied the semen of American Bronze turkeys, reported a mean 6.17 ± 1.47% of abnormal spermatozoon. Froman and Feltmann (1988) examined the spermatozoon morphology of poultry semen and pointed out that the mid-piece is considerably longer than that of other species, approximately one quarter of the head's length, and that this property causes poultry spermatozoa to have more mid-piece bendings than other species. Froman and Feltmann (1988) classified the morphological defect types of semen assessed in vitro as follows:

1- Neck bending (mid-piece bending)

2- Mid-piece damage

3- Acrosome damage:

    a- Bending

    b- Swelling

    c- Knotting or rounding

4- Whole head swelling

5- Tail defects

2.3.1 Semen volume and concentration

Several researchers have studied the effect of semen volume and concentration on fertility. Zahraddeen *et al*. (2005) found no relationship between sperm concentration and fertility. However, Dumpala *et al.* (2006) found a significant relationship between sperm concentration and fertility. However, none of these reports found a significant positive correlation between fertility and semen volume. During a fertility study where hens were artificially inseminated with 0.025 mL semen bi-weekly, Kelso *et al*. (1997a) reported a correlation between fertility and semen volume. In addition, a significant ($P<0.05$) relationship between fertility and sperm concentration was found to exist. The significant correlation between fertility and semen volume found by Hazary *et al*. (2000) suggested that cocks could be selected for fertility midway through the breeding season based on semen volume. Table 2.1 shows ejaculate volume and sperm concentration of semen from different species of poultry, as compiled by Davitan (1996) and Kurbatov *et al.* (1987).

Also, the significant correlation found between fertility and sperm concentration late in the reproductive period is supported by work done by Froman and McLean, (1996). Recently, during a mobility phenotype experiment conducted by Holsberger *et al*. (1998), no significant differences were found among the mean ejaculate volumes or sperm concentrations between high and low mobility

phenotype toms. Donoghue (1999) found no fertility differences among turkey hens when inseminating with 0.025, 0.0125, or 0.010 mL of semen.

Table 2.1 Characteristics of semen from different species

Species strain	Semen volume (ml)	Sperm concentration (billion/ml)
Chicken		
Leghorn	0.2-0.5	2.0-4.0
Cornish	0.82-1.0	1.56-2.0
White Plymouth Rock	0.6-0.8	2.0-2.2
Turkey	0.25-0.4	5.0-8.0
Gander		
Vladimirskie	0.1-0.6	0.8
Kholmogorskie	0.15-0.8	0.8
Chinese	0.1-1.3	1.15
Kubannskie	0.1-1.3	0.3-1.0
Drake		
Pekin	0.1-0.7	1.5-8.0
X11	0.05-0.6	1.5-8.0
Muscovy	0.05-0.5	
Guinea fowl	0.01-0.2	2.1-8.8
Quail	0.005-0.02	1.5-2.5

Source: Davtian (1986) and Kurbatov et al. (1987)

Also, Donoghue *et al.* (1996) found no difference in initial fertility when inseminating with 0.025 mLvs.0.050 mL. However, differences were seen fourteen days after the last insemination. The 0.025 mL amount of semen would appear to be more than sufficient in fertilizing capacity.

2.3.2 Sperm motility

Sperm are vehicles which carry DNA to the ovum (Zahraddeen *et al*., 2005) and as such the sperm motility is a good indicator of sire potential. Sperm motility has been used by several investigators to predict potential fertility. The most obvious variable of a semen sample viewed under a microscope is the motility of its constituent sperm. The extent of the swirling wave motion of sperm movement observed in a hanging drop preparation and scored on a subjective 0 (no movement) to 5 (complete sample vigorously swirling) scale, has been the most widely used method for estimating sperm quality (Graham and Wishart 1994). Historically, the swirling movement of sperm placed on a microscope slide has been used as a subjective estimate of sperm motility (Umesiobi and Iloeje, 1999). The Swirl Method has shown good correlation with fertility in chickens and turkeys (Donoghue, 1999; Zahraddeen *et al*., 2005).

Bayyari *et al*. (1990) made the observation that hens inseminated with less motile sperm produced significantly fewer fertile eggs, compared to hens inseminated with highly motile sperm. Bramwell *et al.* (1995) found a positive correlation between motile sperm and percent fertility, and also that the relationship between the number of motile sperm per ejaculate and fertility was consistent and could be used as a prediction indicator. An important distinction in some of the newer methods of motility assessment is the objectivity of the tests, that is, each semen sample is scored by an instrument instead of a technician, reducing the subjectivity of semen assessment. A very promising approach for objective sperm motility analysis has recently been developed for rooster sperm (Froman and McLean, 1996) and modified for turkeys (Donoghue *et al*., 1998b). The Sperm Mobility Test is based upon the ability of sperm to swim into a dense, inert non-toxic diluent called Accudenz ®. This assay is performed at body temperature (41℃), and requires sperm to be mobile to penetrate into a solution, possibly mimicking some of the environment sperm are exposed to in the hen's reproductive tract. Male-to-male variation in sperm mobility phenotype has been estimated using the Sperm Mobility Test and shown repeatedly to be a normally distributed trait (Froman and McLean, 1996; Froman *et al*., 1997; Froman and Feltmann, 1998; Holsberger *et al*., 1998). Froman *et al*. (1997) contend that sperm mobility is a quantitative trait and most importantly, sperm mobility is a determinant of fecundity. The Sperm Mobility Test has the potential for use as a method of predicting the fertilizing ability of potential sires where other semen evaluation tests have failed. An advantage of the Sperm Mobility Test is that it is

simple, requires little technical expertise and is consistent over the reproductive life of males. Its potential for on-farm use is good, although it needs to be modified for farm application (Zahraddeen *et al*., 2005).

After differentiating toms into minimal and maximal sperm motility categories based upon the Sperm Mobility Test, Froman and McLean (1996) found that the maximal motility groups had greater fertility than the minimal motility groups. These findings were consistent with the research of Froman *et al*. (1997), in which the Sperm Mobility Test was used to separate semen donors from a flock of roosters. Furthermore, Donoghue *et al.* (1998) observed a significant increase in fertility of turkey hens inseminated with high sperm mobility phenotype toms during a 16-wk fertility trial compared to hens artificially inseminated with low sperm mobility phenotypes. To support sperm mobility phenotype use as a sire selection tool, Holsberger *et al*. (1998) tested the initial sperm mobility phenotype for time dependency. They found no significant change over a 5-month period in high mobility phenotype toms and no change at all among low mobility phenotype toms after the first month. Zalata *et al*. (1998) demonstrated a correlation between sperm motility and sperm docosahexaenoic acid in humans and reported that the relationship was not linear but that it could be calculated exponentially. The human ejaculates obtained with poor sperm motility contained lower amounts of docosahexaenoic acid and produced more lipidperoxides, therefore supporting the theory of Umesiobi and Iloeje (1999) and Umesiobi *et al*. (2004) that low motility leads to poor fertility.

2.3.3 Semen pH

Regulation of functions of both mammalian and non-mammalian spermatozoa, such as the initiation of the acrosome reaction or motility, is associated with changes in the internal pH of the spermatozoa (Babcock *et al.*, 1983; Sase *et al.*, 1995; Zeng *et al.*, 1996). A rise in external pH, followed by increased internal pH, initiates motility of boar spermatozoa (Gatti *et al.*, 1993) and alkalinisation of the extracellular fluid alone can induce the re-initiation of motility of human spermatozoa (Saito *et al.*, 1996).

Chicken spermatozoa show a reversible, temperature-dependent, inactivation of motility when the temperature is increased from 30°C to 40℃ (Munro, 1938). At 40℃, inhibition of motility can be released by the addition of calcium (Ashizawa and Wishart, 1987). Alkalinisation of the external pH, with a demonstrable increase in internal pH, (Ashizawa *et al.*, 1989; Ashizawa *et al.*, 1994a and 1994b; Barna and Boldizsár, 1994; Barna and Boldizsár, 1996) was also shown to stimulate chicken sperm motility at 40℃. Temper ature-dependent inhibition of sperm motility has also been found in other avian species, such as turkey and duck (Wilson and Wishart, 1996), although the effect of pH on releasing this inhibition is unknown in these species.

2.4 Fertility of layer chicks

Brillard (2006) defined fertility as the ability to reproduce. This definition should however be more accurately targeted in poultry as it is applicable *per se* to both animal (e.g. hen fertility) and egg, each of which is seen as entity (egg fertility). Indeed, a common practice in poultry species is to restrict the definition of “fertility” to the level of fertilization of laid eggs (fertile eggs/incubated eggs x

100). This criterion can be estimated in eggs at one or the other stage of their development (i.e. before or during incubation). Egg fertility in itself depends on hen fertility as, following mating a hen may or may not store functional spermatozoa in its oviduct.

True fertility is calculated as the number of fertile eggs observed expressed as a percentage of all eggs examined. Wishart *et al*. (2001) described fertility data/percentage fertile as “percentage fertile eggs” laid over a period of time. Such data will be recorded routinely in commercial poultry operations and are usually expressed on a weekly basis. Eggs can also be examined after 5-10 d incubation to determine fertile/ infertile numbers as well as occurrence of deadly germs (see Table 2.2 below). Eggs are carefully broken, and the germinal disc/embryo examined within the broken shell or on the palm of the hand.

Table 2.2 Pattern of embryo mortality related to breeder age

			Expected occurrence(%)		
Description	Days	Embryo	27	45	64
	Incubation	Identification			
Infertile	0	Germinal disc	10	7	15
Early dead					
(1-7d)	1	Primitive streak			
	3	Embryo on left			

		Side	4	2	4
	5	Appearance			
		Elbow/knee joints			
	7	Comb evident			
Mid-dead	11	Tail feathers	0.7	0.5	0.5
(8-14d)	13	Feather down			
Late dead	16	Feathers	2.5	2.0	3.5
(15-21d)	18	Head under right			
		wing			
	20	Yolk sac absorbed			

(Leeson and Summers, 1998)

Internal eggs retain the original yolk colour, and there is little difference in appearance compared to fresh eggs. The germinal disc is still quite distinct and small. With embryos dying very young, the germinal disc will be larger in diameter and there will not be a distinct raised disc *per se*. The yolk is often paler in colour and sometimes has a mottled appearance. When candled at 7d, the total number of viable embryos, expressed as a percentage of all ages examined, is often referred to candling fertility. Eggs can be examined after chicks have been taken from the hatch trays to determine general age of embryo mortality. The most common classification is early, mid and late dead germs which refer to 1-7, 8-14 and 15-21d respectively.

For good results in the artificial insemination of chickens, the quality of semen and a successful injection of semen into the female genital tract must be ensured (Adenokun and Sonaiya, 2001). A scientific determination of the fertilizing ability of the semen can be made by motility, live-dead and morphological examinations, and this is called potential fertility (Alkan *et al*., 2002). In addition to hereditary traits, live-weight and semen collection techniques are known to affect semen quality (Gunaratne, 1999; Kingori *et al*., 2003). Therefore, spermatological characteristics are evaluated in semen to be inseminated and the success of artificial insemination is directly dependent on the quality of the collected semen (Froman and Feltmann, 1998; Zahraddeen *et al*., 2005).

Immediately following ovulation, the ovum descends through the infundibulum where fertilization normally takes place (Bramwell *et al*., 1995). If successful fertilization is to occur; the sperm must penetrate the original inner perivitelline layer (PL), directly over the germinal disk (GD) which surrounds the ovum at ovulation (Cecil and Bakst, 1993; Donoghue1996). Additionally, an outer PL encloses the ovum as it nears the portion of the oviduct adjacent to the ovary. Spermatozoa must digest and penetrate the PL in order to reach and unite with the female pronucleus (Donoghue *et al.*, 1998). Bramwell and Howarth (1992a,b) used these holes in the PL to quantify the sperm penetration in recently ovulated ova in vitro. Using their quantifying technique, Bramwell and Howarth (1992a) suggested that sperm preferred to penetrate directly above the GD. Bramwell *et al*. (1995) reported a significant positive correlation between GD sperm

penetration and fertility (r = .89, P<0.0001) in naturally mated birds and a similar positive correlation between GD sperm penetration and fertility in AI birds (r = .90,P<0.0001). Furthermore, Bramwell *et al*. (1995) observed an increase in fertility in relation to sperm penetration (SP) of the GD as sperm dose increased from 25 million sperm to 100 million sperm. When inseminating hens with fresh semen or 24 h in vitro stored semen, Donoghue (1996) observed large variations among treatments with 3 to 381 and 2 to 460 holes per egg, respectively. Due to the hole variation by week, no differences were observed within the treatments, therefore, the data collected over a 10-wk period were combined. Upon combination of the data, the mean number of holes in the PL was significantly greater for the fresh semen than for the 24 hr stored semen. However, there were no differences in fertility or hatchability between the fresh and stored semen when analysis for fertility and hatchability was completed.

When comparing young (25 wk) to old (60 wk) Naked Neck broiler breeders of the same genetic stock, Kelso *et al*. (1996) observed wide differences in their semen characteristics. The spermatozoa phospholipids, free cholesterol, and free fatty acids increased in the older birds whereas triacylglycerols and cholesterol esters decreased. The older birds' sperm concentration was significantly lower than the younger birds' sperm concentration (1.70 ± 0.57 vs. 2.08 ± 0.41). The live spermatozoa percent dropped significantly in the 60-wk birds compared to the 25-wk birds. While the glutathione peroxidase dropped significantly in the older group to just 9%, the younger age group's expression of superoxide

dismutase stabilised. Also, a 75% drop in the metabolic activity of the old birds was evident, when compared to the younger birds. A combination of the spermatozoa lipid changes observed in old males may play a significant role in the decreased fertility observed with older male turkeys. In addition to confirming the results of Kelso *et al*. (1996), Cerolini *et al*. (1997) observed fertility changes that they believed to be linked to the increased proportion of total sperm phospholipids that reached its maximum at 39 wk. Cerolini *et al*. (1997) reported that semen concentrations increased from 24 to 39 wk of age, remained steady at 39 to 54 wk of age, and then dropped significantly at 72 wk of age. The sperm motility also followed this trend, being highest at 24 to 39 wk of age and dropping significantly during the second half of the reproductive period. Fertility remained unchanged, reaching its peak at 39 wk of age and gradually decreasing from this point. Supporting this data, Zalata *et al*. (1998) showed infertile human spermatozoa to have significantly less DHA, PUFA, n-3 monounsaturated fatty acids, total n-3 series fatty acids, and a lower double bonded index.

The main goal of layer management is producing eggs. However, the only good layer egg is a fertilized egg. Fertility, the percentage of eggs laid that are fertilized, is very important in poultry production. If an egg is not fertilized, then, of course, it will not contain an embryo and will not hatch. Simply put, "Hatchability can never be better than fertility." (Umesiobi, 2000; Kingori *et al*., 2003).

Hatchability is around eight percentage points lower than fertility because, usually, many chick embryos are lost during incubation (Kitalyi, 1998). For example, even if 93 percent of the eggs laid are fertilized, then under normal incubation conditions only 85 percent of the eggs will hatch (Kingori *et al*., 2003). This example illustrates how fertility must be very high to achieve above average hatchability.

Chickens need to be kept under ideal conditions for maximum life of flock fertility. The chicken's reproductive system is very sensitive to the bird's environment, and under poor conditions the reproductive system will dwindle (Umesiobi, 2000). For example, the environment can cause a rooster's testes to increase or decrease in size several hundred-fold (MacDonald and Edwards, 1993). Before we can understand which management factors influence fertility, however, we must first examine the fascinating process of fertilization in poultry.

Fertilization in any animal depends on production of eggs from the female, and sperm from the male. A problem with either sperm or egg production can decrease fertility. The rooster's reproductive system is simple when compared to humans or other mammals. The rooster does not have a prostate gland or any of the accessory reproductive glands. Like all other animals, chicken sperm carry the genetic material from the rooster and are produced within the testes. The rooster has two very large testicles within the abdominal cavity on each side of the backbone. After sperm leave the testes, they enter the epididymis, where

they gain the ability to swim. Next, the sperm enter the vas deferens, where they are stored until the rooster mates with a hen (Brillard, 1994; Rodrigues and Lok, 2000; Umesiobi, 2000).

Sperm formation takes about 15 days. The cock's semen contains around 5 billion sperm per ml, about 40 times as much as that of a human (Brillard, 1994). Once a rooster is mature and if properly maintained, it will manufacture about 35,000 sperm every second of its life. However, just like the males of many animal species, the fertilizing potential of roosters varies, even within a flock. For example, some roosters are extremely fertile and create a maximum number of quality sperm; other roosters are sub-fertile and do not make enough good sperm. This variation in rooster quality is caused by management, environment, nutrition, and genetics (Bakst *et al*., 1994; Brillard, 1994).

The hen does not produce nearly as many eggs as the cock produces sperm, but during her 40 weeks of production, the broiler breeder hen lays about 180 eggs. Egg formation requires about 25 hours. Since egg formation requires more than 24 hours, even the best hens cannot lay an egg every day in succession throughout their productive life. As is the case with roosters, some hens are more productive than others, and management has a major impact on variability among hens (Bakst *et al*., 1994).

The hen's reproductive system can be divided into two major components: the ovary and the oviduct. The ovary produces the egg yolk. The oviduct adds the white, shell membranes, and shell to the yolk to complete egg formation. The hen has only one ovary, which is on the left side of her abdomen. The ovary has several thousand ova (egg yolks) in different stages of development and looks like a bunch of grapes. Very immature yolks contain only genetic material from the hen, and as the yolks grow to around 1 mm in diameter, they become white. If the hen is managed properly, many of these developing egg yolks will mature in about 19 days into large, 35 mm, yellow yolks. As the egg yolk develops it will get water, sugars, fats, proteins, vitamins, and minerals from the hen's blood. These are all necessary for the embryo to develop. The egg yolk is surrounded by the perivitelline membrane. This keeps all of these nutrients in a ball-shaped package. One particularly visible region of the perivitelline membrane is the germinal disc. The germinal disc is a small white dot about half the size of a pencil eraser on the surface of the yellow egg yolk. Fertilization takes place here, and embryonic development begins (Lake, 1995; Hammerstedt, 1996).

When the egg yolk is mature, it leaves the ovary, and within 20 minutes it is captured by the infundibulum, the first part of the oviduct. Here fertilization takes place. Following mating, sperm enter the hen's oviduct and are stored within sperm storage glands. Only sperm that can swim will enter these sperm storage sites. These glands can store more than half a million sperm. Sperm can remain

alive in these glands and fertilize eggs for up to 3 weeks (Brillard, 1994; Etches, 1996).

A hen will have maximum fertility for only about 3 to 4 days after one mating. For this reason, the male-to-female ratio in a flock must be enough to ensure mating of every hen every 3 days or so. Sperm do not break through the eggshell. Instead they travel up the oviduct to the infundibulum to join with the egg yolk. The sperm bind to the perivitelline membrane and make a hole as they enter the egg. Hundreds of sperm may enter the yolk. As a matter of fact, the more sperm that enter the yolk, the more likely the egg is to be fertilized. Around 30 sperm must enter the egg near the germinal disc to ensure a 95 percent chance of fertilization. While it is true that only one sperm is necessary to fertilize an egg, the probability of an egg being fertilized by only one sperm reaching and penetrating it is very low (Brillard, 1994; Etches, 1996).

After about 15 minutes, the yolk leaves the infundibulum (fertilized or not) and receives the egg white, shell membranes, and shell over the next several hours from the magnum, isthmus, and uterus sections of the oviduct. When the hen lays a fertilized egg, the chick embryo has already developed, in about 25 hours, into approximately 20,000 embryonic cells and is a live, breathing organism. If this fertilized egg is handled properly before and during incubation, a healthy baby chick is the result (Brillard, 1994; Etches, 1996; Beaumont *et al*., 1998).

2.5 Hatchability of fertile eggs

As mentioned earlier, hatchability is around eight percentage points lower than fertility because, usually, many chick embryos are lost during incubation. For example, even if 93 percent of the eggs laid are fertilized, then under normal incubation conditions only 85 percent of the eggs will hatch. Most data confirm that fertility is maximized with weekly inseminations of about 150 million sperm (Table 2.3).

Table 2.3 Influence of sperm number per insemination on layer chick fertility and hatchability

Sperm number	Fertility	Hatch of fertile	Early dead
(10^6)	(%)	(%)	(%)
25	42a	87a	11.5a
50	70b	91ab	5.2b
100	87c	93b	1.9b
200	94d	94b	2.5b

Source: Eslick and MacDaniel (1992)

The data from Eslick and MacDaniel (1992) confirm many reports on the relationship between sperm number per insemination and fertility. Also of interest

in this study, is the negative effect on hatch of fertile, due to early dead embryos, from having a minimal number of sperm in the oviduct.

2.6 Effects of cock semen viability and insemination procedure on the fertility of layer chicks

Fertility is always a major concern for poultry breeders. Considerable evidence has shown that reduced semen viability impairs sperm numbers, motility, and fertilizing ability in birds (Kelso *et al*., 1996) and mammals (Umesiobi and Iloeje, 1999; Umesiobi, 2006). Previous studies by Umesiobi (2004) and Umesiobi *et al*. (2002) seemingly implicated semen viability as having an important role in sperm functionality and in optimising fertility. Indeed, mammalian spermatozoa lipid composition has been shown to play a major role in the physiochemical modifications leading to fertilization (Kelso *et al*., 1996).

The idea of selecting cocks early in production based on their seminal traits or sperm characteristics has caused several researchers to perform turkey semen evaluation studies over the past few decades. The amount of work invested in understanding the link between the cock and fertility is easily justified when evaluating the impact poor semen quality has on poultry production. Research on artificial insemination in chickens and turkeys has yielded conflicting information regarding optimum semen dosage (Donoghue *et al*., 1996), frequency of insemination (Froman *et al*., 1997), optimal time of insemination (Holsberger *et*

al., 1998; Hazary *et al*., 2000), optimal depth of insemination (Dumpala *et al*., 2006), and semen volume and concentration (Froman and Feltmann, 1998; Roads *et al*., 1998). To ensure high levels of fertility in hens, the industry typically attempts to follow nature's increasingly high levels of mating just prior to the onset of sexual maturity. Commercially, hens undergo AI 3 times in succession, within 3 to 4 days, following onset of lay, after which hens are inseminated at 7 – 14-day intervals throughout the production season (Ogasawara and Rooney, 1996). This procedure is thought to fill the sperm glands of the hen and prevent fertility arrest (Bakst *et al*., 1994; Lake, 1995).The idea of selecting "superior" males has given rise to the development of assays and semen evaluation methods that test the quality of pooled semen (Brillard, 2006).These assays and methods, typically looking at sperm viability (Bayyari *et al*., 1990; Donoghue *et al*., 1995), sperm plasma membrane integrity (Bakst *et al*., 1991; Donoghue *et al*., 1996) and sperm metabolic functions, (Cecil and Bakst,1993) have not been conducive to "in-field testing".

Depth of insemination and variations among AI techniques such as depth of insemination can greatly influence the rate of fertility. Dumpala *et al*. (2006) recommended deep semen deposition, whereas Roads *et al*. (1998) and Dumpala *et al*. (2006) found no fertility differences when inseminating hens at 1.27 cm or 5 cm. Ogasawara and Rooney (1996) reported optimal fertility with insemination at > 5 cm, in which the spermatozoa were being placed close to the storage glands. Furthermore, Brillard (1994) found deep insemination – 8cm – of

hens produced better fertility compared to inseminations 2.5 cm deep. However, work done by Brillard (1994) showed significantly ($P<0.01$) greater fertility following 2 cm inseminations, compared with 7 cm deep insemination. The above findings may be due to the different distances of the cloaca to the shell gland in each line. The site of deposition with both shallow and deep insemination was also evaluated using Rhodamine B dye or red latex. During shallow inseminations of Large White hens, the markers were found in the vagina independent of depth of inseminations. Deep insemination penetrated 8 out of 15 UV junctions and 2 out of 15 vaginal walls (Beaumont *et al*., 1998). However, one thing to note is that the duration of fertility seems to vary among bird lines, although they all fall within the 28-32 day maximum reported for chickens and 35-70 days for turkeys (Donoghue *et al*., 1996; Ogasawara and Rooney, 1996; Beaumont *et al*., 1998; Brillard, 2006).

Chapter 3

Research design and methodology

3 METHODOLOGY

3.1 Experimental birds

This study was conducted at the Poultry Research Unit of the Agricultural Research Council (ARC), Glen, South Africa during the period of May, 2005 to September, 2006. Four different South African indigenous chicken breeds (Naked Necks (NKN), Ovambo (OVB), Venda (VND) and Potchefstroom Koekoek (PK)) were used in this experiment. The indigenous breeds were homogeneous and supplied by the Agricultural Research Council, Glen. The birds were reared to maturity at 20 weeks of age before the commencement of the trials according to the guidelines provided by the primary breeder during their brooder and grown-out periods.

During the pre-experimental period that lasted three weeks, cocks were trained twice daily (morning and evening) for semen collection by the massage technique. The cocks were assigned randomly to two treatment groups consisting of four birds per group. The experimental hens were also assigned randomly to two treatment groups consisting of twenty birds per treatment group. The treatment groups consisted of morning (930 hours) and afternoon (1530 hours) throughout the duration of the experiment following the procedures of

Umesiobi and Iloeje (1999) Abu *et al*. (2006). Data were generated over a period of 68 weeks (May 2005-September 2006). One male (cock) from each breed was randomly selected and allocated to treatments in a completely randomised design (CRD).

3.2 Bird management and experimental design

From each of four South African chicken breeds, 40 hens and 8 cocks were selected randomly for this study. Semen from each cock was used to inseminate 5 hens per breed, in each treatment. A total of 1600 hatching eggs (400 eggs per breed) were collected for incubation in 3 batches. The birds were individually caged in a tri-tier battery system consisting of a cage space of 0.2 m per bird and the cocks were trained for artificial semen collection. The birds were exposed to natural daylight of 12 hours/day. The birds were weighed and selected according to their weights. The birds were marked with rings on their legs to identify the different breeds.

The birds were dewormed, vaccinated and fed *ad libitum* on commercial diets (AgriData Feeds Pty, Brandfort) containing 150 g crude protein, 11.3 MJ metabolisable energy (ME) and 10 g calcium per kg diet for cocks. The hens were fed on a breeder hen diet containing 180 g crude proteins, 11.3 MJ ME and 30 g calcium per kg diet. Water was provided *ad libitum*. The feeding management was carried out using the procedures recommended by Umesiobi

(2000). All birds were randomised soon after they started laying eggs and they were kept in the same environment.

3.3 Cock semen collection

Semen was collected from the 32 matured cocks once a week, in the morning (09:30) and afternoon (15:30) throughout the duration of the experiment, as recommended by Umesiobi and Iloeje (1999) and Abu *et al*. (2006). Each cock was trained for semen collection using the abdominal massage method recommended by Jayaraja, (1992), Ali *et al*. (1993), Salahuddin *et al*. (1995) and Raju *et al*. (1997).

Figure 2.6 Semen collection at the Poultry Unit of the ARC, Glen

The abdominal massage technique involves restraining the male and gently stroking the back of the bird from behind the wings towards the tail with firm rapid strokes. The male responds with tumescence (erection) of the phallus, at which time the handler gently squeezes the cloaca expressing semen through the

external papillae of the ducti deferentis, collecting semen into a funnel. Prior to the abdominal massage, cocks were clean-shaven in the vent area for cloaca to be visible and for semen not to be wasted by feathers. Care was taken to avoid contamination of semen with faeces and urates. All males producing watery, yellow, or no semen were excluded from the experiment. Semen was collected once per week (09:30 and15:30) and poured into a thistle funnel: one operator was holding a cock while the other was holding the funnel.

3.4 Cock semen evaluation and dilution

Semen evaluation involved determination of ejaculate volume, colour, viscosity, sperm motility and concentration. Sperm motility was recorded in percentage according to Umesiobi and Iloeje (1999). Sperm concentration was estimated by haemocytometer counts as described by Iheukwumere and Okere (1990). Sperm morphology was assessed by differential staining according to Butswat (1994), Hafez (1995) and Kelso *et al*. (1997a).

The semen was diluted with a diluent called VIRG-2 to make a semen extender. The VIRG-2 is a buffered salt solution containing various substrates for the purpose of diluting semen and supporting the viability of the spermatozoa. The diluent consisted of:

Sodium glutamate 2.8 g

Glucose 1.8g

Water 100ml

Ph 6.85-6.95

Semen was diluted with the diluent in the ratio of 1:1 or 1:2. The diluted semen was kept at 5^{o}C. Diluent was prepared by dissolving all the ingredients in 100ml of distilled and dionised water. Each semen sample was divided into two equal volumes. The macroscopic evaluation was used immediately after collection in the heated stage microscope, semen quality was assessed immediately after collection by macroscopic and microscopic evaluation to determine the colour, percentage of live and progressive motile spermatozoa. Only samples with a minimum of 50% live sperm and 40% progressive motility were accepted for further observation as recommended by Umesiobi *et al*. (2004).

3.5 Artificial insemination and incubation

The artificial insemination technique used in this study is referred to as cracking, venting or everting the hen. In this technique, semen was deposited 2 to 4 cm into the vaginal orifice concurrently with the release of pressure on the hen's abdomen. Inseminations were accomplished with straws, syringes or plastic tubes.

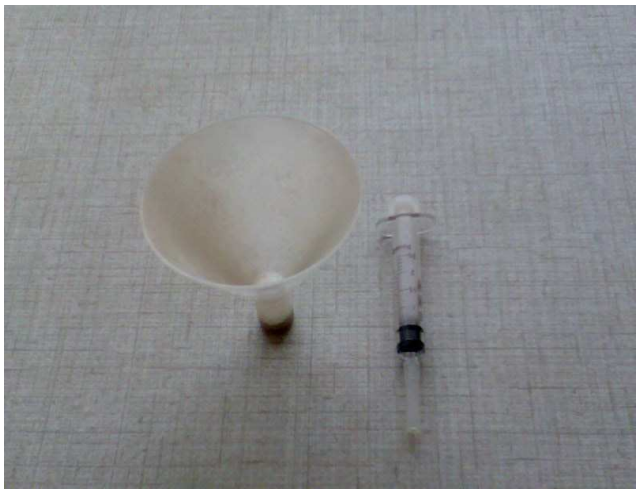

Figure 2.7 Apparatus for Artificial insemination at the Poultry Unit, ARC, Glen

(a) Thistle funnel & (b) Syringe

The females were inseminated intra-vaginally with the syringe to a depth of 8 cm, at between 12:00 and 15:00 hours South African local time, when the uteri of most of them were free of hard-shelled eggs. The dosage that was used was 0.5ml of semen.

Figure 2.8 During artificial insemination hens are partially removed from the cage to provide the opportunity for rapid insemination for caged birds

Each hen was inseminated twice a week throughout the days of incubation. During the experimental period, each hen was inseminated with 0.5 ml diluted semen. The artificially inseminated hens were examined for egg production, fertility (percentage fertilized) and hatchability (percentage hatched) in accordance with the procedures of Barbato *et al*. (1998).

Hatching eggs were collected twice a day (8:00-11:00 and 14:00-17:00) and marked for individual cock and hen two days after first insemination. Eggs were stored in the cooler at a temperature of between 12 and 14^{o}C and were set in weekly intervals at a temperature of 37.5^{o}C and relative humidity of 80-85% in the setter.

Well-shaped and sound shell eggs were weighed and dipped in a powerful disinfectant solution. Then eggs were stored over a period of one week in a cool room at 15 to 17℃ and 75 to 80% humidity (RH). Pro per cleaning, disinfection and fumigation were conducted before setting of eggs. Eggs from each pullet were set adjacent to each other on the same tray. The number of eggs set for each individual sire-hen group was recorded. The eggs were turned automatically in a programmed device, at 2-hourly intervals. The following temperature and humidity was maintained during the incubation period:

a) Setting temperature 37.5^{o}C up to 18 days of incubation.

b) Hatching temperature 0.5-1℃ reduced and 36.5 fr om 18 to 21 days of incubation.

c) Setting RH: 80-85% up to 18 days of incubation.

d) Hatching RH: 2-5% increased and 87-90% RH from 18 days of incubation and RH increased to 92% particularly at 21 days.

On days 10 and 18 of incubation, the eggs were candled to identify and remove infertile or clean eggs and eggs with dead embryos (dead in germ) simultaneously. The remaining eggs were transferred from the setting trays to different pedigrees before setting of eggs. Eggs from a compartment of hatching trays according to the breed and pullet were set in the afternoon of the 18th day of incubation. On day 21, the number of hatched chicks including the normal, weak, abnormal and dead chicks after hatch and the unhatched eggs and pips were counted and recorded separately as dead in shell according to breeds and treatments The chicks that were undersized, poorly feathered, parrot-beaked,, blind, lame, open-naveled etc. were considered abnormal chicks. After discarding all abnormal chicks the rest of the chicks were considered normal. The weight of all hatching eggs was measured in grams by using an electronic digital balance. The average was then calculated. Hatchery sanitation was strictly maintained during the experimental period.

3.6 Hatchability tests

Hafez (1993) defined hatchability as the number of offspring hatched divided by the number of fertile eggs at candling. Factors that depress hatchability include

farm management (improper handling, holding, transport, and storage of eggs before incubation and the improper management of the hatchery), disease transmitted within the egg, the genetics, nutritional status and age of the hen. Not all eggs fertilized by sperm develop during incubation. The eggs were candled on day 18 of incubation using the chicken egg candler which involved transmitting of an intense beam of light through the eggs to highlight vascular development of the embryo. The eggs that were identified as clear were broken open and examined for signs of embryonic development. The eggs without signs of development were declared infertile. Fertile eggs were transferred to the hatcher for the last three days, at a temperature of 37.5^{o}C and relative humidity of 80-85%.

3.7 Management of day-old chicks

The day-old chicks were hatched and removed, vaccinated and transferred to the brooding pens, then reared in battery cages. The hatched chicks were recorded and observed for quality and survivability.

3.8 Statistical analysis

Data on semen viability were analysed using the general linear model procedure of Statistical Analysis System, Version 9.1 (SAS, 2002). The statistical model included semen viability classification of high versus low performing (i.e. HP vs. LP) cocks, and individual cocks (breeding cocks) within treatment groups. All the

fertility and hatchability data were analysed for Analysis of Variance (ANOVA) using a Completely Randomised Design (CRD) (SAS, 2002).

The following model was used during data analysis:

$Y = F + B + (B õ) + e$

Where:

Y is the observation of the k^{th} population of i^{th} breed.

F is the overall mean

B is the fixed effect of i^{th} breed (i = 1, 2, 3, 4)

e is the random error assumed to be distributed (0,ó)

The correlation analysis procedure was used to extrapolate the relationships between the semen viability of cocks and fertility and hatchability traits amongst chicken breeds (Snedecor and Cochran, 1980).

Chapter 4

Results and discussion

4.1 FINDINGS FROM THE QUANTITATIVE NON-EXPERIMENTAL STUDIES

Table 4.1 describes the least squares means (± s.e.) of semen viability traits of South African Naked Necks (NKN), Ovambo (OVB), Potchefstroom Koekoek (PK) and Venda (VND) cock breeds selected for high (HP) or low (LP) breeding performance following 5 min massage technique before semen collection. Comparative assessment of semen viability amongst the various cock breeds is displayed in Figure 4.1. From Table 4.1, significant differences ($P < 0.01$) were found in semen volume amongst the cock breeds. The ejaculate volume ranged from 0.05 to 0.95 ml. The HP cocks in all the breeds studied gave higher semen volume than the LP cocks, with the highest ejaculate volume (0.72 ± 0.02 ml) obtained from PK cocks. The lowest ejaculated volume (0.09 ± 0.005 ml) was harvested from the Venda (VND) cock breed. The values obtained in this study are in conformity with the reports of Umesiobi and Iloeje (1999) and Butswat *et al*. (2002), who reported that the quantity of semen produced by male animals is not only dependent on the amount of sexual excitement but also partly dependent upon a number of factors which include their physical temperaments and sexual adjustment period such as stimulus changes (changes of teaser, semen collection or both).

The percentage sperm motility ranged from 12.32 to 98.11%. The HP cocks were superior to the LP cocks in sperm motility, values being 0.31 ± 0.02 vs. 0.10 ±

0.03% in NKN, 77.15 ± 0.59% vs. 54.50 ± 0.75% in OVB, 92.03 ± 0.65 vs. 60.18 ± 1.07% PK and 50.80 ± 1.13 vs. 47.12 ± 0.92% in VND.

Table 4.1 Least square means (± s.e.) for semen viability exhibited by high-performing or low-performing South African Naked Neck (NKN), Ovambo (OVB), Potchefstroom Koekoek (PK) and Venda (VND) breeds following exposure to 5 minutes sexual massage (5SM) technique

	Cock breeds			
	NKN	**OVB**	**PK**	**VND**
Semen volume (ml)				
HP	0.31 ± 0.02[a]	0.44 ±0.04[a]	0.72 ± 0.02[a]	0.24 ± 0.03[a]
LP	0.10 ± 0.03[b]	0.29 ± 0.03[b]	0.54 ± 0.01[b]	0.09 ± 0.005[b]
Sperm motility (%)				
HP	65.19 ± 1.33[a]	77.15 ± 0.59[a]	92.03 ± 0.65[a]	50.80 ± 1.13[a]
LP	50.33 ± 0.31[b]	54.50 ± 0.75[b]	60.18 ± 1.07[b]	47.12 ± 0.92[b]
Live sperm (%)				
HP	54.55 ± 0.55[a]	72.91 ± 0.9[a]	88.35 ± 0.72[a]	50.25 ± 0.62[a]
LP	38.86 ± 0.71[b]	51.01 ± 0.64[b]	63.14 ± 0.38[b]	42.89 ± 0.86[b]
Total sperm ($x10^9$/ml)				
HP	1.32 ± 0.01[a]	2.10 ± 0.07[a]	3.51 ± 0.04[a]	2.08 ± 0.01[a]
LP	1.04 ± 0.01[b]	1.26 ± 0.01[b]	2.10 ± 0.16[b]	0.76 ± 0.17[b]

[a, b] Values with different superscripts in a column within main effects are different ($P < 0.01$)

Where:
HP = High performing cocks
LP = Low performing cocks

Interestingly, there were significant differences in progressive motility in the four breeds of cocks, with the HP cocks of the PK breed recording the highest (92.03

± 0.65%) sperm motility. These findings agree with the studies of Aganga *et al*. (2000) and Adenokun and Sonaiya (2001) who reported that the superior reproductive potential exhibited by the various indigenous cock breeds showed that differences exist between genotypically different breeds as similarly reported by Butswat *et al*. (2002). The significant differences in most of the variables also suggest that the indigenous cocks are adapted to this environment and could be exploited further.

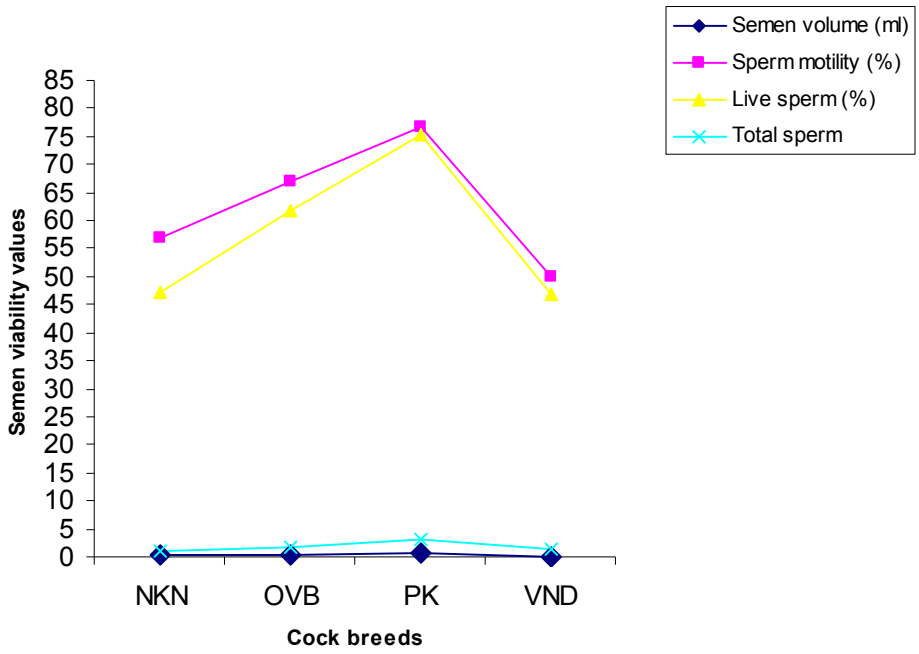

Figure 4.1 Comparative assessment of semen viability amongst cock breeds following 5 min massage technique

Results from the various cock breeds show that seminal traits such as percentage live spermatozoa were significantly higher ($P < 0.01$) in both the HP and LP when semen was harvested after 5 min of sexual massage (see Table 4.1, Figure 4.1). The percentage live spermatozoa ranged from 30.7 to 91.5%.

The HP cocks were superior to the LP cocks in live sperm cells, values being 54.55 ± 0.55 vs. 38.86 ± 0.71% in NKN, 72.91 ± 0.9 vs. 51.01 ± 0.64% in OVB, 88.35 ± 0.72 vs. 63.14 ± 0.38% in PK and 50.25 ± 0.62 vs. 42.89 ± 0.86% in VND. However, the highest percentage of live sperm cells (88.35 ± 0.72%) was obtained from the PK cock and the lowest values (38.86 ± 0.71%) were observed in the NKN cock semen. Similarly, total sperm ($x10^9$/ml) showed significant difference ($P < 0.01$) when semen was collected after 5 min of sexual massage. The values for the total sperm per ejaculate per ml ($x10^9$/ml) were significantly better ($P<0.01$) in the HP than the LP in all the indigenous cock breeds studied. (see Table 4., Figure 4.1).

The percentage range of live sperm cells in the four breeds was much wider than the value (50.7 to 97.1%) reported by Bayley and Phororo (1992). This range difference is probably attributed to genotypes and management. Total sperm ($x10^9$/ml) obtained in this study was higher than the findings of Dessie and Ogle, (1996) and Kumer *et al*. (2002). High fertility could therefore be achieved with the breeds studied, since Donaghue and Walker-Simmons (1999) reported a very high positive correlation between total sperm and fertility.

Tables 4.2 to 4.5 depict the correlations of semen viability traits ($P < 0.05$) amongst the NKN, OVB, PK and VND cock breeds recorded in this study.

Table 4.2 The correlations of different semen viability traits amongst cocks of the Naked Neck (NKN) breed

	1	2	3	4
1				
2	0.91*			
3	0.86*	0.95**		
4	0.91**	0.95**	0.98**	

Where: 1 = Semen volume (ml), 2 = Sperm motility (%), 3 = Live sperm (%) and 4 = Total sperm ($x10^9$/ml)
* = P < 0.05
** = P < 0.01

Naked Neck (NKN) cocks: Positive correlation was found between semen volume and sperm motility (r = 0.91, P < 0.05), semen volume and live sperm cells (r = 0.86, P < 0.01), semen volume and total sperm (r = 0.91, P < 0.01), sperm motility and live spermatozoa (r = 0.95, P < 0.01), sperm motility and total sperm (r = 0.95, P < 0.01), live sperm and total sperm (r = 0.98, P< 0.01).

Table 4.3 The correlations of different semen viability traits amongst cocks of the Ovambo (OVB) breed

	1	2	3	4
1				
2	0.97*			
3	0.93*	0.99**		
4	0.95**	0.98**	0.97*	

Where: 1 = Semen volume (ml), 2 = Sperm motility (%), 3 = Live sperm (%) and 4 = Total sperm ($x10^9$/ml)
* = P < 0.05
** = P < 0.01

Ovambo (OVB) cocks: Significant positive relationships exist between semen volume and sperm motility (r = 0.97, P < 0.05), semen volume and live sperm cells (r = 0.93, P < 0.01), semen volume and total sperm (r = 0.95, P < 0.01), sperm motility and live spermatozoa (r = 0.99, P < 0.01), sperm motility and total sperm (r = 0.98, P < 0.01), live sperm and total sperm (r = 0.97, P< 0.01).

Table 4.4 The correlations of different semen viability traits amongst cocks of the Potchefstroom Koekoek (PK) breed

	1	2	3	4
1				
2	-0.35*			
3	-0.35*	0.99**		
4	-0.41*	0.95**	0.94**	

Where: 1 = Semen volume (ml), 2 = Sperm motility (%), 3 = Live sperm (%) and 4 = Total sperm ($x10^9$/ml)
* = P < 0.05
** = P < 0.01

Potchefstroom Koekoek (PK) cocks: Negative correlations (P < 0.05) were found between semen volume and sperm motility (r = -0.35), semen volume and live sperm cells (r = -0.35), semen volume and total sperm (r = -0.41). However, positive relationships (P < 0.01) were recorded between sperm motility and live spermatozoa (r = 0.99), sperm motility and total sperm (r = 0.95), live sperm and total sperm (r = 0.94).

Table 4.5 The correlations of different semen viability traits amongst cocks of the Venda (VND) breed

	1	2	3	4
1				
2	0.40*			
3	0.26*	0.89**		
4	0.44*	0.82**	0.84**	

Where: 1 = Semen volume (ml), 2 = Sperm motility (%), 3 = Live sperm (%) and 4 = Total sperm ($x10^9$/ml)
* = P < 0.05
** = P < 0.01

Venda (VND) cocks: Positive correlations were noticed between semen volume and sperm motility (r = 0.40, P < 0.05), semen volume and live sperm cells (r = 0.26, P < 0.05), semen volume and total sperm (r = 0.44, P < 0.05), sperm motility and live spermatozoa (r = 0.89, P < 0.01), sperm motility and total sperm (r = 0.82, P < 0.01), live sperm and total sperm (r = 0.84, P< 0.01). This finding of significant correlations on semen viability agree with the findings of Merat (1996) and Missohou *et al*. (2002), who found significant relationships in semen volume and sperm motility in local cock breeds in Southern Senegal. Nevertheless, significant variations in correlations observed for semen viability traits amongst the cock breeds studied seem contradictory to the general belief of higher relationships in most African indigenous cock breeds. Such a difference however, suggests that semen viability in relation to breed may vary according to individual males in a population (Umesiobi, 2006).

Table 4.6 describes the least squares means (± s.e.) of the different fertility and hatchability traits as influenced by South African layer breeds Naked Neck (NKN), Ovambo (OVB), Potchefstroom Koekoek (PK) and Venda (VND) following artificial insemination with semen from high-performing (HP) or low-performing (LP) cocks genotypes. The comparative assessment of fertility and hatchability traits of South African indigenous layer breeds is provided in Figure 4.2. Significant differences ($P < 0.05$) were observed in fertility and hatchability amongst the layer breeds used in this study. The semen viability of HP and LP cocks elicited significant differences ($P < 0.05$) in fertility and hatchability of artificially inseminated (AI) layers. The HP cocks were significantly superior ($P < 0.05$) to the LP cocks in the rate at which their semen sired offspring, as evidenced by the higher fertility and hatchability of the various layer breeds.

Table 4.6 Least square means (± s.e.) for fertility traits in South African Naked Neck (NKN), Ovambo (OVB), Potchefstroom Koekoek (PK) and Venda (VND) breeds following artificial insemination with semen from high-performing or low-performing cocks

	Layer breeds			
	NKN	**OVB**	**PK**	**VND**
Average egg weight (g)				
HP	40.75 ± 0.1[a]	56.81 ± 0.41[a]	58.04 ± 0.85[a]	54.25 ± 0.91[a]
LP	37.10 ± 0.49[b]	40.40 ± 0.96[b]	55.52 ± 0.62[b]	51.22 ± 0.5[b]
Fertility (%)				
HP	60.77 ± 1.26[a]	70.55 ± 0.13[a]	74.29 ± 0.16[a]	60.80 ± 0.46[a]
LP	50.60 ± 0.32[b]	52.06 ± 0.3[b]	68.20 ± 0.45$_b$	53.75 ± 0.33[b]
Hatchability on set eggs (%)				
HP	83.33 ± 0.75[a]	77.50 ± 0.63[a]	84.03 ± 0.64[a]	66.50 ± 0.59[a]

LP	71.09 ± 0.63[b]	70.15 ± 0.66[b]	78.60 ± 0.54[b]	60.85 ± 1.09[b]
Live chicks (%)				
HP	95.66 ± 0.44[a]	79.05 ± 1.1[a]	71.02 ± 0.48[a]	75.91 ± 0.24[a]
LP	80.24 ± 0.45[b]	75.41 ± 0.43[b]	66.01 ± 0.66[b]	70.20 ± 0.18[b]
Normal chicks (%)				
HP	87.90 ± 0.63[a]	87.75 ± 0.45[a]	98.14 ± 0.67[a]	76.85 ± 0.46[a]
LP	75.82 ± 0.86[b]	71.45 ± 0.32[b]	80.10 ± 0.25[b]	61.40 ± 0.88[b]
Chick weight (g)				
HP	23.50 ± 0.11[a]	32.81 ± 0.49[a]	37.90 ± 0.28[a]	26.90 ± 0.36[a]
LP	23.50 ± 0.11[a]	32.81 ± 0.49[a]	37.90 ± 0.28[a]	26.90 ± 0.36[a]

[a, b] Values with different superscripts in a column within main effects are different ($P < 0.05$)

Where:
HP = High performing cocks
LP = Low performing cocks

The average egg weight ranged from 24.34 to 61.5 g. The HP cocks were superior to the LP cocks in average egg weight, values being 40.75 ± 0.1 vs. 37.10 ± 0.49 g in NKN, 56.81 ± 0.41 vs. 40.40 ± 0.96 g in OVB, 58.04 ± 0.85 vs. 55.52 ± 0.62 g PK and 54.25 ± 0.91 vs. 51.22 ± 0.5 g in VND. Nevertheless, PK layers produced eggs that gave the highest (54.25 ± 0.91 g) average egg weight (see Table 4.6, Figure 4.2). Fertility of the layer breeds ranged from 49.01 to 82.26 %. Semen from HP cocks gave higher values compared to those of LP cocks in percent fertility (60.77 ± 1.26 vs. 50.60 ± 0.32% in NKN; 70.55 ± 0.13 vs. 52.06 ± 0.3% in OVB; 74.29 ± 0.16 vs. 68.20 ± 0.45% in PK; 60.80 ± 0.46 vs. 53.75 ± 0.33% in VND) of layer breeds. Similarly, PK layer breeds recorded the

highest (74.29 ± 0.16%) fertility. OVB layers ranked second in fertility (70.55 ± 0.13%) values. The lowest fertility of 52.06 ± 0.3% was obtained from the NKN.

Hatchability of set eggs ranged from 49.07 to 87.02% amongst all the layer breeds studied. The HP cocks were superior to the LP cocks in percent hatchability and values recorded were 83.33 ± 0.75 vs. 71.09 ± 0.63% in NKN, 77.50 ± 0.63 vs. 70.15 ± 0.66% in OVB, 84.03 ± 0.64 vs. 78.60 ± 0.54% in PK and 66.50 ± 0.59 vs. 60.85 ± 1.09% in VND. The PK gave the highest percent (84.03 ± 0.64%) of hatched chicks (see Table 4.6, Figure 4.1). The lowest hatchability of 60.85 ± 1.09% was obtained from the VND layers.

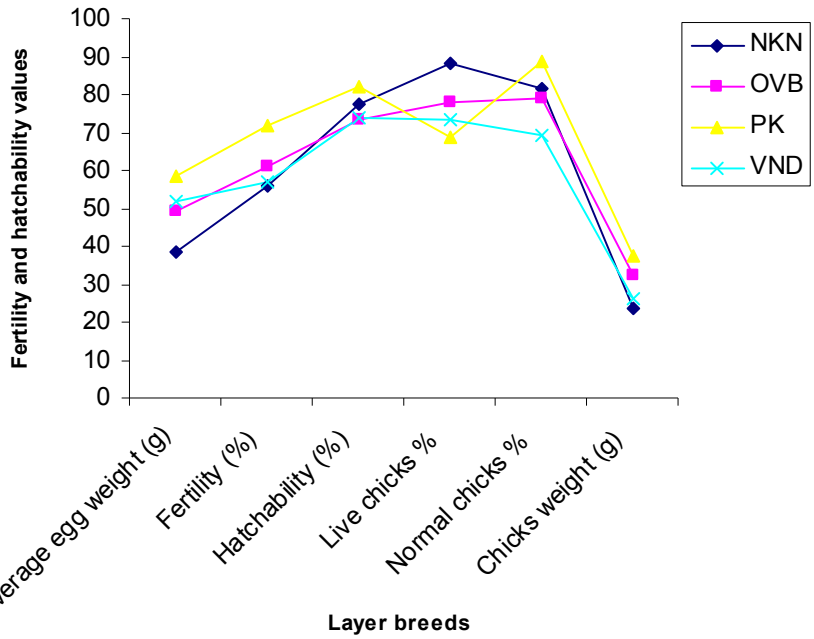

Figure 4.2 Comparative assessment of fertility and hatchability traits amongst layer breeds

Significant differences existed between layer breeds and percent live chicks. The HP cocks were superior to LP cocks in obtaining live chicks (see Table 4.6). Interestingly the NKN which expressed the lowest fertility (52.06 ± 0.3%) in this study gave the highest percent (83.33 ± 0.75%) of hatched chicks (see Table 4.6, Figure 4.1). The normal chicks ranged from 55.08 to 98.62%. Semen from the HP cocks produced a significantly higher ($P < 0.05$) percentage of normal chicks compared to the LP cocks, the values being 87.90 ± 0.63 vs. 75.82 ± 0.86% in NKN, 87.75 ± 0.45 vs. 71.45 ± 0.32% in OVB, 98.14 ± 0.67 vs. 80.10 ± 0.25% in PK and 76.85 ± 0.46 vs. 61.40 ± 0.88% in VND layer breeds. Overall, the PK layers produced the highest (98.14 ± 0.67%) percentage of normal chicks. The NKN ranked second in percentage of normal chicks.

Since no known change or procedure was introduced during the time of semen collection and insemination, these outstanding and consistent improvements in hatchability and fertility of AI layers tend to support earlier evidence (Umesiobi and Iloeje, 1999; Umesiobi, 2004; Umesiobi *et al*., 2004; Umesiobi, 2006a,b) that the ability of the sperm cells to survive during capacitation in a female reproductive tract may be dependent on a male's ability in donating optimum quality of semen following servicing capacity tests. The correlations of different fertility traits amongst layers of South African indigenous chickens are summarised in Tables 4.7 to 4.10.

Table 4.7 The correlations of different fertility traits amongst layers of the Naked Neck (NKN) breed

	1	2	3	4	5	6
1						
2	0.94*					
3	0.90**	0.96**				
4	0.93*	0.95**	0.97**			
5	0.97**	0.95**	0.94*	0.95**		
6	0.01	0.14	0.02	0.02	0.02	

Where: 1 = Average egg weight (g), 2 = Fertility (%), 3 = Hatchability of set eggs (%), 4 = Total chicks (%), 5 = Normal chicks (%) and 6 = Chick weight (g)
* = $P < 0.05$
** = $P < 0.01$

Necked neck (NKN) layers: Significant positive correlation was found between egg weight and fertility ($r = 0.94$, $P<0.05$)), egg weight and hatchability of set eggs ($r = 0.90$, $P<0.01$), egg weight and total chicks ($r = 0.93$, $P<0.05$) and egg weight and normal chicks hatched ($r = 0.97$, $P<0.01$). Also, some significant positive correlations were observed between fertility and hatchability on fertile eggs ($r = 0.96$,), fertility and total chicks hatched ($r = 0.95$, $P<0.01$), fertility and normal chicks hatched ($r = 0.95$, $P<0.01$), hatchability in set eggs and total chicks hatched ($r = 0.97$, $P<0.01$), hatchability in set eggs and normal chicks ($r = 0.94$, $P<0.01$) and total chicks and normal chicks ($r = 0.95$, $P<0.01$).

Table 4.8 The correlations of different fertility traits amongst layers of the Ovambo (OVB) breed

	1	2	3	4	5	6
1						
2	0.97*					
3	0.92**	0.96**				
4	0.82*	0.84**	0.84**			
5	0.98**	0.99**	0.97*	0.85**		
6	-0.11	-0.01	0.22	-0.08	0.05	

Where: 1 = Average egg weight (g), 2 = Fertility (%), 3 = Hatchability of set eggs (%), 4 = Total chicks (%), 5 = Normal chicks (%) and 6 = Chick weight (g)
* = $P < 0.05$
** = $P < 0.01$

Ovambo (OVB) layers: Significant positive relationship was found between egg weight and fertility ($r = 0.97$, $P<0.05$), egg weight and hatchability of set eggs ($r = 0.92$, $P<0.01$), egg weight and total chicks ($r = 0.82$, $P<0.05$), egg weight and normal chicks hatched ($r = 0.98$, $P < 0.01$), fertility and hatchability on fertile eggs ($r = 0.96$, $P<0.01$), fertility and total chicks hatched ($r = 0.84$, $P < 0.01$), fertility and normal chicks hatched ($r = 0.99$, $P<0.01$), hatchability in set eggs and total chicks hatched ($r = 0.84$, $P<0.01$), hatchability in set eggs and normal chicks ($r = 0.97$, $P<0.01$) and total chicks and normal chicks ($r = 0.85$, $P<0.01$). Some negative correlations were observed between egg weight and chick weight ($r = -0.11$), fertility of set eggs and chick weight ($r = -0.01$) and total chicks and chick weight ($r = -0.08$).

Table 4.9 The correlations of different fertility traits amongst layers of the Potchefstroom Koekoek (PK) breed

	1	2	3	4	5	6
1						
2	0.86*					
3	0.88**	0.88**				
4	0.67*	0.79**	0.80**			
5	0.83**	0.98**	0.92**	0.85**		
6	-0.35*	0.07	-0.15	-0.19	0.07	

Where: 1 = Average egg weight (g), 2 = Fertility (%), 3 = Hatchability of set eggs (%), 4 = Total chicks (%), 5 = Normal chicks (%) and 6 = Chick weight (g)
* = $P < 0.05$
** = $P < 0.01$

Potchefstroom Koekoek (PK) layers: Significant positive correlations were found between egg weight and fertility ($r = 0.86$, $P<0.05$), egg weight and hatchability of set eggs ($r = 0.88$, $P<0.01$), egg weight and total chicks ($r = 0.67$, $P<0.05$), egg weight and normal chicks hatched ($r = 0.83$, $P<0.01$), fertility and hatchability of fertile eggs ($r = 0.88$, $P<0.01$), fertility and total chicks hatched ($r = 0.79$, $P<0.01$), fertility and normal chicks hatched ($r = 0.98$, $P<0.01$), hatchability in set eggs and total chicks hatched ($r = 0.80$, $P<0.01$), hatchability in set eggs and normal chicks ($r = 0.92$, $P<0.01$) and total chicks and normal chicks ($r = 0.85$, $P<0.01$). Some negative relationships were observed between egg weight and chick weight ($r = -0.35$, $P<0.05$), hatchability of set eggs and chick weight ($r = -0.15$) and total chicks and chick weight ($r = -0.19$).

Table 4.10 The correlations of different fertility traits amongst layers of the Venda (VND) breed

	1	2	3	4	5	6
1						
2	0.22					
3	0.08	0.79**				
4	0.19	0.96**	0.90**			
5	0.13	0.97**	0.91**	0.98**		
6	0.14	0.13	-0.38*	0.01	-0.05	

Where: 1 = Average egg weight (g), 2 = Fertility (%), 3 = Hatchability of set eggs (%), 4 = Total chicks (%), 5 = Normal chicks (%) and 6 = Chick weight (g)
* = P < 0.05
** = P < 0.01

Venda (VND) layers: Significant positive correlations were found between fertility and hatchability of fertile eggs (r = 0.79, P<0.01), fertility and total chicks hatched (r 0.96, P<0.01), fertility and normal chicks hatched (r = 0.97, P<0.01), hatchability of set eggs and total chicks hatched (r = 0.90, P<0.01), hatchability of set eggs and normal chicks (r = 0.91, P<0.01) and total chicks and normal chicks (r = 0.98, P<0.01). Some negative correlations were found between hatchability of set eggs and chicks weight (r = -0.38, P<0.05) and normal chicks and chick weight (r = -0.05).

Effects of layers of different breeds on fertility and hatchability traits: In this study, little variation was observed in egg weight amongst the breeds. However, an apparent lower egg weight in NKN than in OVB, PK and VND seems to conform to the general notion of lower egg weight in lighter breeds (Aganga *et al.*, 2000;

2003). Highest fertility recorded for the heavy breed PK than for other breeds in the current study contradicts the reports by Aini (1990), who observed high fertility in light breeds. This difference could be because the PK has better inherent adaptation potential than any of the local breeds studied. Differences in fertility among batches (in different periods) agreed with Dessie and Ogle (1996). Differences in hatchability of total eggs set among breeds signify that in this study hatchability of total eggs recorded was just a function of fertility. Furthermore, this finding of hatchability of total eggs set agrees with that of Gueye (1998), who found significant differences in hatchability of fertile eggs between breeds. It also agrees with Kumer *et al*. (2002), who found that the hatchability for indigenous breeds on set eggs was higher.

The number of sound normal chicks is an indication of success of hatchability. The results showed significant differences among breeds. As well as this, significant deviation in abnormal chicks was recorded among breeds, leading to an assumption that genetic background may have significant influence on chick abnormality rather than being a function of management and environment. Chick weight variation for genetic background recorded is supported by Missohou *et al*. (2002) who noted that day-old chick weight increased significantly with increase in egg weight, which may also be as a result of differences in breeds. The results showed that comparatively large sized eggs always resulted in heavier chicks, and suggested that breed may play a significant role. Thus the result also signifies that chick weight is not just a function of egg weight, but is altered by

genetic background, and that it is favoured in heavier breeds (Pitel *et al*., 2000; Mosoeunyane and Nkebenyane, 2001).

Correlation among fertility and hatchability traits: In the present study, significant positive correlation was shown for all breeds between fertility and hatchability of set eggs, egg weight and chick weight. Positive correlation was shown for NKN, OVB and PK for fertility and egg weight; fertility and chick weight; fertility and normal chicks; egg weight and hatchability of set eggs; hatchability of set eggs and chick weight; hatchability of set eggs and egg weight; hatchability of set eggs and chick weight; egg weight and chick weight; egg weight and percent chick weight. Only VND, however, showed no correlation between egg weight and fertility, egg weight and hatchability of set eggs, egg weight and total chicks and egg weight and normal chicks.

Some negative correlations among NKN, OVB and PK were observed between egg weight and chick weight ($r = -0.35$, $P<0.05$), hatchability of set eggs and chick weight ($r = -0.15$) and total chicks and chick weight ($r = -0.19$). Furthermore, for VND, negative correlations were found between hatchability of set eggs and chick weight ($r = -0.38$, $P<0.05$) and normal chicks and chick weight ($r = -0.05$).

The positive correlations found between egg weight and normal chicks regardless of breed are well understood. Such egg weight related to normal chicks recorded agrees with Adenokun and Sonaiya (2001), who obtained

heavier normal chicks from larger eggs for Naked Neck chickens. A peculiarity of the findings of this experiment is that there were positive correlations between egg weight and fertility; egg weight and total chicks and egg weight and normal chicks hatched in NKN, OVB and PK, while the same correlations were not found in case of VND, indicating an interaction of breed and egg weight in terms of the above parameters. Such information is not available in literature, so comparisons cannot be made with the results of the current findings.

There was a negative correlation of normal chicks with chick weight for VND, while fertility had little relation to chick weight. This result partially agrees with Adenokun and Sonaiya (2001), who recorded higher fertility and hatchability of heavier eggs in Naked Neck chickens. Moreover, this study suggests that such a relationship may not be applicable to all breeds. It is evident from Tables 4.7 to 4.10 that chick weight had a positive correlation with fertility and hatchability for NKN, OVB and PK, but not for VND, indicating that such a relation may differ according to breed. The correlation figures also indicate that in OVB, the hatchability and egg weight were highly endowed with the advantage of producing more normal chicks.

Chapter 5

Conclusions and recommendations

5.1 GENERAL CONCLUSION

In conclusion, the results obtained in this study on the relationships between cock semen viability and the fertility of artificially inseminated South African indigenous layer breeds showed that the use of high performing (HP) cocks following 5 minutes of sexual massage prior to semen collection and artificial insemination of layers is a practical method for optimising sperm viability and subsequent fertility of hens. The results of this study suggest that the Potchefstroom Koekoek (PK) cocks are superior to those of the Naked Neck (NKN), Ovambo (OVB) and Venda (VND) breeds. The Ovambo and Naked Neck cocks ranked second in donating quality semen as well as improving the fertility and hatchability traits of the indigenous chicken breeds. Breed differences exist with respect to semen viability. This means that the Potchefstroom Koekoek cocks have highest potential for use in natural mating and AI programmes since seminal traits could be a viable index for selection of breeding cocks. This is an important consideration because studies on semen viability tests in which the semen viability of potential cocks is determined avails the breeder of the opportunity to assess the fertility proficiency of cocks before their use in AI programmes. The presence of cocks with low reproductive performance in a pen increases the total number of cocks required and limits genetic contributions from individual cocks.

Considering the overall fertility and hatchability traits it is concluded that breed has a significant effect on fertility and hatchability of fertile eggs in indigenous layer breeds. Fertility and hatchability of set eggs is significantly higher in the Potchefstroom Koekoek (PK), compared to Naked Necks, Ovambo and Venda under South African conditions. The Potchefstroom Koekoek layers appear to be superior to other breeds in terms of fertility and hatchability parameters.

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