The effect of temperature on semen characteristics of beef bulls in South Africa.

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Declaration

I, Chrizell Cilliers, do hereby declare that the research presented in this dissertation, was executed by myself, and apart from the normal guidance from my supervisor, I have received no assistance.

Neither the substance, nor any part of this dissertation has been submitted in the past or is to be submitted for a degree at this University or any other University.

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Abstract

The aim of the study was to determine the effect of temperature on semen characteristics of beef bulls over a seven-year period. Data analysis was performed using ANOVA GLM Proc for 22 099 fresh semen data records used from eight provinces and nine bull breeds while 3 003 thawed semen data records was used from seven provinces and six bull breeds in South Africa. The semen characteristics analysed included semen volume, percentage live sperm, sperm count, sperm viability, linear movement, non-linear movement, major defects, minor defects, five individual major defects and three individual minor defects. The percentage semen records per province varied from 3.02 % to 31.59 %. Breed had a significant effect on some fresh and frozen-thawed semen characteristics. The Drakensberger bulls had superior ($P < 0.05$) fresh semen characteristics compared to Bos Indicus, Bos taurus and composite breed bulls. Bos Indicus bulls had higher quality semen characteristics than Bos taurus bulls. The composite breed bulls had superior ($P < 0.05$) frozen-thawed semen characteristics. Seasonal differences were observed with the highest ($P < 0.05$) quality semen characteristics from semen produced during spring collected in summer. Temperature had significant effect with lower quality semen above 32 °C. Sperm motility in fresh semen was significantly reduced at temperatures >38 °C compared to temperatures between 32 °C to 37 °C. At temperatures > 38 °C sperm maturation exhibited significantly higher abnormal loose heads. Frozen-thawed semen had increasingly poor sperm count, major defects and minor defects when bulls produced semen while exposed to temperatures > 38 °C. Frozen-thawed semen tended to have lower significant differences between individual major defects but was still in the acceptable range. Temperature is a factor that requires more research, especially in regions with temperatures above 32 °C. Results indicated that a range of factors influence semen characteristics which need to be considered before bulls are included in planned breeding programs to ensure high fertility.

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LIST OF ABBREVIATIONS

AI	Artificial insemination
ALH	Amplitude of lateral head displacement
ANOVA	Analysis of variance
AR	Acrosome reacted
ATCH	Adrenocorticotropic hormone
ATP	Adenosine triphosphate
AV	Artificial vagina
BCF	Beat/cross frequency
CASA	Computer assisted sperm analysis
cm	Centimeter
CTC	Chlortetracycline staining
DAFF	Department of Animal Forest and Fisheries
DNA	Deoxyribonucleic acid
EE	Electro-ejaculation
Fig	Figure
GnRH	Gonadotropin-releasing hormone
GLM	Generalised linear model
GPx	Glutathione peroxidase
GSH	Glutathione
GSH-Px	Glutathione peroxidase
HOS	Hypo-osmotic swelling
HS	Hancock's solution
ISCS	Internal Statistical Consultation Service
LH	Luteinizing hormone
LIN	Linearity
LPO	Lipid peroxidation
Max	Maximum
MDA	Melondialdehyde
Min	Minimum
mL	Millilitre
mm	Millimetres
PM	Progressive motility
Proc	Program
PUFA	Polyunsaturated fatty acids
RM	Transrectal massage
RH	Relative humidity
ROS	Reactive oxygen species
SA	South Africa

SAS	Statistical Analysis System
SAWS	South African Weather Service
SCA	Sperm Class Analyser
SD	Standard deviation
SOD	Superoxide dismutase activity
STR	Straightness
T	Thermometer temperature
TBARS	Thiobarbituric acid reactive substance
T_{db}	Dry bulb temperature
THI	Thermo-humidity index
TM	Total motility
TNZ	Thermo-neutral zone
U/g or U/mg	Microgram or Micromilligram
USA	United States of America
VAP	Average path velocity
VCL	Curvilinear velocity
VSL	Straight line velocity
%	Percentage

Chapter 1: Introduction

1.1 Introduction

South Africa (SA) is a country with a well-established red meat industry, growing rapidly every year with the demand estimated to increase with 9% by 2025 (Schutte, 2016). Beef cattle population in South Africa consists of 4.82 million cows/heifers and 0.19 million bulls, which produce approximately 1.56 million calves per annum (DAFF, 2016). According to SA Stud Book Association annual report 2016 (Logix beef) there are 245 791 cows/ heifers and 86 066 bulls registered in SA, providing the industry with approximately 83 298 calves per annum. These values indicate that only 40% of all stud cows registered produced registered offspring and 33.8% of commercial cows produced recorded offspring, keeping in mind that these values do not consider the calves that were not recorded. Nonetheless, the overall productivity of commercial and stud breeders in SA are perceived as lower, which can be attributed to several factors including low reproduction efficiency (DAFF, 2016; SA Stud Book Association, 2016).

The fertility of bulls is dependent on genetic and non-genetic environmental factors both known to influence sperm quality. Breeding soundness plays a direct role in the reproduction performance of bulls and is defined as the bull's potential to breed naturally (Entwistle & Fordyce, 2003; Hopper, 2014). Functional traits include feet and leg soundness as both may affect the longevity and serving ability of bulls (Entwistle & Fordyce, 2003). The scrotum must have the ability to stretch for cooling of testis and the testicles should be soft and without fibrosis. The bull's internal reproductive genitalia include the seminal vesicles, urethra and ampullae that should have no signs of tumours, fibrosis or swelling which pose a risk to semen quality (Entwistle & Fordyce, 2003; Mitchell & Doak, 2004). The scrotal circumference should be within a range directly linked to the weight or age of the bull, which directly affect the semen volume. Scrotal circumference of bulls may range between 32 cm and 34 cm for bulls older than 18 months of age (Entwistle & Fordyce, 2003; Mitchell & Doak, 2004; Kastelic & Thundathil, 2008; Hopper, 2014). The penis and prepuce should be free of any injuries or fibrosis that may cause pain to the bull or result in redirection of the penis during erections (Entwistle & Fordyce, 2003; Hopper, 2014).

Low fertility rates can also be attributed to non-genetic environmental factors, indirectly affecting semen quality. Factors include age, handling, nutrition, season and most importantly, environmental temperatures (Gholami *et al.*, 2011; Takahashi, 2012; Snoj *et al.*, 2013; Ntemka *et al.*, 2016). Temperature is the second most influential factor, after nutrition that effects semen quality. The warming trend in SA is consistent with global increased temperatures in combination with years of below-normal rainfall becoming more frequent. The resultant drought events per year place additional stress on animals less adapted to high temperatures (Frayene *et al.*, 2012). The effect of high temperatures on semen quality have been researched in countries such as USA, Arizona (o'Brien *et al.*, 2010), Brazil (Nichi *et al.*, 2006), Iran (Gholami *et al.*, 2011), Japan (Kadokawa *et al.*, 2012) and the subtropical-Mediterranean zones, including Spain, South of France, Italy and Greece (Silanikove, 2001). These studies all have concluded that high temperatures have negative effects on semen quality.

Parameters used to evaluate semen quality in studies include consistency, tested directly after collection, which is an approximate estimate of the semen concentration (Entwistle & Fordyce, 2003; Zanganeh *et al.*, 2013). The second evaluation is mass motility, the wave motion or gross motility, identified through observation under the microscope, although it is bias indication of bull fertility (Entwistle & Fordyce, 2003). Progressive motility is then analysed by identifying the percentage of spermatozoa that is motile (Entwistle & Fordyce, 2003; Zanganeh *et al.*, 2013). The rest of the motility characteristics are analysed using internationally accepted computer assisted sperm motility analysis (CASA). That includes variables of total motility (TM, %), average path velocity (VAP, m/s), straight line velocity (VSL, m/s), curvilinear velocity (VCL, m/s), amplitude of lateral head displacement (ALH, m), beat/cross frequency (BCF, Hz), straightness (STR, %) and lastly, the linearity (LIN, %) (Zanganeh *et al.*, 2013).

Morphological examinations of spermatozoa aid in identifying testicular and duct system dysfunction, where abnormal morphologies are classified into major and minor defects that indicate the cause of the observed defect (Entwistle & Fordyce, 2003; Bukac *et al.*, 2010; Chhillar *et al.*, 2012). The evaluation of spermatozoa cell concentration indicates the number of dead or alive cells per ml, or sperm viability (Beardon *et al.*, 2004; Zanganeh *et al.*, 2013). Less common spermatozoa analysis include DNA damage, plasma membrane integrity, status of capacitation, the acrosome integrity, melondialdehyde (MDA) concentration, GSH (nmol/mg protein), GSH-Px, SOD activities and lastly, the mitochondrial activity (Beardon *et al.*, 2004; Bucak *et al.*, 2010; Tuncer *et al.*, 2010; Chhillar *et al.*, 2012; Tasdemir *et al.*, 2013; Zanganeh *et al.*, 2013).

Effective decisions with regards to bull fertility and management for increased reproduction efficiency can only be made if all the factors influencing semen quality are considered by the producer. The fact that bulls contribute 50% to the genetic pool of progeny, highlights the importance of using fertile bulls (Fortes *et al.*, 2012; 2013). Bulls produce 1.56 million offspring per annum, contributing 20% replacement calves per annum (DAFF, 2016). A bull is expected to produce more than one calf per annum, indicating that one bull will have a much higher genetic influence per year compared to a cow. This gives breeders the ability to improve the genetics of his or her herd, by breeding with superior bulls for the traits of interest (Fordyce *et al.*, 2006; Fortes *et al.*, 2013). Therefore, when the semen quality of a bull is compromised, it affects the improvement in production efficiency and the overall production of the red meat industry in SA. Fertility testing is therefore done at least once a year prior to the breeding season and before stud bull sales. Breeders may also choose to monitor the semen quality of the bulls in the herd. Fertility reports are available to potential buyers, breeder societies and cattle producers to add to their array of selection tools and reproductive trait selection (Entwistle & Fordyce, 2003; Fordyce *et al.*, 2006). Although much has been done to evaluate and monitor semen quality and bull fertility, several authors have commented that, the science of bull fertility is far from complete and further research is required to provide solutions to unanswered problems (Entwistle & Fordyce, 2003; Fordyce *et al.*, 2006).

In SA there are limited published data on the effect of South African temperature on semen quality. South African temperatures show an increasing trend similar to global observations, thus placing more strain on high production bulls (DEA, 2013). This study was performed to determine the effect of SA temperature on

semen quality in SA beef bulls. The data applied in this study has been collected from eight provinces for fresh semen and seven provinces for frozen semen, namely the North West, Gauteng, Limpopo, Mpumalanga, Kwazulu-Natal, Free State, the Eastern Cape and the Northern Cape. For this study data has been made available of semen quality evaluations performed on stud and commercial bulls at least once a year over a period of seven years. Data consists of both evaluation of fresh and frozen semen.

1.2 Aim and objectives

The aim of the study was to determine the effect of temperature on semen characteristics of beef bulls in South Africa, over a seven year period on farms in Gauteng, North West, Limpopo, Mpumalanga, Kwazulu-Natal, Free State, Eastern Cape and the Northern Cape provinces.

The following objectives were set:

1. Determine the effect of temperature on fresh semen quality and major spermatozoa defects from eight provinces in South Africa collected from nine beef bull breeds.
2. Determine the effect of temperature on thawed semen quality and major spermatozoa defects from seven provinces in South Africa collected from seven beef bull breeds.

Chapter 2: Literature review

2.1 Introduction

The selection of superior sires aims to improve traits of economic importance, production and quality of products as desired by consumers (Steverink *et al.*, 1999; Driancourt *et al.*, 2013). Often artificial insemination (AI) is used as reproductive biotechnology tool to transfer superior genetics, improving reproduction efficiency (Jemal, 2015; Kirkwood & Kauffold, 2015). The success of AI is highly dependent on the quality of the semen used. Several factors affecting semen quality play an important role in semen processing and preservation (Snoj *et al.*, 2013). These factors include breed, age, handling, nutrition, season and environmental temperatures (Gholami *et al.*, 2011; Takahashi, 2012; Snoj *et al.*, 2013; Ntemka *et al.*, 2016). The aim of this section was to review concepts of adaptation, SA climate, bull fertility and semen characteristics.

2.2 Adaptation and homeostasis

Homeostasis is the term used to describe the simple physio-chemical state in animals with closed systems, where known forces are balanced. Changes to animal surroundings stimulate or influence reactions within the animal that will directly result in internal disturbances to the animal (Cannon, 1932; Lawrence & Fraley, 2011; Betts *et al.*, 2013). Automatic adjustments within the animal keep the internal conditions fairly constant causing disturbances to be kept within narrow limits. Constant conditions can be described by the term “equilibrium”. When altering the equilibrium through temperature, the body will initiate and create thermoregulation by altering the blood in its respiratory services. Due to these alterations an integrated cooperation of organs are brought into action, including the brain and nerves, heart, lungs, kidney and spleen (Guyton 1966; Duarte & Grutter, 2012).

The known factors that alter the constant state in animals are water, oxygen, temperature and nutrients (salts, fat and sugar) (Cannon, 1932; Cooper 2008). Temperature is well-known to have an effect on cellular activity within the body. Cattle have a normal body temperature range of 38.6˚C to 39.1˚C, therefore when the cattle’s body temperature is increased ever so slightly it causes coagulation of certain proteins in nerve cells (Cannon, 1932; Collier & Gebremedhin, 2015). Low body temperature is not fatal and has less damaging effects on body cells compared to high body temperature. In cases where the body temperature of a bull reaches an above normal body temperature, the body will immediately aim to maintain homeostatic conditions within the body that is known as thermoregulation (Cannon, 1932; Rodolfo, 2000). Two mechanisms regulate homeostasis namely, the regulation of supplies and the regulation of processes. Thermoregulation in the body provides favourable conditions for maintaining the chemical equilibrium in the body. If the equilibrium was altered through low body temperature, the body will immediately try to maintain the temperature through constricting peripheral vessels and by erection of hairs. Erect hairs trap a layer of warm poorly conducted hot air near the skin and in doing so it warms the body. Once all these actions are insufficient, shivering is the final automatic protection against temperature drop (Cannon, 1932; Frandson *et al.*, 2009; Collier & Gebremedhin, 2015).

During high body temperatures the body responds by relaxation of the peripheral vessels. This assists the excessive heat to escape to a colder environment, through exposing warm blood to the surface of the skin. If this method is ineffective sweating will occur, cooling the skin trough evaporation (Cannon, 1932; Rodolfo, 2000; Cooper 2008; Collier & Gebremedhin, 2015). Methods of heat loss in animals are known as conduction, convection, radiation and evaporation (Silanikove, 2001; Marai *et al.*, 2007; O'Brien *et al.*, 2010; Collier & Gebremedhin, 2015). Therefore, the highly efficient arrangement for maintaining homeostasis of body temperature involves only the acceleration or retardation of processes that create thermoregulation controlled by the sympathetic system (Cannon, 1932; Cooper, 2008).

Animals in tropical and sub-tropical areas are often exposed to extreme ambient temperatures during summer, causing the animals to undergo hyperthermia, commonly known as heat stress (Marai *et al.*, 2007; Gholami *et al.*, 2011; Takahashi, 2012). Heat stress is induced by several extreme climatic factors such as ambient temperature, humidity, radiation and wind. It has been reported that animals suffering from heat stress have decreased growth rates, decreased milk production and low fertility rates (Silanikove, 2001; Marai *et al.*, 2007; O'Brein *et al.*, 2010; Kadokawa *et al.*, 2012; Takahashi, 2012). This occurs when the energy of an animal is used for thermoregulation rather than production. The temperature humidity index (THI) is a parameter widely used to describe the heat load on animals under different environmental conditions. The formulas used are shown in Table 2.1a associated with heat stress (Silanikove, 2001; Gholami *et al.*, 2011; Kadokawa *et al.*, 2012; Menegassi *et al.*, 2015).

Table 2.1a Summary of the different THI formulas used.

Formula	Abbreviations	Reference
THI = (1.8 x T (^{0}C) + 32) – [(0.55 – 0.0055 x RH%) x (1.8 x T (^{0}C) – 26)]	T(^{0}C)–Thermometer temperature RH% - Relative humidity	The National Research Council (1971): (Al-Kanaan *et al.*, 2015)
THI = (0.8 x T_{db}) + [(RH/100) x (T_{db} – 14.4)] + 46.4	T_{db}- Dry bulb temperature (^{0}C) RH - Relative humidity	(Menegassi *et al.*, 2015)

Table 2.1b display the ranges of THI associated with heat stress (Gholami *et al.*, 2011; Kadokawa *et al.*, 2012; Menegassi *et al.*, 2015).

Table 2.1b Summary of the THI classes given for the specific ranges.

Class	THI range	Production/Fertility rates
1 - Comfortable	< 70	High rate.
2 – Begin heat stress	70 - 72	No adverse effects.
3 – Heat stressed	73 - 78	Severely affected.
4 – Acute heat stress	>80	Life threatening/all energy used for thermoregulation.

Heat stress can be controlled when the night temperatures decreases below 21 ºC for at least three to six hours. This provides the animal with a chance to lose all their heat gained during the day (Silanikove, 2001).

Another parameter used to describe temperature load on animals is the thermo-neutral zone (TNZ) (Schaefer *et al.*, 2018). The TNZ describes the relationship between animal and its environment as illustrated in figure 2.1. It can also be used to give an alternative measurement of animal adaptation and wellbeing. The zone of optimal thermal comfort is the ideal environmental condition for animals to perform at an optimum growth rate, maximise production and increase fertility rates. Stage one (Fig 2.1) indicates the lower critical ambient temperature range, where heat production at resting is used to maintain thermal balance. Stage two is known as the aversive stage that represents the upper critical ambient temperature range at which the metabolic rate and evaporative cooling starts increasing (24 – 26 ºC). At stage three, the noxious stage, a THI of >70 (>27ºC), the attempts of thermoregulation by animal is unsuccessful, resulting in impairing of fitness and wellbeing of animal. Stage four is called the extreme stage as animal enters the acute phase of heat stress and animal's wellbeing is severely affected. During this stage the THI of >80 causes animals to experience heavy panting and sweating (Silanikove, 2001; Schaefer *et al.*, 2018).

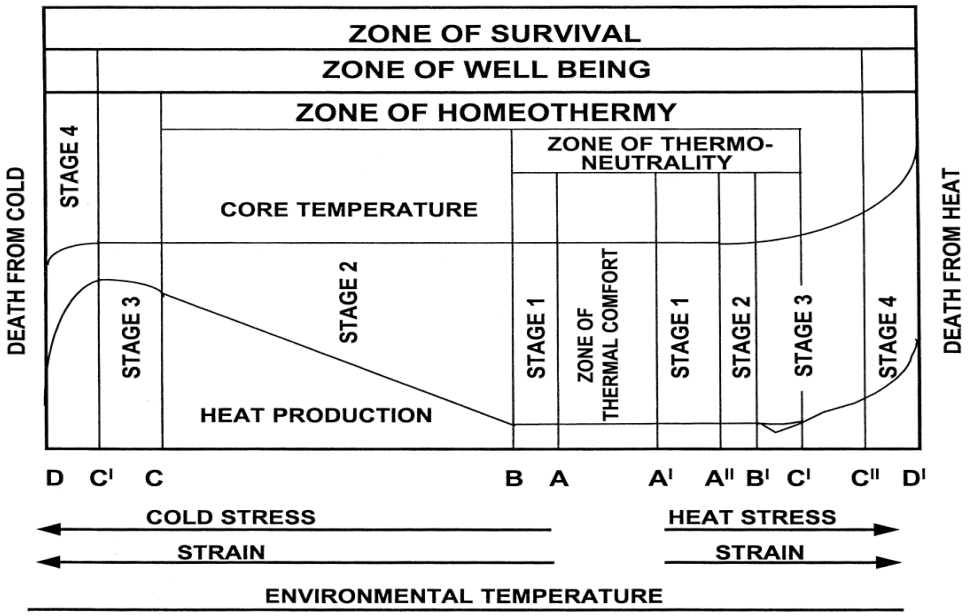

Figure 2.1 Schematic presentation of the zones of survival, wellbeing, and homeothermy in respect to environmental conditions in ruminants (Silanikove, 2001).

Therefore, it has been shown that the external environmental stimulus on an animal is closely correlated with its production efficiency. The ability of an animal to grow, produce effectively and regularly reproduce is identified as an adapted animal (Scholtz *et al.*, 2008; Canario *et al.*, 2012; Boettcher *et al.*, 2015). The late Prof Bonsma stated "with the aid of homeostasis an animal that is in perfect harmony with its environment are known as an adaptive animal". The adaptability of an animal is observed to be highly dependent on its difference in hereditary characteristics inherited from its ancestors (Bonsma, 1949; Scholtz *et al.*, 2008; Canario *et al.*, 2012; Boettcher *et al.*, 2015). Hereditary characteristics cause the reaction of animals to its environment to be different between different breeds or a type within a breed. A good example is cattle with coat felting qualities, causing animals to have difficulty regulating their body temperature in hot environments. It has also been observed that older animals can tolerate heat better compared to younger animals (Bonsma,

1949; Collier & Gebremedhin, 2015). However, young animals with higher tolerance for high temperatures within its first year of life have the ability to maintain performance under warmer conditions when older. These young animals will be ideal for breeding to ensure that their offspring will have some resistance against tropical and subtropical conditions (Bonsma, 1949; Canario *et al.*, 2012; Boettcher *et al.*, 2015). It has been reported that poorly adaptable animals have lower reproductive abilities as a result of retarded growth, therefore causing repressed sexual activity. Thus, selecting animals based on fertility will be an effective method of breeding adaptability (Bonsma, 1949; Scholtz *et al.*, 2008; Canario *et al.*, 2012; Boettcher *et al.*, 2015).

2.3 Thermoregulation of testes

The ability to regulate temperature is an adaptation that allows animals to function despite the extreme variation in ambient temperatures. Thermoregulation of animals allows temperature to be used as a signal to control physiological processes (Bonsma, 1949; Silanikove, 2001; Hafez & Hafez, 2013). During summer low quality semen can be a result of the inability of the body cooling mechanism to keep the bull's testes at four ºC below body temperature (38.6 ºC) (Bearden *et al.*, 2004; Hafez & Hafez, 2013). High body temperatures cause degeneration of the cell lining of the seminiferous tubules walls, resulting in the infertility in males. Spermatogenesis terminates as soon as the testes temperature reaches body temperature (Bearden *et al.*, 2004; Marai *et al.*, 2007; Takahashi, 2012). Ambient temperatures between five ºC and 21 ºC are ideal for cattle to maintain a normal body temperature. When ambient temperatures reach 38 ºC (THI 75 -78) it will cause both the body and testes temperatures to become elevated, reducing the difference between testes and body temperature by half (2 ºC). These elevated temperatures within the testes will result in termination of spermatogenesis (Bearden *et al.*, 2004; Menegassi *et al.*, 2015). Therefore, sufficient thermoregulation is a prerequisite to ensure fertility in males.

Two muscles are involved in the response to environmental temperature called the tunica dartos and the external cremaster muscle. The tunica dartos muscle is smooth muscle lying in the scrotum, where the external cremaster muscle is striated muscle around the spermatic cord that is known to be sensitive to temperatures. During cold ambient temperatures both muscles contact to draw the testes closer to the body. The opposite occurs during hot ambient temperatures when both muscles relax causing the scrotum to stretch, increasing the surface area for increased evaporation, and the spermatic cord to lengthen causing the testes to move further away from the body. Heat tolerance can therefore be measured using the tunica dartos muscle that reflects the magnitude of vascular heat exchange (Marai *et al.*, 2007; Hafez & Hafez, 2013; Simoni & Huhtaniemi, 2017). The two mechanisms that directly cool the testes is evaporative cooling and heat exchange. The skin of the scrotum contains both sweat and sebaceous glands that is activated during hot weather, causing gland excretions that is evaporated, cooling the scrotum. The second mechanism, heat exchange, takes place in the circulatory system where arteries have convoluted folds passing through a network of veins called the pampiniform venous plexus, as indicated in figure 2.2. The arteries transport the blood at internal body temperature descending along the spermatic cord. The veins will then transport cooler blood back towards the heart indirectly cooling the testes (Bearden *et al.*, 2004; Hafez & Hafez, 2013; Hopper, 2014; Simoni & Huhtaniemi, 2017).

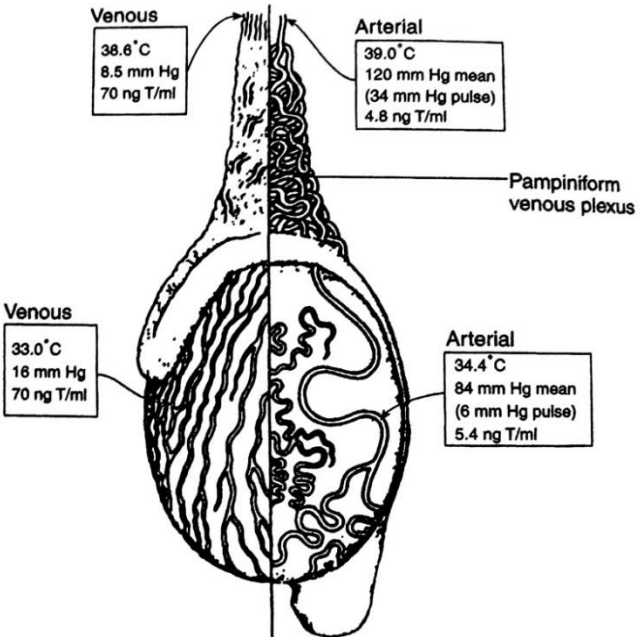

Figure 2.2 Cooling of the testes by heat exchange through the circulatory system (Bearden *et al.*, 2004).

These cooling mechanisms are sufficient to control testes temperature during the normal ambient temperatures but are less effective during extremely high ambient temperatures. Therefore, it is crucial to select for animals with the ability to effectively regulate its own body temperature. The evolutionary adaptation that allow homeotherms to function despite variations in ambient temperatures (Silanikove, 2001).

2.4 The South African climate

The South African environment is one of the primary factors causing stress to animals on a daily basis. South Africa (SA) is a semi-arid country consisting of temperate forests, subtropical grasslands, fynbos and savanna (Acocks, 1988; Cowling, 1999; Crétat *et al.*, 2012). In SA large amounts of rainfall occur in austral summer during November - February, with summer starting in December and ending in February, autumn stretching from March to May, winter starting in June until August and spring starts in September until November (Crétat *et al.*, 2012). Extreme temperatures are associated with maximum temperature higher than or equal to 35 ºC in summer versus three to five days with a minimum temperature of 5 ºC or lower in winter respectively (Kruger & Shongwe, 2004).

Records of the SAWS over 43 years show that the average temperatures, hot extremes have increased with 1^0C that clearly indicates climate change in SA (Bryan *et al.*, 2009; DEA, 2013). From data collected by The South African Weather Service (SAWS), 2010 to 2017, the average maximum temperatures and average minimum temperatures during summer months are indicated for all nine provinces in SA in Table 2.2.

Table 2.2 Summary of South African maximum and minimum temperatures in summer for the period 2010 - 2017.

Province	Average max temperature (^{0}C)	Highest maximum temperature (^{0}C)	Average min temperature (^{0}C)
Gauteng	25.77-30.3	42.7	16.07-17.03
Mpumalanga	26.52-28.75	44.7	15-17.01
Limpopo	29.52-32.67	44.7	19.08-20.21
North West	28.98-33.46	46	17.13-19.06
Free Sate	26.8-31.15	42.9	15.24-16.96
Northern Cape	31.64-33.79	53	16.90-19.03
Western Cape	27.98-30.29	47.7	15.63-18.25
Eastern Cape	26.61-29.21	44.4	14.92-15.57
Kwazulu-Natal	26.75-31.45	41.6	17.59-18.62

SA annual rainfall occurs mostly during summer months from October-March, but the Western Cape receives most of its rain during the winter months (Thomas *et al.*, 2007). The average annual rainfall in SA vary from 464 to 564 mm, with a large variation among provinces as indicated in Table 2.3 (Bryan *et al.*, 2009).

Table 2.3 Summary of South African average annual rainfall (SAWS, 2010-2017).

Province	Average annual rainfall (mm)
Gauteng	329 – 964
Mpumalanga	300 – 1857
Limpopo	186 – 700
North West	136 – 500
Free State	150 – 486
Northern Cape	0 – 286
Western Cape	64 – 750
Eastern Cape	171 – 1457
Kwazulu-Natal	343 – 1929

A decrease in the suspected annual rainfall with increased intensity has been identified with a significant decrease in precipitation over the whole SA (Bryan *et al.*, 2009; DEA, 2013; Daron, 2018; Nyoni *et al.*, 2019). Decreased precipitation is classified as one of the results of climate change that poses a threat to food and water security and natural resources used for South African agriculture. Therefore, adaptation of animals to small temperature variations will benefit their adaptation to long term climate change (Bryan *et al.*, 2009).

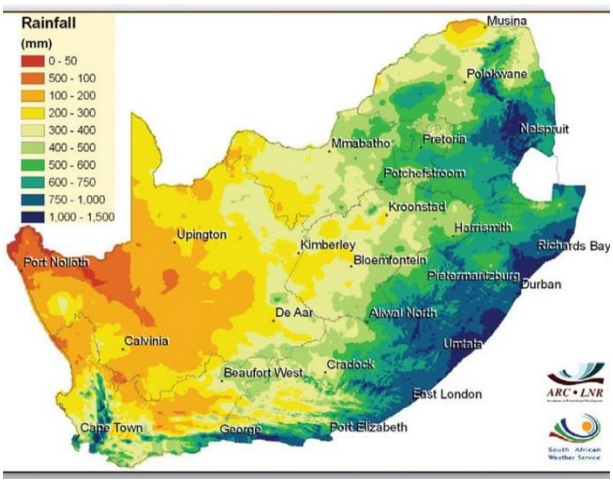

Figure 2.3 Total rainfall (mm) for the period of July 2011 to June 2012 (SAWS, 2012).

2.5 Factors influencing semen quality.

Important factors that influence semen quality include ambient temperature, breed of bull, season, age and level of nutrition.

2.5.1 Heat Stress

The environment is unpredictable and uncontrolled and one of the most significant factors influencing semen quality. Heat stress in cattle is defined by Kadokawa *et al.* (2012), as 'an external force on the animal that displaces the body temperature from a set-point temperature'. Heat in the tropical belt and arid areas put a major constraint on animal productivity and reproduction (Silanikove, 2001; Pareek *et al.*, 2016). It has been established that ambient temperatures of above 27 ºC will lower semen quality due to heat stress affecting the endocrine and biochemical conditions in males. Hyperthermia inhibits spermatogenesis that ultimately decreases semen quality (Gholami *et al.*, 2011; Takahashi, 2012).

During heat stress in cattle adrenocorticotropic hormone (ATCH) from its anterior pituitary is released that stimulates the secretion of cortisol and glucocorticoids from the adrenal cortex. Glucocorticoids have the ability to inhibit pulsatile release of luteinizing hormone (LH) that results in a reduced libido in bulls (Silanikove, 2001; Nichi *et al.*, 2006; Marai *et al.*, 2007; Gholami *et al.*, 2011). Another negative outcome of elevated testicular temperature reported is biochemical changes that increase the production of reactive oxygen species (ROS), which indicates testicular oxidative stress. High levels of ROS are very destructive towards lipids present within the structural membrane of bull spermatozoa. ROS causes lipid peroxidation which damages spermatozoa morphology, spermatozoa DNA and chromatin structure of spermatozoa, which increase the number of head and intermediary piece and tail defects of spermatozoa. Evidence shows that X and Y spermatozoa are affected differently by heat stress, where the sex ratio of embryos shifted towards female cattle (Nichi *et al.*, 2006; Takahashi, 2012).

A biochemical reaction that indicates heat stress in cattle is a high level of thiobarbituric acid reactive substances (TBARs). TBARs decreases glutathione peroxidase (GPx) level which is an antioxidant enzyme in bovine seminal plasma that protects the spermatozoa cells from oxidative stress (Takahashi, 2012). The testicular tissue affected by heat is the sertoli and leydig cells, killing spermatocytes in the meiotic prophase, where spermatozoa have metabolic and structural abnormalities (Hopper, 2014). Studies of male ruminants concluded that heat stress induced by environmental conditions increases abnormal spermatozoa morphology and decreases spermatozoa progressive motility, semen volume, spermatozoa concentration and total spermatozoa production. This phenomenon is known as "summer sterility" (Marai *et al.*, 2007; Gholami *et al.*, 2011; Al-Kanaan *et al.*, 2015). Thus, it can be concluded that the effect of heat stress on ruminant semen quality is quite severe.

Spermatozoa motility is mainly affected by heat in the caput of the epididymis as well as in spermatids of the testes producing abnormal spermatozoa morphology. Abnormal morphology defines spermatozoa

which is decapitated, have abnormal acrosomes, abnormal tails and protoplasmic droplets (Hopper, 2014; Simoni & Huhtaniemi, 2017). In some studies, the scrotum has been insulated for up to eight hours to induce heat stress during spermatogenesis. Heat stress induced through bovine testes insulation produced particular spermatozoa abnormalities including pyriform heads due to lack of chromatin protamination, nuclear vacuoles, microcephalic spermatozoa and abnormal DNA condensation. Insulation also reduced the proportion of progressively motile and the proportion of live spermatozoa. A significant decrease in spermatozoa motility, acrosome integrity and membrane integrity can be observed during the insulation technique (Januskauskas *et al.*, 1995; Hopper, 2014). Insulation of the scrotal neck of bulls represents accumulation of fat in the scrotal neck that mostly affects spermatozoa within the epididymis (acrosome phase), explaining the increases in number of mid-piece defects and cytoplasmic droplets. The epididymis has been observed to be cooler compared to the testes for the purpose of storing spermatozoa. This method of heat stress evaluation is less accurate due to the fact that the natural mechanisms are inhibited. Nevertheless, when scrotum temperature increases, the increased cauda temperature impairs the absorptive secretory function resulting in change in cauda fluid composition that causes the passage rate in epididymis to increase. Increased passage rate means, hastening sperm maturation that results in a smaller number of spermatozoa present in ejaculate (Hopper, 2014; Al-Kanaan *et al.*, 2015; Simoni & Huhtaniemi, 2017). Therefore, the above results can be used to indicate heat stress in beef and dairy bulls.

Consequences of heat stressed rams are reduced sexual behaviour, semen volume, mass motility and semen concentration (Maurya *et al.*, 2016; Dias *et al.*, 2017). In another study performed on rams, Dias *et al.* (2017) determined that high ambient temperatures influence semen quality the most. The results included compromised structures of the plasmatic membrane of spermatozoids due to altered epididymal maturation process. High temperatures increased the seminiferous tubules, testicular volume and scrotal circumference in goat rams, therefore indirectly influencing semen morphological and seminal changes (Dias *et al.*, 2017). Stressed cavies have a lower number of spermatozoa present in the cauda of the epididymis and decreased testicular mass and individual motility. Results of the increase in the percentage minor abnormal shaped spermatozoa and increased rate of Malondialdehyde (MDA) and glutathione (GSH) levels where found (Ngoula *et al.*, 2017). Ngoula *et al.* (2017) concluded that cavies experiencing heat stress have significant reductions in their spermatozoa characteristics with increased oxidative stress parameters. In addition, rodents had reduced testicular germ cell proliferation causing an increase in cell apoptosis, directly lowering the testicular weight. The rest of the results were similar to the results found by Ngoula *et al.* (2017) and Kanter *et al.* (2013). In the review completed by Santiago-Moreno *et al.* (2016), the conclusion focussed on heat stress reducing testicular germ cell proliferation, causing increased cell apoptosis. A review discussing heat stress in broilers, concluded that thiobarbituric acid reactive substance (TBARS) and MDA levels increased during heat stress, but can be decreased by supplementation of antioxidants (Slimen *et al.*, 2016).

2.5.2 Other factors

Season also play a significant role in bull fertility. During the hot summer and spring months when mating season of bulls are underway, bulls get exposed to environmental variations that may influence bull fertility. A number of studies have been conducted over several years with similar results where only a few will be mentioned. Season was observed to have an influence on semen volume, where a significantly higher semen volume was observed in summer than in winter (Javed *et al.* 2000; Snoj *et al.*, 2013; Al-Kanaan *et al.*, 2015). Different seasons have a significant effect on semen colour, where the colour (milk-creamy) was observed to be better in the autumn months (Javed *et al.* 2000). The pH of semen was observed to be significantly lower in autumn compared to winter, that indirectly effects sperm motility (Javed *et al.* 2000). The total spermatozoa output was significantly higher in spring and autumn and the highest in summer, indicating seasonal affects (Snoj *et al.*, 2013; Al-Kanaan *et al.*, 2015). Sperm concentration was however not significantly affected by seasons (Javed *et al.* 2000; Snoj *et al.*, 2013). Season also has an influence on sperm motility, where semen has a significantly higher mass motility during autumn and spring compared to winter and summer. Spermatozoa have significantly higher total motility and progressive motility in spring including humid summers (Stälhammar *et al.*, 1989; Javed *et al.*, 2000; Sabés-Alsina *et al.*, 2017). During spring spermatozoa velocity parameters were found to be significantly higher versus winter and summer. The proportion of viable spermatozoa with non-reactive acrosomes and live spermatozoa not producing ROS is significantly higher in spring than winter (Sabés-Alsina *et al.*, 2017). Spermatozoa morphology was the only semen quality not affected by season (Menegassi *et al.*, 2015; Sabés-Alsina *et al.*, 2017). The results discussed concluded that spermatozoa collected in spring may have better chance at fertilizing an oocyte compared to spermatozoa collected in any other season (Sabés-Alsina *et al.*, 2017).

Nutrition is one of the primary factors that can be used directly and indirectly to improve fertility (Bucak *et al.*, 2010; Surai & Fisinin, 2015). Nutrition has a direct effect on the development of spermatozoa and the fertility of spermatozoa in the testes and epididymis. Nutrition also influences the production of hormones and therefore indirectly influencing hormone concentrations. Among nutrition lipids and antioxidants play crucial roles in male fertility. Lipids are consumed mainly as an energy source, but it also acts as component in membranes of spermatozoa. Omega-3 fatty acids are the polyunsaturated fatty acids (PUFA) found in phospholipids of spermatozoa primarily found in the tail. PUFA are important for membrane integrity, spermatozoa motility, spermatozoa viability and cold sensitivity. High levels of PUFA increase spermatozoa tail flexibility, compressibility, deformability and elasticity, directly improving flagellar movement and stress tolerance of fresh semen (Gholami *et al.*, 2011; Surai & Fisinin, 2015; Pareek *et al.*, 2016). Nutrients fed including antioxidants play a crucial role in protecting spermatozoa against reactive oxygen species (ROS) during oxidative stress. Antioxidants include cysteine, methionine, selenium and vitamin B12. Intracellular antioxidants in cytoplasm of sperm are secreted in the epididymis of the male reproduction tract. The main function of these antioxidants is decreasing the production of hydrogen peroxide and balancing out ROS produced by spermatozoa cells. These actions prevent lipid peroxidation of plasma membranes and decreasing the damage to spermatozoa (Bansal *et al.*, 2010; Bucak *et al.*, 2010; Pareek *et al.*, 2016). Antioxidants protect the acrosome, mitochondria and membrane integrity, increasing spermatozoa motility,

viability and fertility. Thus, antioxidants and omega-3 fatty acids are essential in diets of male animals (Coyan *et al.*, 2011; Ari *et al.*, 2012).

Non-genetic effects include characteristics of the animal that cannot be changed. The effects discussed include the age and breed of bulls. Bulls at the age of 12 months up to 84 months are kept at the AI centres and used for semen collection. It was found that the age of bulls had significant effects on semen quality and should be considered before their semen is used for inseminations. Although age has no significant effect on the ejaculate volume, the ejaculate volume tends to be higher in adult bulls (>24 months) and peaks at 84 months (Stälhammar *et al.*, 1989; Javed *et al.*, 2000; Snoj *et al.*, 2013). Semen pH in adult bulls were significantly lower compared to older bulls (Javed *et al.*, 2000). Age significantly affect semen mass activity, where it was significantly higher in adult versus older bulls (Stälhammar *et al.*, 1989; Javed *et al.*, 2000). Spermatozoa concentration is widely known to be significantly higher in young bulls (Javed *et al.*, 2000), where the total spermatozoa output and semen quality increased with age (Stälhammar *et al.*, 1989; Snoj *et al.*, 2013). The low spermatozoa concentration and low mass activity in older bulls was assumed by Javed *et al.* (2000) that the cause can be senility. The pH of semen has a negative correlation with the colour, spermatozoa concentration and mass motility (Javed *et al.*, 2000). Older bulls are more environmentally sensitive and thus can contribute to their semen having a lower quality (Al-Kanaan *et al.*, 2015).

Limited studies are available on the effect of breed on semen quality, with few studies comparing Bos indicus to Bos taurus bulls. The gross, total and progressive motility of bull semen are essential measurements to predict bull fertility, due to most spermatozoa damage result in decreased spermatozoa motility. The progressive motility and spermatozoa abnormalities were found to be significantly lower in the Belgian Blue breed compared to the rest of the Bos taurus bulls in this study (Kathiravan *et al.*, 2011; Ntemka *et al.*, 2016). The spermatozoa morphology differed significantly between bulls of the Bos Taurus breeds as observed in Ntemka's *et al.* (2016) study. In thus study head abnormalities were significantly higher in some Bos taurus breeds where tail abnormalities were significantly higher in other Bos taurus breeds, but both abnormalities would not be high in the same Bos taurus breed. There was no significant difference in sperm DNA damage and sperm membrane damage between breeds (Ntemka *et al.*, 2016). In some studies, it was found that seasonal effects were stronger on semen characteristics in Bos indicus compared to Bos taurus breeds. The peak ejaculate volume was reached at different ages between different breeds (Snoj *et al.*, 2013). Most of the semen fertility parameters were found to be lower in crossbred and Bos indicus bulls versus Bos taurus bulls (Kathiravan *et al.*, 2011). Therefore, breed needs to be considered when looking at semen quality (Ntemka *et al.*, 2016).

Semen quality will also be affected using incorrect semen collection and handling procedures. Proper semen handling is essential in preserving sperm with optimal fertility for insemination to ensure the optimum conception rate (Simoni & Huhtaniemi, 2017). Handling mistakes include exposing spermatozoa to extreme temperatures, contamination and thawing that impact the survival of spermatozoa. Extreme temperature changes cause functional, structural and biochemical damage to spermatozoa decreasing the proportion of motile spermatozoa per straw (Ntemka *et al.*, 2016). The handling as well as successful collection of semen

is important to ensure optimal semen quality. Collection methods include the artificial vagina (AV), electro-ejaculation (EE) and transrectal massage (RM) (Sarsaifi *et al.*, 2013). EE produces a large volume of semen but a lower number of spermatozoa per ejaculate, therefore producing a lower concentration of semen. RM produces a significantly higher concentration semen. However, the collection method does not affect the motility, viability, acrosome integrity and morphology of spermatozoa (Sarsaifi *et al.*, 2013). Thawing does affect spermatozoa motility and acrosome integrity. The AV collection method had the highest % of intact acrosomes of spermatozoa and viable spermatozoa cells. The EE method provides a more acceptable post-thawed semen quality permitting a faster reproduction rate (Sarsaifi *et al.*, 2013), while the quality of frozen semen will always vary between different inseminators (Ntemka *et al.*, 2016).

2.6 Parameters for evaluating semen quality

There are multiple methods to evaluate sperm abnormalities and immobility to supply fertile spermatozoa for AI, this process is called semen evaluation. Table 2.4 presents the sperm parameters analysed during semen quality evaluation of bulls indicating the averages that are acceptable for high quality spermatozoa (Entwistle & Fordyce, 2003; Bearden *et al.*, 2004; Mitchell & Doak, 2004; Kasimanickam *et al.*, 2006; Bukac *et al.*, 2010; Coyan *et al.*, 2011; Chhillar *et al.*, 2012; Tasdemir *et al.*, 2013; Zanganeh *et al.*, 2013).

The first step in semen evaluation is the gross evaluation is where the volume and colour of the semen are evaluated. A low volume may indicate a disease or infection present in the animal, it can just be the collection procedure that was not effective and need to be repeated (Zanganeh *et al.*, 2013). The ejaculate is weighed in a 15 ml or 25 ml tube and the weight is converted to millilitres by computer. The overall colour of the ejaculate was described as thick creamy or white in colour, indicating if semen had high concentration of spermatozoa or does the ejaculate have more water, or when low concentration of spermatozoa is present the appearance of ejaculate will be less opaque (Zanganeh *et al.*, 2013).

Since 1988 the preferred method used all over the world to evaluate semen quality is computer aided sperm analysis (CASA) (Farrell *et al.*, 1998; Mycroptic, 2014). Sperm class analyser (SCA) is the most advanced modular CASA, that is a modular automatic system for evaluation of concentration, motility, morphology, DNA fragmentation and viability in variables. These evaluations can be obtained by placing some of the sample on a slide into the microscope that scans the microscopic fields as outlined by the world health organisation (WHO) three times (Microptic, 2014). This includes the variables total motility (TM,%), progressive motility (PM,%), average path velocity (VAP, m/s), straight line velocity (VSL, m/s), curvilinear velocity (VCL, m/s), amplitude of lateral head displacement (ALH, m), beat/cross frequency (BCF, Hz), straightness (STR, %) and lastly the linearity (LIN, %) (Zanganeh *et al.*, 2013). The evaluation done is of progressive motility by assessing individual spermatozoa under a microscope at magnification of 400X with at least 300 cells (Bukac *et al.*, 2010), indicating the amount of spermatozoa that is fully motile by identifying the amount of spermatozoa that can move in a more or less straight line. This method of evaluation can also indicate spermatozoa with abnormal tails and damage to flagella, mitochondria or acrosome. Spermatozoa

abnormalities are assessed under phase contrast microscope (magnification of 1000x) on a slide where the percentage of total acrosome and other abnormalities are identified (Bucak *et al.*, 2010). The motile spermatozoa are identified by moving flagellums and non-motile spermatozoa are identified by flagellums that are stationary (Chhillar *et al.*, 2012).

Table 2.4 Criteria for semen parameters used to categorize bulls for breeding potential.

Parameters	Unsatisfactory	Questionable	Satisfactory
Scrotal circumference (cm)	< 28	28 – 30	> 36
Gross evaluation	1 – Clear - cloudy	3 - Milky	4 - 5 - Thick creamy
Volume (ml)	< 2	3	4 - 6
Total sperm (Billion)	< 4	5 - 7	8 - 15
Total Motility (%)	< 45	45 - 60	65 - 80
Progressive motility (%)	< 30	35 - 55	> 60
Average path velocity (VAR) (µm/s)	< 75	80 - 100	> 120
Straight line velocity (VSL) (µm/s)	< 65	70 - 90	> 100
Curvilinear velocity (VCL) (µm/s)	< 103	155 - 185	> 210
Amplitude of lateral head displacement (ALH) (µm/s)	< 5	6 - 8	> 9
Beat/cross frequency (BCF) (Hz)	-	22 - 28	> 30
Straightness (STR) (%)	-	-	> 80
Linearity (LIN) (%)	-	37 - 42	> 53
Morphology – normal sperm (%)	< 30	30 – 70	> 70
Concentration (sperm x 10^6/ml)	< 1000	1 200 - 2000	3 000 – 8 000
Sperm viability (%)	< 40	50 - 70	> 80
Membrane integrity (%)	< 40	50 - 70	> 80
Acrosome integrity (%)	< 45	50 - 60	> 70
Lipid peroxidation (MDA) (nmol/ml)	> 2	1.2 – 1.4	< 0.66
GSH activity (µm/ml)	< 16	35 - 50	> 60
SOD activity (nmol/mg)	< 0.15	0.23 – 1.3	> 1.8
pH	< 5	5.5 - 6	6.5 - 7

The evaluation of spermatozoa cell concentration indicates the number of cells per ml, or it can be done where spermatozoa cells are counted using a Hemocytometer for a direct cell count. The visible spectrophotometry and electrical particle counter are used to identify if enough spermatozoa cells are present in ejaculate for maximum breeding units, it can also identify if spermatozoa apoptosis occurred followed by phagocytosis (Zanganeh *et al.*, 2013).

The evaluation of spermatozoa viability is done to identify viable and non-viable sperm and is the application of differential staining, where eosin is the differential stain that can only pass through dead spermatozoa membranes. Eosin is used with background stain nigrosin to aid in the identifying of unstained

living spermatozoa by turning them opal blue or fast green, thus the combination staining used is called the eosin-nigrosin (Bearden *et al.*, 2004; Zanganeh *et al.*, 2013). The viable and non-viable stained spermatozoa are identified by counting spermatozoa under phase-contrast microscopy at 400X with 200 cells per slide including three slides (Chhillar *et al.*, 2012). The phospatidylser in translocation assay evaluate spermatozoa viability if spermatozoa is apoptotic or dead, they are then divided into three groups viable non-apoptotic cells, spermatozoa that indicate early cell apoptosis and dead spermatozoa (Bearden *et al.*, 2004; Zanganeh *et al.*, 2013).

The evaluation of spermatozoa morphology is usually done once a month for each male animal at an AI station to cryopreserve semen, as it is the time required for the evaluation of two independent ejaculates. The spermatozoa are evaluated by using the Han-cock's solution (HS), where three drops of semen are mixed with 1 mL HS. One drop is then taken from the mixture and placed on a slide and is then covered. The evaluation is completed by counting 200 spermatozoa under phase-contrast microscopy of 1000X and identifying the percentage of abnormal spermatozoa (Tuncer *et al.*, 2010; Zanganeh *et al.*, 2013; Souza *et al.*, 2018), such as the spermatozoa presented in figure 2.4.

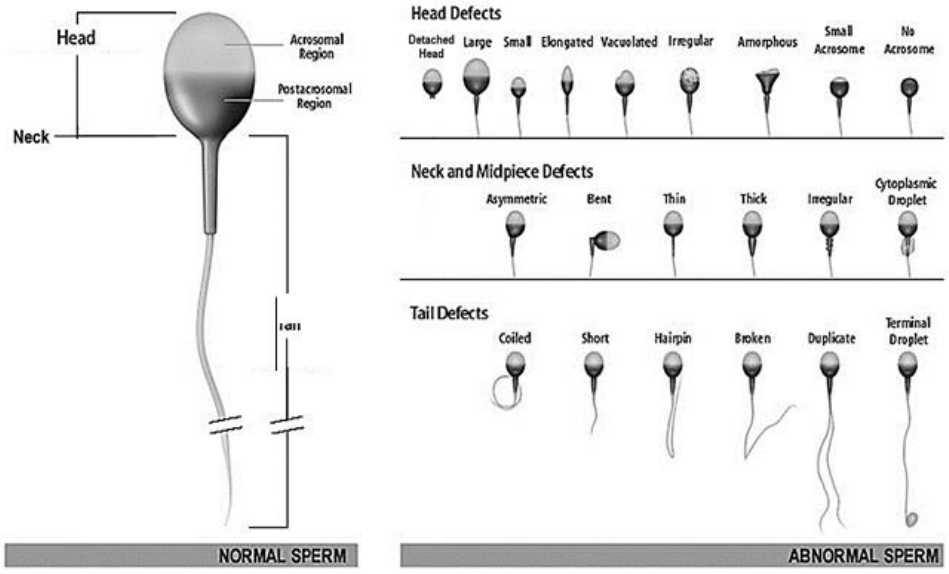

Figure 2.4 Illustration of a normal sperm versus abnormal sperm with different types of defects (Fertility solutions, 2015).

The severity of damage to spermatozoa is the assessment of DNA of spermatozoa and is implemented as standard procedure at cutting-edge AI centres. DNA damage is assessed by removing seminal plasma of spermatozoa using the method of diluting semen samples and centrifuging it for 10 min, the DNA damage is identified using single cell gel electrophoresis assay with high alkaline conditions for DNA to unwind (Tuncer *et al.*, 2010; Souza *et al.*, 2018). This process is done under dimmed light to prevent further DNA damage due to light. As the DNA unwind under electrophoresis forming "comet tails", the DNA damage can be identified by the intensity of DNA reflecting breakage (Tasdemir *et al.*, 2013).

The evaluation of the plasma membrane integrity is also important, due to weak plasma membranes negatively effects spermatozoa morphology and motility (Bearden *et al.*, 2004; Tasdemir *et al.*, 2013). Membrane integrity evaluation is conducted using the hypo-osmotic swelling test (HOS). Spermatozoa with functional plasma membranes had a swollen appearance and coiled tails. HOS is done by using hypo-osmotic solution and incubating semen for 60 minutes that is then assessed under phase microscopy at 400X. The number of spermatozoa that appeared swollen and had coiled tails were abnormal out of 200 sperm (Chhillar *et al.*, 2012; Tasdemir *et al.*, 2013).

The evaluation used to identify the status of capacitation and the acrosome integrity is chlortetracycline staining (CTC). Spermatozoa is evaluated under an epifluoresent microscope and assigned to a category to differentiate between acrosome-reacted and the non-reacted spermatozoa as observed in figure 2.5 (Chhillar *et al.*, 2012). Categories include F-pattern where fluorescence is seen over whole area of spermatozoa head and it indicates that spermatozoa acrosome is intact and functional. The B-pattern is where fluorescence is detected in whole head except in post-acrosome area this indicate that spermatozoa had undergone capacitation. Lastly is the AR-pattern where no fluorescence is observed in head, but a bright band is observed in equatorial segment, this indicates that the acrosome reacted (Bearden *et al.*, 2004; Zanganeh *et al.*, 2013). Figure 2.6 displays schematic sperm acrosome as sperm was aging or when sperm was injured.

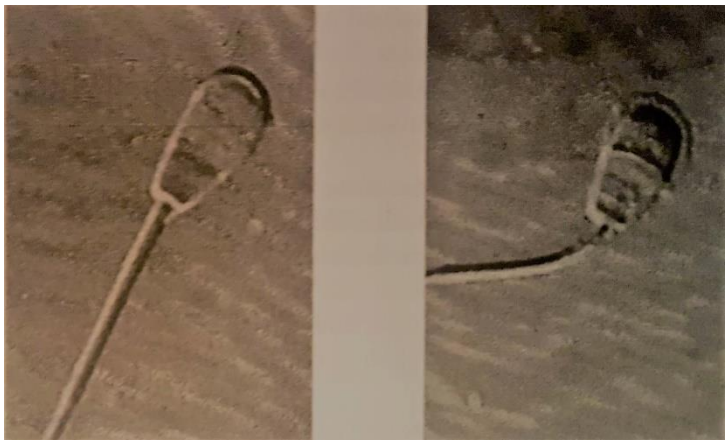

Figure 2.5 Photomicrographs of postthawed spermatozoa; intact acrosome (left) and damaged acrosome (right) (Bearden *et al.*, 2004).

The test for melondialdehyde (MDA) concentration nmol/mL is also used for semen evaluation where it indicates the index of lipid peroxidation (LPO) in semen by a spectrophotometer only. The MDA influence spermatozoa morphology thus the higher the LPO the greater the abnormalities of spermatozoa shape (Zanganeh *et al.*, 2013). MDA was measured using the thiobarbituric acid reaction that was produced comparing malondialdehyde equivalents that express the MDA levels (nmol/mL) in semen (Bucak *et al.*, 2010).

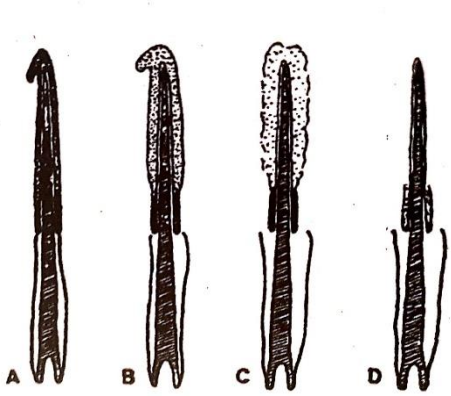

Figure 2.6 Schematic representation of sagittal section through the bovine sperm head by use of electron microscope. A through D show sequential alterations of the acrosome due to aging or injured sperm (Mitchell & Doak, 2004).

The assessment of GSH (nmol/mg protein), GSH-Px and SOD activities are also done where semen samples are centrifuged for five minutes and are then read by a spectrophotometer only. The GSH-Px activity is determined by incubating the sample for five minutes and the absorption is determined by spectrophotometer, the activity is calculated and expressed as U/g protein. The SOD activity is also measured by spectrophotometric absorbance and expressed as U/mg protein (Bucak *et al.*, 2010).

Mitochondrial activity is also an important evaluation that is used in spermatozoa evaluation. The mitochondria activity should be high enough to indicate that there is no damage to the mitochondria and sufficient ATP should be produced for spermatozoa to be motile (Zanganeh *et al.*, 2013).

2.7 Summary

Bull fertility plays an essential role in the livestock industry. Beef bulls may breed 20 cows naturally under extensive production systems that can be negatively affected by high ambient temperatures. Environmental temperature is a primary factor affecting bull fertility that requires consideration. Therefore, to maximise reproduction efficiency during summer, strategies that will alleviate the negative effects of heat stress will have to be implemented.

Chapter 3: Material and Methods

3.1 Introduction

The focus of this study was to determine the effect of temperature on semen quality in nine provinces of South Africa. The data was collected during routine bull fertility testing by the company Vriesit (682 Wattle street, Doornpoort, Pretoria, 0186). Fresh and frozen semen data was evaluated on semen quality characteristics. Ethical approval was granted by the University of Pretoria (EC171106-168) for the use of external data.

3.2 Methodology

Data on bull semen characteristics was received from Vriesit consisting of 32 488 records of fresh and frozen-thawed semen. The data included recordings over a period of 2010 - 2016. Bull semen was collected from bulls during routine fertility evaluations that represented different beef breeds in all nine provinces of South Africa. For each bull the following information was received; bull number, farm address where bull resides, day, month and year when semen was collected, results of reproductive gland evaluations and the semen quality characteristics. For fresh semen collections, seven parameters associated with bull semen quality namely volume, consistency, mass motility, linear motility, non-linear motility, major and minor defects. All bulls with frozen-thawed semen records that had five parameters analysed using the computer assisted sperm analysis (CASA) system and two count parameters were used namely; percentage live sperm (CASA), linear motility (CASA), non-linear motility (CASA), major and minor defects, number of sperm per ml (CASA) and the viability of sperm (CASA).

Data was filtered to create a file with all the bulls that have complete records of all semen quality parameters. Therefore, the number of bulls per breed must be above 120 for a breed to be included. This resulted in only nine breeds being included for fresh semen and seven breeds being included for frozen-thawed semen (Table 3.1). The same principle was applied to provinces that had to have more than a 100 bull records per province to be included in this study. The Western Cape was excluded from the fresh semen data due to insufficient amount of bull records that was below. The Northern Cape and Western Cape were excluded from the frozen semen data due to the number of bull records that were lower than the inclusion amount, as indicated in Table 3.1.

Table 3.1 Summary of the number of fresh and frozen-thawed semen records available per province, breed and year of collection.

	Number of records	
Province	**Fresh semen**	**Frozen semen**
Limpopo	3415	354
North West	6613	566
Mpumalanga	3198	476
Gauteng	2005	294
Free State	5017	969
KwaZulu-Natal	696	122
Eastern Cape	505	145
Northern Cape	611	28
Western Cape	39	49
Total	**22 099**	**3 003**
Breed		
Beefmaster (Composite Breed)	4626	400
Bonsmara (Composite breed)	7722	688
Boran (Composite breed)	584	404
Brahman (Bos Indicus)	2545	772
Drakensberger (Indigenous breed)	686	
Limousin (Bos Taurus)	747	
Nguni (Bos Taurus)	260	
Holstein (Bos Taurus)		273
Simbra (Composite breed)	1792	204
Simmentaler (Bos Taurus)	3137	262
Total	**22 099**	**3 003**
Year		
2010	1011	210
2011	3735	515
2012	3479	489
2013	3288	493
2014	3580	456
2015	3089	359
2016	2959	344
Total	**21 141**	**2 866**

Every record per semen parameter in fresh and frozen-thawed data sets were included when within the acceptable ranges or excluded due to human error, missing or untrustworthy results as seen in table 3.2. The data was edited thoroughly according to outliners per parameters or the reasons stated in table 3.2.

Table 3.2 Data-editing based on semen parameters to produce a reliable fresh and frozen-thawed semen dataset.

Parameters	Levels	Included / Excluded	Reason
Volume (ml)	0 and > 20ml	Excluded	No record and Human error
Consistency (class)	1 - 7	Included	Human error
Mass Motility	All records	Excluded	Bias and untrustworthy observations
Percentage live sperm	0	Excluded	Sperm are dead
Sperm count (no/ml)	0	Excluded	Not done
Sperm viability (%)	0	Excluded	Not done
Stress	All	Excluded	Lack of measurements
Linear motility (%)	0	Excluded	Sperm are dead
Non-Linear motility (%)	0 - 90	Included	Human error
Scrotal circumference (cm)	All	Excluded	Most records predicted
Major and minor defects (%)	0 - 100	Included	Human error
Individual major defects (counted)	All	Included	Read by CASA
Individual minor defects (counted)	All	Included	Read by CASA

All the major and minor defects recorded are listed in Addendum A1 and Addendum A2 for fresh semen and for frozen semen respectively. In table 3.3 the numbers of non-zero and zero measurements per defect are identified in the preliminary analysis to ensure the data were sufficient to perform statistical analysis.

Table 3.3 Number of individual major and minor spermatozoa defects for fresh semen.

	Fresh Semen			
Major defects	**Number of non-zero measurements**	**Number of zero measurements**	**Total number of measurements**	**Sufficient measurements for statistical analysis?**
Mid-piece abnormalities	14224	1194	15418	Yes
Tail abnormalities	11479	3939	15418	Yes
Head abnormalities	289	15129	15418	No
Periform heads	6873	8545	15418	Yes
Other major defects	7265	8153	15418	Yes
Proximal protoplasmic drops	11664	3754	15418	Yes
Abnormal loose heads	2001	13417	15418	No
Craters	754	14664	15418	No
Minor defects				
Minor mid-piece abnormalities	6031	9387	15418	Yes
Minor loose heads	11207	4211	15418	Yes
Minor gebuigdehoofstuk	3804	11614	15418	No
Minor gekruidedistel	4257	11161	15418	No
Minor distal protoplasmic drops	11286	4132	15418	Yes
Abaksialeinplanting	661	14757	15418	No
Beskadigdeakrosoom	314	15104	15418	No
Other minor defects	35	15383	15418	No

In table 3.4 the data for non-zero and zero measurements per defect are listed for frozen-thawed semen indicating sufficient numbers to perform statistical analysis. General linear models were only performed for those defects for which the data set had a sufficient number of observations. The number of zero measurements should be below 60 % for each defect to be included.

Table 3.4 Number of individual major and minor sperm defects in frozen-thawed semen.

	Frozen semen			
Major defects	**Number of non-zero measurements**	**Number of zero measurements**	**Total number of measurements**	**Sufficient measurements for statistical analysis?**
Mid-piece abnormalities	2089	96	2185	Yes
Tail abnormalities	1755	430	2185	Yes
Head abnormalities	73	2112	2185	No
Pyriform heads	883	1302	2185	Yes
Other major defects	1083	1102	2185	Yes
Proximal protoplasmic drops	1353	832	2185	Yes
Abnormal loose heads	270	1915	2185	No
Craters	177	2008	2185	No
Minor defects				
Minor mid-piece abnormalities	1203	982	2185	Yes
Minor loose heads	1631	554	2185	Yes
Minor bended head	376	1809	2185	No
Minor curled distal	476	1709	2185	No
Minor distal protoplasmic drops	1356	829	2185	Yes
Abaxial implant	156	2029	2185	No
Damaged acrosome	37	2148	2185	No
Other minor defects	6	2179	2185	No

The South African Weather Service (SAWS) provided average monthly maximum temperatures and humidity data from 89 weather stations in SA, from 2010 to 2016. The home location of each bull was linked to the nearest SA weather station, as indicated in Addendum B. Bulls received a temperature and humidity value, individually, that was linked to the town, the month and year when bull semen was collected for evaluation of the effect of temperature on semen quality at time of collections. Each bull received a specific temperature and humidity value from 60 days before semen collection, to evaluate the influence of temperature on sperm production. The temperature and humidity data per bull was used to calculate the thermo-humidity index (THI) specific to each bull, using the following equation:

THI = (1.8 x T + 32) – ((0.55 – 0.0055 x HR) x (1.8 x T – 26)).

The impact of the THI on ruminant reproduction was categorised as follows:

- bulls are regarded as fertile: THI is <41 to < 71,
- thermal stress causing fertility to decrease: THI is ≥ 71 to ≤ 73
- sudden drop in fertility where bulls can become infertile: THI >73.

The months allocated to each season were as follows:

- Summer: December to February
- Autumn: March to May
- Winter: June to August
- Spring: September to November

3.3 Statistical Analysis

Statistical Analysis System 9.4 (SAS) (SAS Institute Inc., Cary, NC, USA. 2009) was used for statistical analyses in consultation with the Internal Statistical Consultation Service (ISCS) at the University of Pretoria. The dependant and independent variables included in the analyses are indicated in Table 3.5. An analysis of variance (ANOVA) method was used to calculate the descriptive statistics and to perform significance testing of the fixed effects; year, season, breed, province and temperature (THI) for each of the different parameters.

Table 3.5 Summary of dependent and independent variables used during analyses.

Dependent variables	**Independent variables**
Volume (ml)	Province
Live spermatozoa (%)	Year
Spermatozoa count (no/ml)	Season
Spermatozoa viability (%)	Breed
Linear motility (%)	Temperature and humidity (THI)
Non-Linear motility (%)	
Major defects (%)	
Minor defects (%)	
Individual major defects (counted)	
Individual minor defects (counted)	

The analysis was performed, using six ANOVA PROC GLM statistical models which includes all nine semen parameters, five major defects, three minor defects per THI category as dependant variables as well as the corresponding independent variables province, breed, THI, season and year. All models are displayed in table 3.6.

Table 3.6 ANOVA PROC GLM models used for statistical analysis on fresh and frozen-thawed semen dataset.

Model	PROC GLM	Dependant variables
I	Fresh Semen parameters = F(Provinces, Breed, THI, Season, Year)	Volume Linear movement Non-linear movement Major defects Minor defects
II	Fresh Major defects = F(Province, Breed, THI, Season, Year)	Proximal protoplasmic droplets Mid-piece abnormalities Tail abnormalities Abnormal Loose heads Pyriform heads Other major defects
III	Fresh Major defects per THI category = F(Province, Breed, Season, Year)	Proximal protoplasmic droplets Mid-piece abnormalities Tail abnormalities Abnormal Loose heads Pyriform heads Other major defects
IV	Thawed Semen parameters = F(Provinces, Breed, THI, Season, Year)	% Live sperm Linear movement Non-linear movement Count/mL Viability % Major defects Minor defects
V	Thawed Major defects = F(Province, Breed, THI, Season, Year)	Proximal protoplasmic droplets Mid-piece abnormalities Tail abnormalities Pyriform heads Other major defects
VI	Thawed Major defects per THI category = F(Province, Breed, Season, Year)	Proximal protoplasmic droplets Mid-piece abnormalities Tail abnormalities Pyriform heads Other major defects

Chapter 4: Results

4.1 Descriptive statistics: Fresh and frozen-thawed bull semen

In table 4.1 and 4.2 descriptive statistics for fresh and frozen-thawed bull semen characteristics were summarised. Analysis for breed, province, season and THI were summarised in this chapter.

Table 4.1 Summary of descriptive statistics of the fresh and frozen-thawed bull semen characteristics used for analysis.

Bull semen	Traits	Number of observations	Mean	Median	Std. Dev.	Min	Max
Fresh	Volume (%)	14566	5,24	5	1,66	1	20
	Linear Movement (%)	14560	81,19	86	17,78	0	99
	Non-Linear Movement (%)	14559	5,52	5	2,26	0	90
	Major Defects (%)	14546	14	12	9,98	0	86
	Minor Defects (%)	14546	6,86	6	5,27	0	80
Frozen	Live sperm (%)	2029	52,4	52,54	12,81	4,64	82,25
	Linear movement (%)	2029	47,14	47,3	13,31	1,82	78,82
	Non-Linear movement (%)	2029	5,26	5,05	2,69	1,16	66,35
	Major Defect (%)	2029	12,5	10	8,17	0	60
	Minor Defect (%)	2029	5,81	5	4,42	0	68
	Count/mL	2029	79,16	77,7	24,76	2,37	266,1

The percentages for fresh and frozen-thawed semen per province is presented in table 4.2. The results show that the highest percentage of collections was in the North West for fresh semen and Free State for frozen-thawed semen.

Table 4.2 Summary of percentages for fresh and frozen-thawed semen records per province.

	Eastern Cape	Free State	Gauteng	KwaZulu -Natal	Limpopo	Mpumalanga	North West	Northern Cape
Fresh semen	3.02	22.56	9.59	3.74	15.64	14.36	27.85	3.24
Frozen-thawed semen	5.42	31.59	9.66	4.63	12.47	15.57	20.65	0

The percentages for fresh and frozen-thawed semen per breed is presented in table 4.3 showing the highest percentage in Bonsmara bulls and the lowest percentage in Nguni bulls for fresh semen. The highest percentage of collections for frozen-thawed semen was in Brahman bulls and the lowest percnetage in Holstein-Fries bulls.

Table 4.3 Summary of percentages for fresh and frozen-thawed semen records per breed.

	Beefmaster	Bonsmara	Boran	Brahman	Drakensberger	Limousin	Nguni	Simbra	Simmentaler	Holstein - Fries
Fresh semen	19.0	32.69	2.68	13.07	3.39	3.25	1.30	8.70	15.87	0
Frozen-thawed semen	13.95	24.05	13.06	25.68	0	0	0	17.20	0	6.06

The highest percentage of bulls were tested in spring and the lowest was tested in summer as illustrated in figure 4.1.

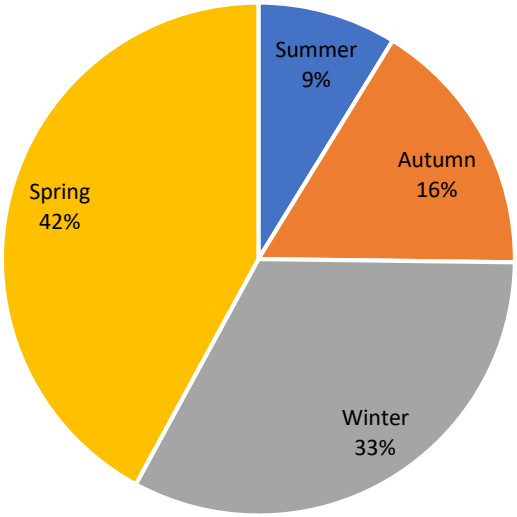

Figure 4.1 Summary of the percentage fresh bull semen records per season.

The percentage of collections in figure 3.2 illustrated that spring has the highest percentage and summer the lowest percentage for frozen-thawed bull semen.

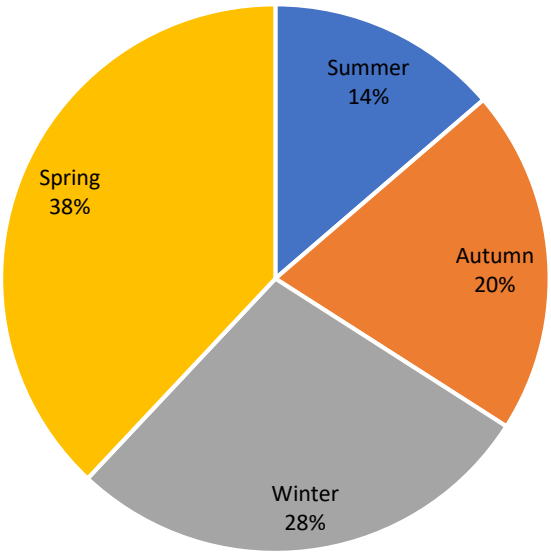

Figure 4.2 Summary of the percentage frozen-thawed bull semen records per season.

4.2 Fresh semen results

The results for fresh semen characteristics per province are shown in Table 4.4. Significant differences (P <0.05) between provinces were observed for the various semen characteristic per province over 7 years. Eastern Cape and the Northern Cape had some of the best semen characteristics, where KwaZulu-Natal had the worst semen characteristics observed.

Table 4.4 Summary of means for each fresh semen characteristic per province at semen collection over seven years.

	Volume (ml)	Linear movement (%)	Non-Linear movement (%)	Major Defects (%)	Minor defects (%)
Eastern Cape	5.99 ± 0.10 ^{a}	83.37 ± 0,80 ^{ab}	5.20 ± 0.10 ^{bc}	13.59 ± 0.45 ^{abc}	6.49 ± 0.25 ^{cd}
Free State	5.24 ± 0.038 ^{bd}	80.15 ± 0,29 ^{c}	5.77 ± 0.04 ^{a}	14.20 ± 0.16 ^{bc}	7.19 ± 0.09 ^{bcd}
Gauteng	5.48 ± 0.068 ^{bc}	80.55 ± 0,52 ^{ac}	5.66 ± 0.07 ^{ab}	15.21 ± 0.29 ^{ab}	7.72 ± 0.16 ^{ab}
Kwazulu-Natal	4.96 ± 0.094 ^{d}	78.53 ± 0,71 ^{c}	5.96 ± 0.09 ^{a}	14.39 ± 0.40 ^{abc}	8.71 ± 0.22 ^{a}
Limpopo	5.38 ± 0.046 ^{bc}	82.14 ± 0,35 ^{ab}	5.39 ± 0.05 ^{bc}	14.63 ± 0.20 ^{abc}	7.31 ± 0.11 ^{bc}
Mpumalanga	5.26 ± 0.048 ^{bd}	80.12 ± 0,36 ^{c}	5.90 ± 0.05 ^{a}	15.39 ± 0.21 ^{a}	7.17 ± 0.11 ^{bcd}
North West	5.23 ± 0.035 ^{bd}	82.96 ± 0,26 ^{b}	5.33 ± 0.03 ^{c}	13.85 ± 0.15 ^{c}	6.75 ± 0.08 ^{d}
Northern Cape	5.76 ± 0.101 ^{ac}	82.33 ± 0,76 ^{abc}	5.16 ± 0.10 ^{c}	15.38 ± 0.43 ^{abc}	6.51 ± 0.24 ^{cd}

All results are expressed as mean ± SD.
^{abcd} Subscripts are used to indicate that the values in each column which have different letters are significantly different (p <0.05).

Table 4.5 presents the significant differences between different breeds for each semen characteristics at semen collection. Variation was observed for the different semen characteristics among the different breeds, with some significant breed differences for specific semen traits.

Table 4.5 Summary of fresh semen characteristics per breed over seven years.

	Breed type	Volume (ml)	Linear movement (%)	Non-Linear movement (%)	Major Defects (%)	Minor Defects (%)
Beefmaster	Composite	5.19 ± 0.042 ^{bd}	78.70 ± 0.32 ^{d}	5.95 ± 0.04 ^{a}	14.71 ± 0.18 ^{b}	7.54 ± 0.10 ^{a}
Bonsmara	Composite	5.31 ± 0.032 ^{abcd}	82.13 ± 0.24 ^{bc}	5.47 ± 0.03 ^{bc}	14.65 ± 0.14 ^{b}	7.27 ± 0.08 ^{a}
Boran	Composite	5.65 ± 0.11 ^{bc}	81.33 ± 0.84 ^{abcd}	5.60 ± 0.11 ^{abc}	14.11 ± 0.48 ^{bcd}	6.80 ± 0.26 ^{abc}
Brahman	Bos Indicus	5.51 ± 0.05 ^{ac}	80.40 ± 0.38 ^{bd}	5.62 ± 0.05 ^{b}	14.37 ± 0.22 ^{bc}	7.24 ± 0.12 ^{ab}
Drakensberger	Indigenous	5.77 ± 0.10 ^{a}	83.98 ± 0.75 ^{ac}	5.34 ± 0.10 ^{bc}	11.28 ± 0.43 ^{e}	5.73 ± 0.24 ^{c}
Limousin	Bos Taurus	4.84 ± 0.12 ^{d}	81.90 ± 0.87 ^{abcd}	5.49 ± 0.11 ^{abc}	13.50 ± 0.50 ^{abcde}	6.70 ± 0.27 ^{abc}
Nguni	Bos Taurus	4.99 ± 0.16 ^{bcd}	82.56 ± 1.20 ^{abcd}	5.20 ± 0.16 ^{bc}	11.60 ± 0.69 ^{cde}	6.74 ± 0.38 ^{abc}
Simbra	Composite	5.38 ± 0.06 ^{abc}	79.97 ± 0.47 ^{d}	5.67 ± 0.06 ^{ab}	17.69 ± 0.27 ^{a}	7.14 ± 0.15 ^{ab}
Simmentaler	Bos Taurus	5.18 ± 0.05 ^{bc}	84.05 ± 0.35 ^{a}	5.26 ± 0.05 ^{c}	13.16 ± 0.20 ^{d}	6.74 ± 0.11 ^{bc}

All results are expressed as mean ± SD.
^{abcde} Subscripts are used to indicate that the values in each column which have different letters are significantly different (p <0.05).

The effect of THI on bull semen characteristics is displayed in Figure 4.3 and 4.4. No differences were observed between THI groups for bull semen volume, sperm major and minor defects. The temperatures in all results were classified as follows: THI 1 is 41 – 71 when bulls are fertile; THI 2 is 71 – 73 when thermal stress decreased fertility and THI 3 is >73 causing a sudden drop in fertility.

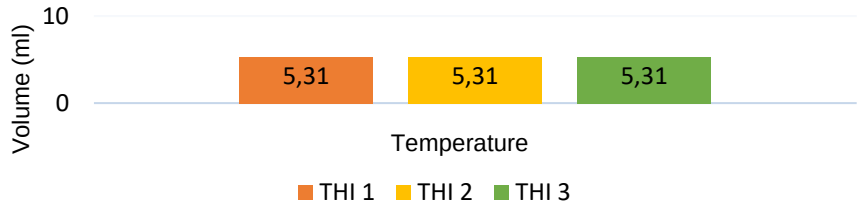

Figure 4.3 Semen volume per temperature group when semen was collected.

Significant differences were observed for linear and non-linear movement in figure 4.4. Linear movement at THI 1 was higher that than THI 2 and THI 3.

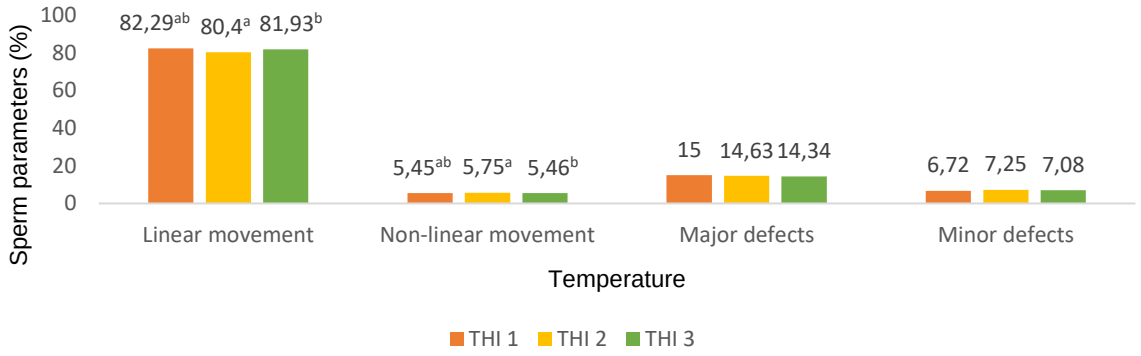

Figure 4.4 Linear and non-linear sperm movement, per temperature group when semen was collected (a and b were used to indicate that columns with different letters are significantly different; p <0.05).

The temperature 60 days prior to semen collection for THI groups and the effect of THI on bull semen characteristics are displayed in Figure 4.5 and 4.6. There are no results for TH1 group due to the low number of records that could not be statistically analysed. Temperature at sperm production affected minor defects as it was observed to be significantly (P <0.05) lower when bulls are heat stressed at THI 2 than severely heat stressed bulls at THI 3.

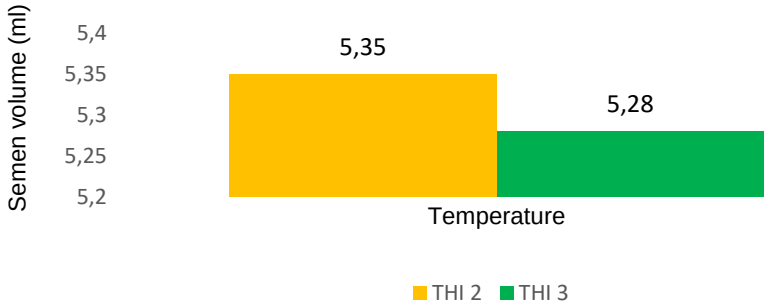

Figure 4.5 Semen volume per temperature group two months prior to semen collection.

In table 4.6 linear and non-linear sperm movement shared significant differences between THI 2 and THI 3. Major defects only had one value in the group, THI 3 as the number of values for the other group was too low for analysis.

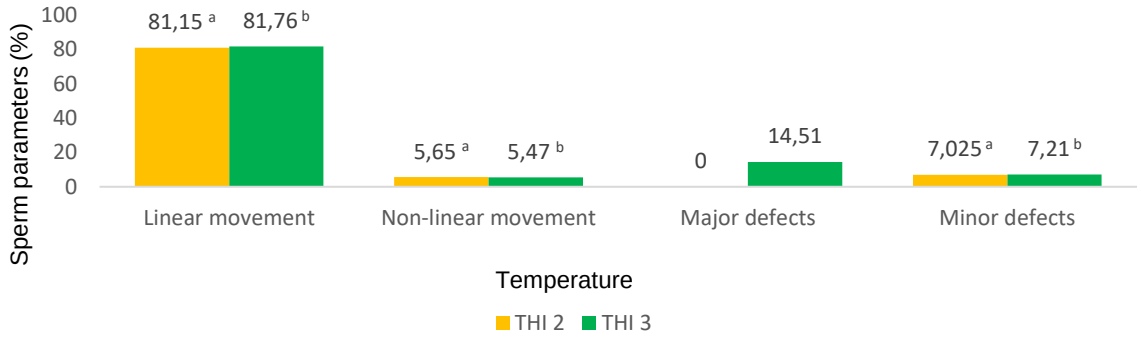

Figure 4.6 Semen characteristics per temperature group two months prior to semen collection (a and b were used to indicate that the column which have different letters are significantly different ($p < 0.05$)).

The effect of season over seven years on semen characteristics and significant difference are shown in Table 4.6. Higher values for semen characteristics were observed for autumn compared to spring and summer. Summer semen collection had higher linear sperm movement and lower fresh semen volume, non-linear sperm movement, major and minor defects of spermatozoa.

Table 4.6 Fresh semen characteristics per season when semen was collected over a seven-year period.

	Volume (ml)	Linear Movement (%)	Non-Linear Movement (%)	Major Defects (%)	Minor Defects (%)
Autumn	5.34 ± 0.043	80.27 ± 0.33 ^{b}	5.55 ± 0.043 ^{a}	14.58 ± 0.19	7.73 ± 0.10 ^{a}
Spring	5.27 ± 0.029	82.48 ± 0.22 ^{a}	5.47 ± 0.028 ^{ab}	14.44 ± 0.13	6.88 ± 0.069 ^{a}
Summer	5.32 ± 0.059	82.88 ± 0.45 ^{a}	5.33 ± 0.058 ^{b}	13.95 ± 0.26	6.92 ± 0.14 ^{ab}
Winter	5.34 ± 0.032	80.23 ± 0.24 ^{b}	5.76 ± 0.032 ^{c}	14.53 ± 0.14	7.19 ± 0.077 ^{b}

All results are expressed as mean ± SD.
^{ab} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table 4.7 shows significant differences between seasons and semen characteristics over seven years. The lowest values for semen characteristics were observed during spring and the highest values in summer. This observation differed from the observation made in Table 4.6.

The significant differences between year and semen characteristics or individual major sperm defects were attached as addendum C1 table C1.1 – C1.8. The results of individual minor defects compared to province, breed, THI, season and year were also displayed in addendum C2 table C2.1 – C 2.28.

Table 4.7 Fresh semen characteristics per season when semen was produced, over a period of seven years.

	Volume (ml)	Linear Movement (%)	Non-Linear Movement (%)	Major Defects (%)	Minor Defects (%)
Autumn	5.29 ± 0.036 [a]	79.98 ± 0.27 [b]	5.78 ± 0.035 [c]	14.94 ± 0.20 [b]	7.52 ± 0.087 [b]
Spring	5.13 ± 0.041 [b]	83.34 ± 0.31 [a]	5.26 ± 0.04 [a]	13.79 ± 0.19 [a]	6.78 ± 0.098 [a]
Summer	5.36 ± 0.054 [a]	80.32 ± 0.41 [b]	5.50 ± 0.053 [b]	14.98 ± 0.24 [b]	7.55 ± 0.13 [b]
Winter	5.42 ± 0.031 [a]	81.94 ± 0.23 [a]	5.55 ± 0.03 [b]	14.62 ± 0.35 [ab]	6.91 ± 0.074 [a]

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column with different letters are significantly different ($p < 0.05$).

Table 4.8 presents major spermatozoa defects per province that is influenced by the temperature at time of semen collection. All three major spermatozoa defects, loose heads, pyriform heads and other major defects had higher values in KwaZulu-Natal and the lowest for the Northern Cape samples.

Table 4.8 Fresh semen individual major spermatozoa defects (%) per province over seven years.

	Proximal Protoplasmic droplets	Mid-piece abnormalities	Tail abnormalities	Loose heads	Pyriform heads	Other major defects
Eastern Cape	4.49 ± 0.24[ab]	1.078 ± 0.06 [bd]	0.61 ± 0.032 [b]	1.56 ± 0.19	0.80 ± 0.066 [b]	0.67 ± 0.048 [abc]
Free State	4.04 ± 0.09 [a]	1.26 ± 0.022 [abc]	0.73 ± 0.012 [ab]	1.50 ± 0.058	0.89 ± 0.023 [b]	0.49 ± 0.017 [a]
Gauteng	4.22 ± 0.16 [ab]	1.31 ± 0.039 [abc]	0.69 ± 0.021 [ab]	1.42 ± 0.095	1.047 ± 0.039 [ab]	0.65 ± 0.031 [bc]
Kwazulu-Natal	4.25 ± 0.23 [ab]	1.47 ± 0.055 [a]	0.76 ± 0.029 [ab]	1.36 ± 0.18	0.75 ± 0.072 [b]	0.45 ± 0.044 [ab]
Limpopo	3.99 ± 0.11 [a]	1.32 ± 0.026 [abc]	0.73 ± 0.014 [ab]	1.49 ± 0.067	0.89 ± 0.029 [b]	0.57 ± 0.021 [abc]
Mpumalanga	3.68 ± 0.11 [a]	1.36 ± 0.027 [ac]	0.78 ± 0.014 [a]	1.52 ± 0.065	1.099 ± 0.027 [a]	0.60 ± 0.022 [bc]
North West	3.62 ± 0.08 [a]	1.25 ± 0.02 [bcd]	0.70 ± 0.01 [b]	1.48 ± 0.047	0.94 ± 0.021 [b]	0.54 ± 0.016 [abc]
Northern Cape	5.08 ± 0.23 [b]	1.028 ± 0.057 [d]	0.72 ± 0.031 [ab]	1.61 ± 0.14	1.34 ± 0.057 [c]	0.71 ± 0.046 [c]

All results are expressed as mean ± SD.
[abcd] Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table 4.9 presents the percentages of major spermatozoa defects per breed when semen was collected. The breed that had the highest percentile major defects was observed to be Simbra. The breeds with the lowest percentile include Boran, Drakensberger and Nguni bulls. No significant difference was found between different breeds concerning abnormal loose heads.

Table 4.9 Fresh semen individual major spermatozoa defects (%) per breed over seven years.

	Frequency	Proximal Protoplasmic droplets	Mid-piece abnormalities	Tail abnormalities	Loose heads	Pyriform heads	Other major defects
Beefmaster	2722	4.20 ± 0.10 ^{ab}	1.26 ± 0.024 ^{a}	0.74 ± 0.013 ^{abc}	1.56 ± 0.064	0.92 ± 0.025 ^{a}	0.61 ± 0.019 ^{ab}
Bonsmara	4752	3.61 ± 0.08 ^{c}	1.42 ± 0.018 ^{b}	0.75 ± 0.01 ^{ab}	1.37 ± 0.05	0.82 ± 0.021 ^{a}	0.58 ± 0.015 ^{abc}
Boran	399	4.10 ± 0.26 ^{abc}	1.27 ± 0.063 ^{abd}	0.67 ± 0.033 ^{abcd}	1.24 ± 0.18	0.82 ± 0.069 ^{ac}	0.59 ± 0.05 ^{abc}
Brahman	1939	4.36 ± 0.12 ^{a}	1.32 ± 0.029 ^{ab}	0.70 ± 0.015 ^{acd}	1.33 ± 0.078	0.88 ± 0.033 ^{a}	0.48 ± 0.023 ^{c}
Drakensberger	513	3.53 ± 0.24 ^{abc}	0.87 ± 0.06 ^{c}	0.57 ± 0.031 ^{d}	1.59 ± 0.14	0.90 ± 0.057 ^{ac}	0.41 ± 0.047 ^{ac}
Limousin	491	3.29 ± 0.27 ^{abc}	1.23 ± 0.066 ^{abd}	0.72 ± 0.035 ^{abcd}	1.40 ± 0.17	1.00 ± 0.072 ^{ac}	0.48 ± 0.052 ^{ac}
Nguni	197	2.66 ± 0.40 ^{bc}	1.099 ± 0.09 ^{abcd}	0.68 ± 0.048 ^{abcd}	1.58 ± 0.20	0.87 ± 0.097 ^{abc}	0.34 ± 0.073 ^{abc}
Simbra	1276	5.27 ± 0.14 ^{d}	1.34 ± 0.036 ^{ab}	0.80 ± 0.019 ^{b}	1.68 ± 0.081	1.40 ± 0.035 ^{b}	0.69 ± 0.028 ^{b}
Simmentaler	2277	3.40 ± 0.11^{c}	1.065 ± 0.026^{cd}	0.68 ± 0.014^{cd}	1.59 ± 0.055	1.077 ± 0.025^{c}	0.49 ± 0.021 ^{c}

All results are expressed as mean ± SD.
^{abcd} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

The results in Table 4.10 represent the different major spermatozoa defects per THI at the time of collection. It can be observed that the lowest values can be observed in the THI 2 group for the major spermatozoa defects. The highest values of major spermatozoa defects were observed in the THI 1 group. No significant differences could be found between groups for spermatozoa proximal protoplasmic droplets, mid-piece abnormalities and tail abnormalities.

Table 4.10 Fresh semen major spermatozoa defects (%) in fresh semen at different THI groups during fresh semen collection across breeds and over a period of seven years.

		Production rate	Proximal Protoplasmic droplets	Mid-piece abnormalities	Tail abnormalities	Loose heads	Pyriform heads	Other major defects
THI 1	< 70 - 72	High	4.24 ± 0.37	1.18 ± 0.087	0.74 ± 0.045	2.083 ± 0.24 ^{b}	1.17 ± 0.087 ^{b}	0.67 ± 0.068 ^{ab}
THI 2	73 - 78	Decreased	3.83 ± 0.07	1.27 ± 0.018	0.72 ± 0.01	1.53 ± 0.045 ^{ab}	1.14 ± 0.018 ^{b}	0.53 ± 0.014 ^{a}
THI 3	79 - > 80	Zero	3.98 ± 0.05	1.29 ± 0.013	0.73 ± 0.007	1.46 ± 0.033 ^{a}	0.84 ± 0.014 ^{a}	0.57 ± 0.010 ^{b}

All results are expressed as mean ± SD.
^{ab} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table 4.11 depicts the summary of major spermatozoa defects per THI group, 60 days before semen collection. The lowest percentages of major spermatozoa defects were observed in the THI 2 group.

Table 4.11 Fresh semen major spermatozoa defects (%) at different THI groups that occurred during semen production across breeds over a seven-year period.

		Production rate	Proximal Protoplasmic droplets	Mid-piece abnormalities	Tail abnormalities	Loose heads	Pyriform heads	Other major defects
THI 1	< 70 - 72	High	-	-	-	-	-	-
THI 2	73 - 78	Decreased	3.87 ± 0.07	1.27 ± 0.017	0.70 ± 0.0088 [a]	1.57 ± 0.041 [b]	0.97 ± 0.017	0.53 ± 0.014 [a]
THI 3	79 - > 80	Low	4.038 ± 0.06	1.29 ± 0.015	0.73 ± 0.0075 [b]	1.45 ± 0.039 [a]	0.94 ± 0.015	0.57 ± 0.012 [b]

All results are expressed as mean ± SD.
[a] Subscripts are used to indicate that the values in each column which have different letters are significantly different (p <0.05).

The effect of season on major spermatozoa defects was summarised in Figure 4.7. There were only two major spermatozoa defects that had no significant differences between seasons namely; tail abnormalities and loose heads. It was observed that spring had the lowest values for major spermatozoa defects. Proximal protoplasmic droplets were significantly higher (P <0.05) when semen collection were in summer, but other major spermatozoa defects were significantly higher (P <0.05) when collected in autumn than in spring and was observed to have significantly lower (P <0.0001) other major defects. The semen when collected in winter had significantly lower (P <0.0001) pyriform heads, where spermatozoa produced in autumn was significantly higher (P <0.05). Mid-piece abnormalities were only significantly higher (P <0.05) when semen was collected in spring. Tail abnormalities were significantly lower when spermatozoa production occurred during winter.

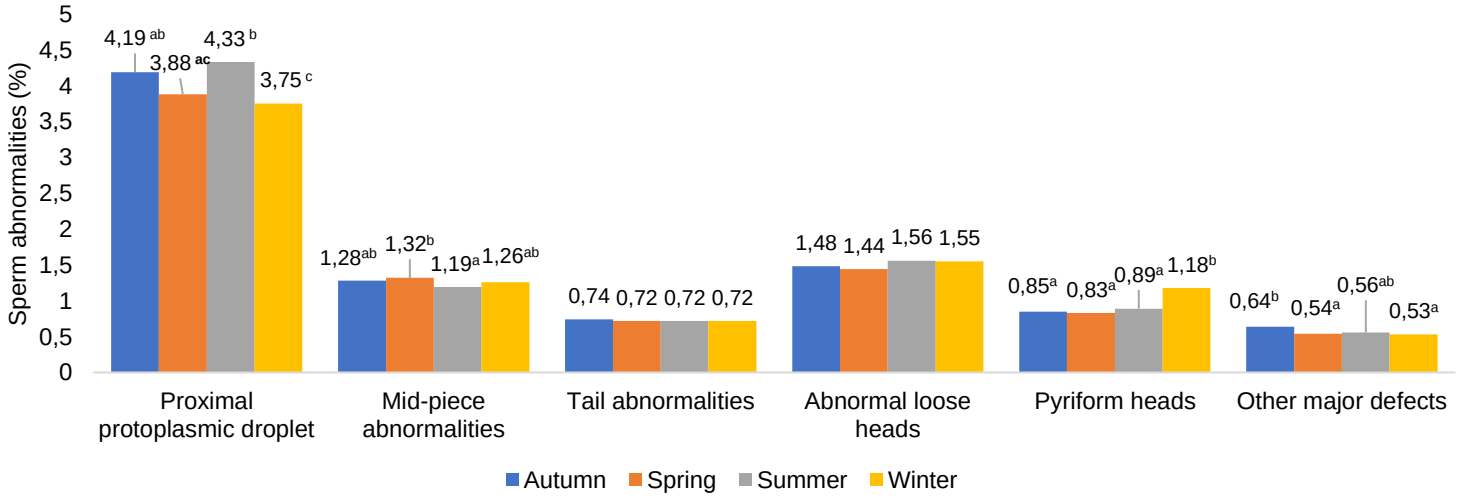

Figure 4.7 Summary of major spermatozoa defects per season during semen collection (a, b and c were used to indicate that the column which have different letters are significantly different (p <0.05)).

In Figure 4.8 the summary of seasonal effect on major defects under the influence of the temperature during spermatogenesis are displayed. The season when semen was produced had higher percentages of major spermatozoa defects; mid-piece abnormalities, loose heads and other major defects was during spring. No significant differences could be observed between production in seasons for the major spermatozoa defects mid-piece abnormalities and loose heads.

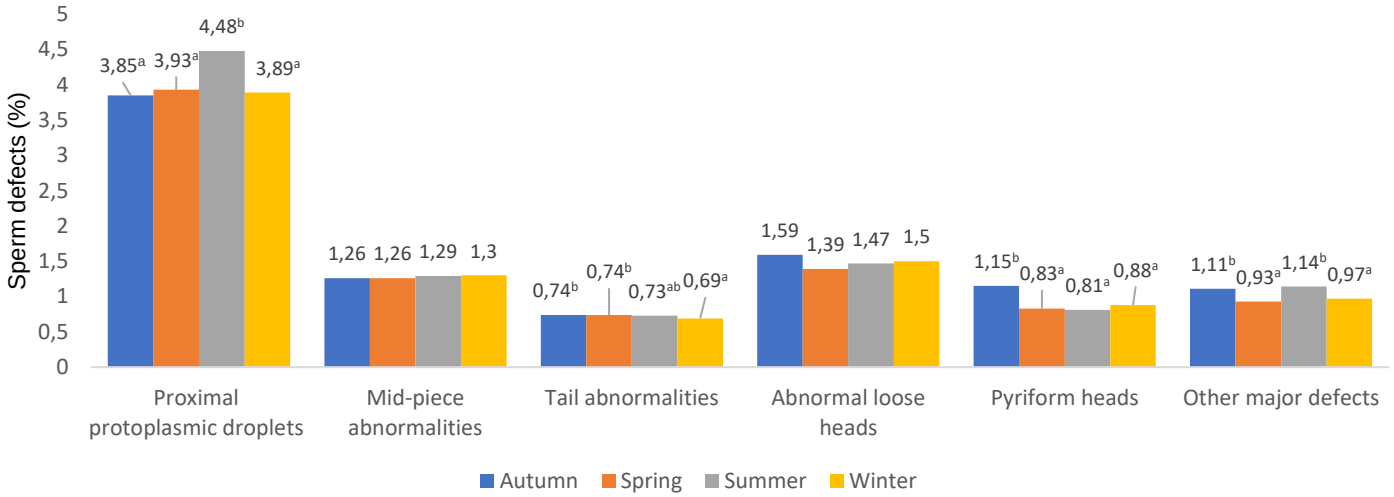

Figure 4.8 A summary of major spermatozoa defects per season during semen production (a and b were used to indicate that the column which have different letters are significantly different (p <0.05)).

The results between major defects per province at THI groups as co-variate at semen collection are shown in Table 4.12. The major defects; proximal droplets, tail abnormalities and other major defects in table 4.12 indicates that most of the values per provinces were higher in the THI 3 group than in the THI 2 group. The highest values were observed in Eastern Cape and KwaZulu-Natal.

Table 4.12 Summary of major spermatozoa defects (%) with significant differences per provinces when fresh semen was collected over seven years.

	Proximal Protoplasmic droplets		**Mid-piece abnormalities**		**Tail abnormalities**		**Pyriform heads**		**Other major defects**	
	THI 2	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**
Eastern Cape	4.43 ± 0.23 ^{ab}	5.24 ± 0.87 ^{ab}	0.99 ± 0.062 ^{b}	2.017 ± 0.21 ^{a}	0.57 ± 0.034 ^{a}	1.053 ± 0.10 ^{a}	0.81 ± 0.081 ^{a}	0.76 ± 0.19 ^{ab}	0.67 ± 0.053 ^{b}	0.67 ± 0.15 ^{abc}
Free State	3.65 ± 0.13 ^{a}	4.33 ± 0.12 ^{b}	1.18 ± 0.031 ^{bc}	1.34 ± 0.03 ^{a}	0.70 ± 0.017 ^{ab}	0.76 ± 0.016 ^{a}	1.00 ± 0.036 ^{a}	0.77 ± 0.03 ^{b}	0.43 ± 0.027 ^{a}	0.55 ± 0.023 ^{c}
Gauteng	4.013 ± 0.29 ^{a}	4.32 ± 0.19 ^{ab}	1.24 ± 0.072 ^{bcd}	1.34 ± 0.047 ^{a}	0.69 ± 0.039 ^{ab}	0.69 ± 0.025 ^{ab}	1.10 ± 0.085 ^{ab}	1.027 ± 0.042 ^{a}	0.48 ± 0.061 ^{ab}	0.72 ± 0.037 ^{a}
Kwazulu-Natal	4.29 ± 0.30 ^{ab}	4.00 ± 0.39 ^{ab}	1.63 ± 0.073 ^{a}	1.27 ± 0.089 ^{ab}	0.82 ± 0.041 ^{bc}	0.73 ± 0.045 ^{ab}	0.70 ± 0.11 ^{a}	0.68 ± 0.11 ^{ab}	0.55 ± 0.064 ^{ab}	0.26 ± 0.066 ^{b}
Limpopo	3.96 ± 0.29 ^{a}	4.00 ± 0.12 ^{ab}	1.45 ± 0.073 ^{acd}	1.30 ± 0.028 ^{a}	0.78 ± 0.042 ^{bc}	0.73 ± 0.014 ^{ab}	1.051 ± 0.092 ^{ab}	0.86 ± 0.027 ^{ab}	0.36 ± 0.065 ^{ab}	0.59 ± 0.021 ^{ac}
Mpumalanga	3.44 ± 0.15 ^{a}	4.001 ± 0.17 ^{ab}	1.37 ± 0.036 ^{ad}	1.36 ± 0.043 ^{a}	0.77 ± 0.019 ^{bc}	0.79 ± 0.022 ^{a}	1.25 ± 0.041 ^{b}	0.89 ± 0.038 ^{ab}	0.59 ± 0.030 ^{b}	0.61 ± 0.033 ^{ac}
North West	3.71 ± 0.18 ^{a}	3.60 ± 0.093 ^{a}	1.25 ± 0.044 ^{bcd}	1.25 ± 0.022 ^{a}	0.66 ± 0.024 ^{ab}	0.71 ± 0.012 ^{ab}	1.28 ± 0.051 ^{b}	0.82 ± 0.021 ^{b}	0.46 ± 0.038 ^{ab}	0.55 ± 0.017 ^{c}
Northern Cape	5.92 ± 0.41 ^{b}	4.74 ± 0.28 ^{b}	1.17 ± 0.097 ^{bcd}	0.95 ± 0.07 ^{b}	0.97 ± 0.052 ^{c}	0.59 ± 0.038 ^{b}	2.33 ± 0.11 ^{c}	0.76 ± 0.063 ^{ab}	1.16 ± 0.081 ^{c}	0.47 ± 0.056 ^{bc}

All results are expressed as mean ± SD.
^{abcd} Subscripts are used to indicate that the values in each column which have different letters are significantly different (p <0.05).

Table 4.13 display the results of major spermatozoa defects per province at a THI 60 days prior to semen collection. The values of major spermatozoa defects per provinces were higher in the THI 3 group compared to the THI 2 group. The province with the lowest values for all the major spermatozoa defects was KwaZulu-Natal.

Table 4.14 present the effect of breed on semen major spermatozoa defects at the THI when semen was collection. It was observed that spermatozoa with mid-piece abnormalities, tail abnormalities, abnormal loose heads and pyriform heads had highest major spermatozoa defect values during THI 2. This observation was the opposite of what was observed between provinces in Table 4.13. The breed with the lowest values over all the different major spermatozoa defects was Drakensberger. It was observed that Simbra semen had higher ($P < 0.05$) values over all major spermatozoa defects.

Table 4.13 Summary of fresh semen major spermatozoa defects (%) with significant differences between THI groups during spermatozoa production per province over seven years.

	Proximal Protoplasmic droplets	Mid-piece abnormalities		Tail abnormalities		Pyriform heads		Other major defects	
	THI 3	THI 2	THI 3	THI 2	THI 3	THI 2	THI 3	THI 2	THI 3
Eastern Cape	4.88 ± 0.75 ^{abc}	1.013 ± 0.061 ^{a}	1.69 ± 0.20 ^{ab}	0.58 ± 0.036 ^{a}	0.83 ± 0.085 ^{ab}	0.80 ± 0.068 ^{a}	0.81 ± 0.20 ^{abc}	0.69 ± 0.05 ^{c}	0.50 ± 0.14 ^{ab}
Free State	4.47 ± 0.15 ^{bc}	1.29 ± 0.028 ^{b}	1.24 ± 0.038 ^{ab}	0.70 ± 0.016 ^{ab}	0.77 ± 0.018 ^{ab}	0.90 ± 0.029 ^{a}	0.92 ± 0.039 ^{abc}	0.48 ± 0.023 ^{ab}	0.49 ± 0.03 ^{a}
Gauteng	4.59 ± 0.25 ^{bc}	1.35 ± 0.054 ^{b}	1.29 ± 0.063 ^{ab}	0.64 ± 0.032 ^{a}	0.75 ± 0.030 ^{ab}	0.91 ± 0.056 ^{a}	1.17 ± 0.059 ^{a}	0.54 ± 0.045 ^{abc}	0.73 ± 0.049 ^{b}
Kwazulu-Natal	4.29 ± 0.36 ^{abc}	1.22 ± 0.098 ^{ab}	1.56 ± 0.086 ^{a}	0.74 ± 0.053 ^{ab}	0.78 ± 0.043 ^{ab}	0.66 ± 0.13 ^{a}	0.69 ± 0.095 ^{b}	0.26 ± 0.074 ^{a}	0.46 ± 0.072 ^{ab}
Limpopo	4.031 ± 0.13 ^{ab}	1.29 ± 0.057 ^{ab}	1.32 ± 0.03 ^{a}	0.76 ± 0.033 ^{ab}	0.73 ± 0.014 ^{ab}	0.93 ± 0.064 ^{a}	0.87 ± 0.031 ^{b}	0.55 ± 0.047 ^{abc}	0.57 ± 0.023 ^{ab}
Mpumalanga	4.028 ± 0.19 ^{ab}	1.40 ± 0.042 ^{b}	1.38 ± 0.046 ^{a}	0.72 ± 0.024 ^{ab}	0.80 ± 0.022 ^{a}	0.98 ± 0.04 ^{a}	1.089 ± 0.044 ^{ac}	0.62 ± 0.034 ^{bc}	0.70 ± 0.036 ^{b}
North West	3.56 ± 0.10 ^{a}	1.23 ± 0.035 ^{ab}	1.25 ± 0.025 ^{ab}	0.69 ± 0.021 ^{ab}	0.70 ± 0.012 ^{b}	0.96 ± 0.036 ^{a}	0.92 ± 0.025 ^{bc}	0.51 ± 0.029 ^{abc}	0.55 ± 0.02 ^{ab}
Northern Cape	5.56 ± 0.32 ^{c}	1.077 ± 0.093 ^{ab}	1.0053 ± 0.078 ^{b}	0.89 ± 0.057 ^{b}	0.67 ± 0.036 ^{ab}	2.10 ± 0.083 ^{b}	0.80 ± 0.078 ^{abc}	0.82 ± 0.08 ^{c}	0.67 ± 0.06 ^{ab}

All results are expressed as mean ± SD.

^{abc} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table 4.14 Summary of fresh semen major spermatozoa defects (%) between THI groups during semen collection per breed over a period of seven years.

	Proximal Protoplasmic droplets		Mid-piece abnormalities		Tail abnormalities	Abnormal Loose heads	Pyriform heads		Other major defects	
	THI 2	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**
Beefmaster	3.88 ± 0.14 ^{ac}	4.46 ± 0.14 ^{a}	1.27 ± 0.034 ^{b}	1.26 ± 0.035 ^{ac}	0.75 ± 0.018 ^{abc}	1.72 ± 0.093 ^{a}	1.016 ± 0.04 ^{a}	0.79 ± 0.033 ^{ac}	0.61 ± 0.028 ^{abc}	0.58 ± 0.027 ^{ab}
Bonsmara	3.55 ± 0.15 ^{ac}	3.64 ± 0.089 ^{c}	1.50 ± 0.036 ^{a}	1.40 ± 0.021 ^{a}	0.76 ± 0.020 ^{ab}	1.27 ± 0.059 ^{b}	0.93 ± 0.044 ^{a}	0.76 ± 0.022 ^{a}	0.49 ± 0.031 ^{abc}	0.61 ± 0.016 ^{ab}
Boran	4.75 ± 0.41 ^{ab}	3.66 ± 0.33 ^{ac}	1.26 ± 0.10 ^{abc}	1.28 ± 0.08 ^{abc}	0.71 ± 0.058 ^{abc}	1.23 ± 0.23 ^{ab}	0.98 ± 0.13 ^{ab}	0.73 ± 0.076 ^{ac}	0.80 ± 0.091 ^{ab}	0.49 ± 0.06 ^{ab}
Brahman	4.14 ± 0.17 ^{ab}	4.54 ± 0.16 ^{ab}	1.20 ± 0.042 ^{bc}	1.42 ± 0.04 ^{a}	0.64 ± 0.023 ^{bc}	1.28 ± 0.10 ^{ab}	1.022 ± 0.055 ^{a}	0.74 ± 0.041 ^{ac}	0.46 ± 0.036 ^{abc}	0.50 ± 0.031 ^{b}
Drakensberger	3.087 ± 0.32 ^{ac}	4.017 ± 0.35 ^{abc}	0.90 ± 0.076 ^{c}	0.84 ± 0.089 ^{b}	0.57 ± 0.042 ^{c}	1.32 ± 0.27 ^{ab}	0.97 ± 0.09 ^{a}	0.83 ± 0.073 ^{ac}	0.33 ± 0.066 ^{c}	0.52 ± 0.069 ^{ab}
Limousin	3.09 ± 0.50 ^{abc}	3.36 ± 0.32 ^{abc}	1.28 ± 0.12 ^{abc}	1.22 ± 0.08 ^{abc}	0.76 ± 0.064 ^{abc}	1.33 ± 0.22 ^{ab}	1.33 ± 0.14 ^{abc}	0.77 ± 0.082 ^{abc}	0.35 ± 0.10 ^{abc}	0.56 ± 0.062 ^{ab}
Nguni	3.88 ± 0.90 ^{abc}	2.38 ± 0.45 ^{c}	1.61 ± 0.24 ^{abc}	1.016 ± 0.098 ^{abc}	0.60 ± 0.14 ^{abc}	1.63 ± 0.22 ^{ab}	1.00 ± 0.25 ^{abc}	0.83 ± 0.095 ^{abc}	0.33 ± 0.22 ^{abc}	0.34 ± 0.076 ^{b}
Simbra	5.098 ± 0.23 ^{b}	5.38 ± 0.18 ^{b}	1.38 ± 0.059 ^{ab}	1.31 ± 0.045 ^{ac}	0.84 ± 0.031 ^{a}	1.62 ± 0.10 ^{ab}	1.74 ± 0.063 ^{c}	1.18 ± 0.039 ^{b}	0.69 ± 0.049 ^{a}	0.69 ± 0.035 ^{a}
Simmentaler	2.93 ± 0.21 ^{c}	3.55 ± 0.13 ^{c}	0.98 ± 0.05 ^{c}	1.099 ± 0.031 ^{bc}	0.65 ± 0.027 ^{bc}	1.61 ± 0.065 ^{ab}	1.46 ± 0.054 ^{bc}	0.90 ± 0.027 ^{c}	0.43 ± 0.043 ^{bc}	0.50 ± 0.024 ^{b}

All results are expressed as mean ± SD.

^{abc} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Individual major defects for each breed at the temperature 60 days prior to semen collection can be observed in Table 4.15. Bull semen at THI 3 had the highest values in different major spermatozoa defects. More major spermatozoa defects were observed at THI 3 at semen collection (Table 4.14) which differed from the number of major defects observed per breed during spermatozoa production in Table 4.15. Tail abnormalities and abnormal loose heads had the higher ($P < 0.05$) values at a THI 3 in Table 4.15, but in Table 4.14 they were higher at THI 2.

Table 4.15 Summary of major spermatozoa defects (%) with significant differences in fresh semen at different THI groups during semen production per breed over a period of seven years.

	Proximal Protoplasmic droplets		Mid-piece abnormalities		Tail abnormalities		Pyriform heads		Other major defects	
	THI 2	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**
Beefmaster	4.19 ± 0.14 ^{ab}	4.27 ± 0.15 ^{bc}	1.29 ± 0.035 ^{ab}	1.22 ± 0.038 ^{ac}	0.71 ± 0.02 ^{ab}	0.74 ± 0.018 ^{ab}	0.95 ± 0.035 ^{b}	0.86 ± 0.037 ^{a}	0.56 ± 0.028 ^{ab}	0.60 ± 0.03 ^{ab}
Bonsmara	3.46 ± 0.14 ^{ac}	3.68 ± 0.095 ^{b}	1.47 ± 0.035 ^{a}	1.41 ± 0.023 ^{b}	0.74 ± 0.02 ^{ab}	0.75 ± 0.011 ^{ab}	0.81 ± 0.038 ^{b}	0.79 ± 0.025 ^{a}	0.55 ± 0.028 ^{ab}	0.60 ± 0.018 ^{ab}
Boran	4.22 ± 0.31 ^{abc}	3.79 ± 0.46 ^{abc}	1.30 ± 0.079 ^{abcd}	1.29 ± 0.11 ^{abc}	0.62 ± 0.046 ^{ab}	0.74 ± 0.052 ^{ab}	0.84 ± 0.084 ^{b}	0.78 ± 0.12 ^{ac}	0.62 ± 0.065 ^{ab}	0.61 ± 0.086 ^{ab}
Brahman	4.29 ± 0.18 ^{ab}	4.47 ± 0.17 ^{c}	1.23 ± 0.045 ^{bd}	1.34 ± 0.04 ^{ab}	0.69 ± 0.027 ^{ab}	0.69 ± 0.019 ^{ab}	0.79 ± 0.052 ^{b}	0.90 ± 0.043 ^{ac}	0.49 ± 0.037 ^{b}	0.46 ± 0.032 ^{a}
Drakensberger	3.41 ± 0.27 ^{ac}	3.83 ± 0.46 ^{abc}	0.92 ± 0.064 ^{c}	0.74 ± 0.12 ^{c}	0.58 ± 0.038 ^{a}	0.57 ± 0.059 ^{ab}	0.92 ± 0.062 ^{b}	0.85 ± 0.11 ^{ac}	0.38 ± 0.053 ^{b}	0.50 ± 0.099 ^{ab}
Limousin	3.18 ± 0.43 ^{abc}	3.65 ± 0.47 ^{abc}	1.42 ± 0.10 ^{abd}	1.12 ± 0.12 ^{abc}	0.63 ± 0.059 ^{ab}	0.68 ± 0.056 ^{ab}	1.037 ± 0.097 ^{ab}	0.83 ± 0.15 ^{abc}	0.64 ± 0.083 ^{ab}	0.60 ± 0.094 ^{ab}
Nguni	2.46 ± 0.48 ^{ac}	2.98 ± 0.68 ^{abc}	1.19 ± 0.12 ^{abcd}	1.00 ± 0.14 ^{abc}	0.61 ± 0.076 ^{ab}	0.74 ± 0.061 ^{ab}	0.82 ± 0.12 ^{b}	0.93 ± 0.15 ^{abc}	0.16 ± 0.11 ^{b}	0.50 ± 0.10 ^{ab}
Simbra	5.063 ± 0.20 ^{b}	5.61 ± 0.22 ^{a}	1.44 ± 0.05 ^{ab}	1.24 ± 0.054 ^{abc}	0.79 ± 0.029 ^{b}	0.81 ± 0.025 ^{a}	1.44 ± 0.05 ^{a}	1.37 ± 0.048 ^{b}	0.72 ± 0.041 ^{a}	0.67 ± 0.042 ^{b}
Simmentaler	3.18 ± 0.16 ^{c}	3.67 ± 0.17 ^{bc}	1.046 ± 0.038 ^{cd}	1.085 ± 0.039 ^{c}	0.65 ± 0.022 ^{ab}	0.68 ± 0.019 ^{b}	1.0024 ± 0.037 ^{b}	1.099 ± 0.036 ^{c}	0.46 ± 0.031 ^{b}	0.52 ± 0.031 ^{ab}

All results are expressed as mean ± SD.
^{abcd} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

The summary in Table 4.16 displays the results between major spermatozoa defects and seasons when semen was collected at different THI groups. The THI 3 had higher major spermatozoa defect values for mid-piece abnormalities, tail abnormalities, abnormal loose heads and other major defects. When semen was collected in spring lower values of major spermatozoa defects were observed. Semen collected in autumn were observed to have higher values of major spermatozoa defects. All the major sperm defects, except mid-piece abnormalities had no values during summer at THI 2, due to the low number of observations for statistical analysis that could not be done.

Table 4.16 Summary of major spermatozoa defects (%) in fresh semen with temperature as a co-variate during semen collection per seasons over seven years.

	Proximal Protoplasmic droplets	Mid-piece Abnormalities	Pyriform heads	Other major defects
	THI 3	**THI 3**	**THI 2**	**THI 3**
Autumn	4.21 ± 0.12 [ab]	1.29 ± 0.029 [ab]	0.89 ± 0.063 [a]	0.67 ± 0.022 [b]
Spring	3.86 ± 0.07 [b]	1.32 ± 0.017 [a]	0.83 ± 0.083 [a]	0.54 ± 0.013 [a]
Summer	4.37 ± 0.15 [a]	1.19 ± 0.035 [b]	-	0.56 ± 0.028 [a]
Winter	3.46 ± 0.25 [b]	1.18 ± 0.06 [ab]	1.20 ± 0.023 [b]	0.52 ± 0.046 [a]

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table 4.17 shows major spermatozoa defects in different seasons with temperature as co-variate 60 days prior to semen collection. Only two major spermatozoa defects, mid-piece abnormalities and tail abnormalities had higher values in the THI 3 group than the remaining defects that was high at a THI 2, between seasons. Autumn was observed to have the highest values of major spermatozoa defects (Table 4.16). It was observed that winter had lower values of major spermatozoa defects. The values for major spermatozoa defects; tail abnormalities, abnormal loose heads, pyriform heads and other major defects, are missing due to lack of sufficient number of samples for statistical analysis.

Table 4.17 Summary of major spermatozoa defects (%) in fresh semen when temperature was used as co-variate during spermatozao production per season over a period of seven years.

	Proximal Protoplasmic droplets	Mid-piece Abnormalities	Tail abnormalities	Pyriform heads	Other major defects	
	THI 3	**THI 2**	**THI 2**	**THI 2**	**THI 3**	**THI 3**
Autumn	3.97 ± 0.11 [a]	1.16 ± 0.035 [a]	0.75 ± 0.02 [b]	1.20 ± 0.033 [a]	1.12 ± 0.025 [b]	1.11 ± 0.031 [a]
Spring	3.90 ± 0.11 [a]	1.28 ± 0.081 [ab]	0.71 ± 0.046 [ab]	0.97 ± 0.095 [ab]	0.82 ± 0.027 [a]	0.93 ± 0.03 [b]
Summer	4.48 ± 0.13 [b]	0.40 ± 1.19 [ab]			0.81 ± 0.034 [a]	1.14 ± 0.034 [a]
Winter	3.77 ± 0.19 [a]	1.30 ± 0.019 [b]	0.68 ± 0.011 [a]	0.88 ± 0.02 [b]	0.88 ± 0.043 [a]	0.92 ± 0.047 [b]

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

4.3 Frozen-thawed semen results

Table 4.18 shows semen characteristics and provinces when temperature at semen collection was considered. Only major spermatozoa defects had significant differences between Free State and Limpopo (P <0.05).

Table 4.18 Frozen-thawed semen characteristics per province over a period of seven years.

	% Live sperm	Linear movement (%)	Non-linear movement (%)	Count/ml	Viability (%)	Major defects (%)	Minor defects (%)
Eastern Cape	53.63 ± 1.15	48.60 ± 1.20	5.01 ± 0.25	78.25 ± 2.24	49.26 ± 1.44	13.86 ± 0.75 [ab]	6.10 ± 0.40
Free State	52.86 ± 0.48	47.51 ± 0.50	5.28 ± 0.10	80.37 ± 0.94	49.23 ± 0.59	11.67 ± 0.31 [a]	5.81 ± 0.17
Gauteng	52.56 ± 0.98	47.80 ± 1.01	5.22 ± 0.21	78.49 ± 1.90	47.59 ± 1.26	12.44 ± 0.63 [ab]	5.62 ± 0.33
Kwazulu-Natal	53.62 ± 1.23	48.37 ± 1.28	5.21 ± 0.26	80.50 ± 2.39	51.22 ± 1.66	11.50 ± 0.79 [ab]	5.92 ± 0.42
Limpopo	50.63 ± 0.76	45.05 ± 0.79	5.78 ± 0.16	75.99 ± 1.48	48.063 ± 0.97	14.41 ± 0.49 [b]	6.012 ± 0.26
Mpumalanga	52.07 ± 0.70	46.73 ± 0.73	5.32 ± 0.15	79.42 ± 1.36	48.51 ± 0.88	12.97 ± 0.45 [ab]	6.13 ± 0.24
North West	52.28 ± 0.59	47.74 ± 0.60	5.12 ± 0.12	80.18 ± 1.12	48.35 ± 0.71	12.79 ± 0.37 **[ab]**	6.01 ± 0.20

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column which have different letters are significantly different (p <0.05).

Table 4.19 display the significant differences of semen characteristics between breeds. Holstein semen had significantly higher values for linear sperm movement and significantly lower minor defects. Composite breeds had significantly higher % live sperm, sperm count/ml, and sperm viability, with significantly lower major defects.

Table 4.19 Frozen-thawed semen characteristics per breed over seven years.

	Breed type	% Live sperm	Linear movement (%)	Non-Linear movement (%)	Count/ml	Viability (%)	Major Defects (%)	Minor Defects (%)
Beefmaster	Composite	50.73 ± 0.72 [a]	45.41 ± 0.75 [a]	5.33 ± 0.15	81.89 ± 1.40 [ab]	48.84 ± 0.96 [ab]	11.98 ± 0.47 [abc]	5.54 ± 0.25 [ab]
Bonsmara	Composite	52.09 ± 0.56 [ab]	46.95 ± 0.57 [ab]	5.28 ± 0.12	85.29 ± 1.07 [a]	50.36 ± 0.67 [a]	13.14 ± 0.36 [cb]	6.27 ± 0.19 [b]
Boran	Composite	54.27 ± 0.78 [b]	49.08 ± 0.81 [b]	5.27 ± 0.17	77.99 ± 1.51 [b]	49.97 ± 0.91 [ab]	10.60 ± 0.51 [a]	5.93 ± 0.27 [ab]
Brahman	Bos Indicus	52.53 ± 0.54 [ab]	47.15 ± 0.55 [ab]	5.45 ± 0.11	71.89 ± 1.04	46.38 ± 0.68 [b]	13.89 ± 0.34 [c]	6.04 ± 0.83 [ab]
Holstein	Bos Taurus	54.77 ± 1.09 [ab]	50.08 ± 1.12 [b]	4.61 ± 0.23	81.95 ± 2.10 [ab]	47.23 ± 1.32 [ab]	11.16 ± 0.70 [ab]	4.75 ± 0.37 [a]
Simbra	Composite	51.73 ± 0.67 [ab]	47.05 ± 0.68 [ab]	5.29 ± 0.14	80.27 ± 1.27 [ab]	49.28 ± 0.84 [ab]	12.55 ± 0.42 [abc]	6.07 ± 0.22 [ab]

All results are expressed as mean ± SD.
[abc] Subscripts are used to indicate that the values in each column with different letters are significantly different (p <0.05).

Figure 4.9 summarises the significant differences between semen characteristics and THI groups at semen collection. The only significant difference was observed between THI 2 and THI 3 for non-linear sperm movement. The highest values were found in THI 3 group.

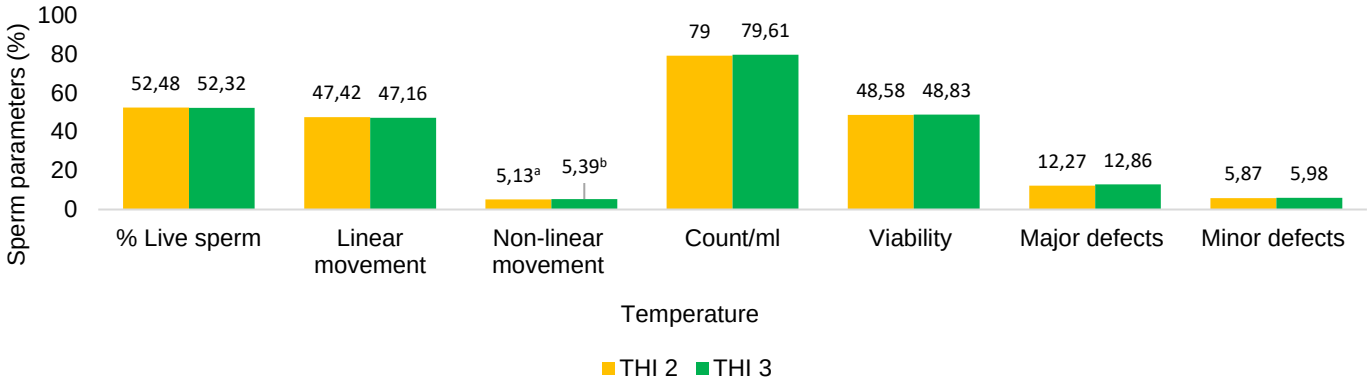

Figure 4.9 Frozen-thawed semen characteristics per THI at semen collection over a period of seven years (a and b were used to indicate that the column with different letters are significantly different (p <0.05)).

Figure 4.10 displays the means of semen characteristics per THI group 60 days prior to semen collection. It was observed that the highest values were observed at THI 2 that differed from figure 4.9. The significant differences in figure 4.10 increased by two compared to figure 4.9. Temperature during semen production had significant effect (P <0.05) on sperm count, major and minor sperm defects.

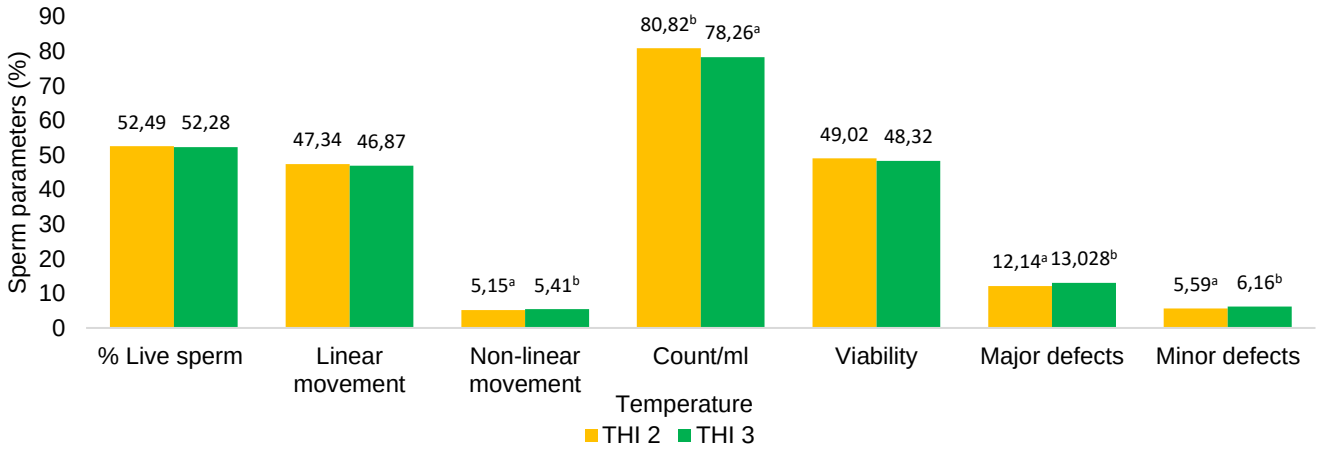

Figure 4.10 Semen characteristics in frozen-thawed semen at each THI group when semen production occurs (a and b were used to indicate that the column with different letters are significantly different (p <0.05)).

Table 4.20 shows the effects of season when semen collection occurred on semen characteristics. The highest values for semen characteristics that were collected in autumn and the lowest values when semen were collected in summer. Semen quality in spring semen collection was significantly (P <0.05) higher in spermatozoa count/ml and spermatozoa viability with significantly (P <0.05) lower minor defects.

Table 4.20 Frozen-thawed semen characteristics per season when semen collection occurred, over seven years.

	% Live sperm	Linear movement (%)	Non-linear movement (%)	Count/mL	Viability (%)	Major defects (%)	Minor defects (%)
Autumn	52.48 ± 0.60	47.69 ± 0.59	5.50 ± 0.12 [b]	77.26 ± 1.11 [b]	49.45 ± 0.65 [ab]	13.074 ± 0.37	6.57 ± 0.20 [b]
Spring	52.86 ± 0.45	47.46 ± 0.48	5.40 ± 0.099 [b]	81.79 ± 0.89 [a]	50.13 ± 0.59 [a]	12.82 ± 0.30	5.48 ± 0.16 [a]
Summer	50.61 ± 0.75	45.59 ± 0.77	5.30 ± 0.16 [ab]	76.53 ± 1.44 [b]	46.30 ± 0.95 [b]	12.57 ± 0.48	6.12 ± 0.25 [ab]
Winter	52.54 ± 0.52	47.46 ± 0.54	4.98 ± 0.11 [a]	79.42 ± 1.01 [ab]	47.47 ± 0.66 [b]	12.078 ± 0.34	5.90 ± 0.18 [ab]

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table 4.21 shows the effect of season during spermatozoa production 60 days prior to semen collection on semen characteristics. It was observed that the lowest values were in summer just as observed in Table 4.20 and the highest values were observed when spermatozoa production occurred in winter. The percentage minor spermatozoa defects were significantly ($P < 0.05$) lower during winter than autumn when considering the season at sperm production.

Table 4.21 Frozen-thawed semen characteristics per season when semen production occurred over a period of seven years.

	% Live sperm	Linear movement (%)	Non-linear movement (%)	Count/mL	Viability (%)	Major defects (%)	Minor defects (%)
Autumn	52.71 ± 0.55	47.49 ± 0.57	5.22 ± 0.12	79.22 ± 1.032	48.67 ± 0.66	12.92 ± 0.36	6.31 ± 0.19 [a]
Spring	51.73 ± 0.66	46.33 ± 0.69	5.40 ± 0.15	77.67 ± 1.27	48.14 ± 0.81	13.56 ± 0.43	5.95 ± 0.23 [ab]
Summer	51.65 ± 0.72	46.21 ± 0.75	5.44 ± 0.16	79.14 ± 1.33	48.60 ± 0.92	12.021 ± 0.47	6.17 ± 0.24 [ab]
Winter	52.73 ± 0.45	47.49 ± 0.47	5.24 ± 0.099	80.36 ± 0.87	48.81 ± 0.59	12.25 ± 0.29	5.51 ± 0.16 [b]

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

The significant differences between semen characteristics or individual major sperm defects between years can be observed in addendum C1. The results of individual minor sperm defects per province, breed, THI, season and year were displayed in addendum C2.

Table 4.22 display the significant differences between province of major spermatozoa defects. The province with the lowest major spermatozoa defects percentage was KwaZulu-Natal and the province with the highest percentages of major spermatozoa defects was Limpopo. Limpopo with higher temperatures had significantly higher mid-piece abnormalities ($P < 0.05$) than the Free State.

Table 4.22 Major frozen-thawed spermatozoa defects (%) per province over a period of seven years.

	Proximal Protoplasmic droplets	Mid-piece abnormalities	Tail abnormalities	Pyriform heads	Other major defects
Eastern Cape	2.59 ± 0.39	1.38 ± 0.11 [ab]	0.68 ± 0.050	0.62 ± 0.13	1.017 ± 0.12
Free State	2.75 ± 0.17	1.087 ± 0.044 [a]	0.67 ± 0.022	0.86 ± 0.058	0.93 ± 0.051
Gauteng	2.85 ± 0.31	1.091 ± 0.09 [ab]	0.70 ± 0.045	1.02 ± 0.11	1.00 ± 0.10
Kwazulu-Natal	2.38 ± 0.45	1.21 ± 0.11 [ab]	0.66 ± 0.057	0.83 ± 0.17	0.84 ± 0.13
Limpopo	3.081 ± 0.26	1.45 ± 0.069 [b]	0.75 ± 0.034	0.76 ± 0.093	1.12 ± 0.077
Mpumalanga	2.98 ± 0.24	1.14 ± 0.064 [ab]	0.74 ± 0.031	0.91 ± 0.085	1.15 ± 0.071
North West	2.80 ± 0.20	1.20 ± 0.053 [ab]	0.68 ± 0.026	1.024 ± 0.066	0.90 ± 0.06

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table 4.23 shows the significant differences of major sperm defects between breeds. The breed with the least damaged semen that had the lowest sperm defects values was Beefmaster.

Table 4.23 Major frozen-thawed spermatozoa defects (%) per breed over seven years.

	Breed type	Proximal Protoplasmic droplets	Mid-piece abnormalities	Tail abnormalities	Pyriform heads	Other major defects
Beefmaster	Composite	3.22 ± 0.26	1.063 ± 0.066 [ac]	0.63 ± 0.032	0.71 ± 0.084 [a]	1.00 ± 0.069 [ab]
Bonsmara	Composite	2.35 ± 0.19	1.33 ± 0.050 [ab]	0.71 ± 0.024	0.72 ± 0.069 [a]	1.16 ± 0.055 [a]
Boran	Composite	2.84 ± 0.28	0.94 ± 0.073 [c]	0.69 ± 0.036	0.73 ± 0.10 [a]	0.78 ± 0.093 [b]
Brahman	Bos Indicus	3.15 ± 0.18	1.41 ± 0.049 [b]	0.73 ± 0.024	0.84 ± 0.068 [a]	1.0061 ± 0.057 [ab]
Holstein	Bos Taurus	2.39 ± 0.36	0.91 ± 0.10 [c]	0.74 ± 0.054	1.00 ± 0.11 [ab]	0.80 ± 0.12 [ab]
Simbra	Composite	2.83 ± 0.23	1.04 ± 0.06 [c]	0.68 ± 0.028	1.24 ± 0.067 [b]	0.88 ± 0.065 [ab]

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column with different letters are significantly different ($p < 0.05$).

Figure 4.11 show that the higher values of major spermatozoa defects were mostly found in the THI 3 group, but no significant differences could be observed between THI groups.

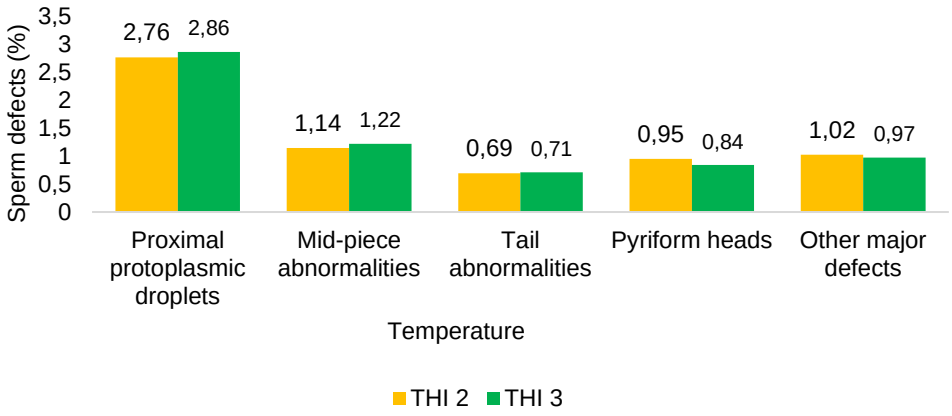

Figure 4.11 Frozen-thawed semen major spermatozoa defects per THI group during semen collection (a and b were used to indicate that the column which have different letters are significantly different (p <0.05)).

Figure 4.12 displays the effect of temperature on sperm major defects 60 days before semen collection. It was observed that the higher values per major sperm defects were in THI 3, with only one significant difference between THI 2 and THI 3 for tail abnormalities.

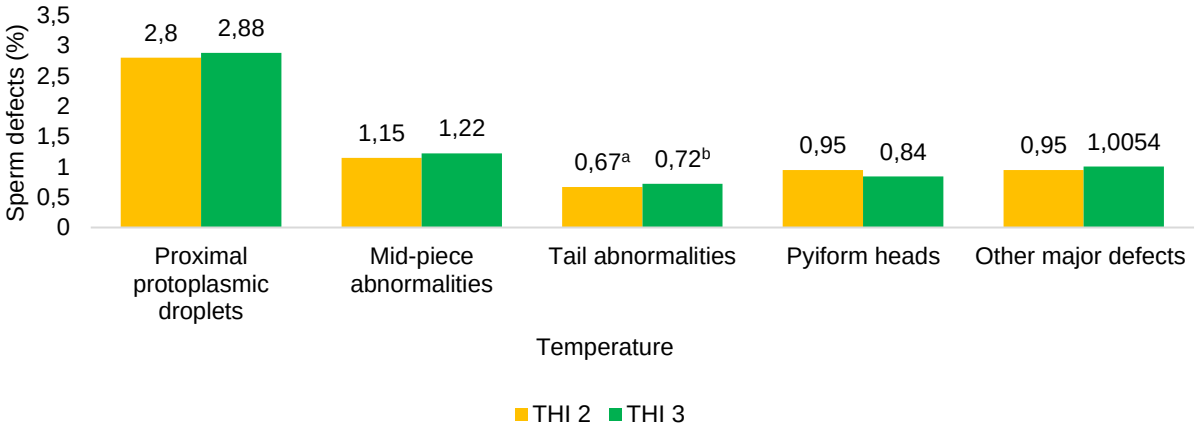

Figure 4.12 Frozen-thawed major spermatozoa defects per THI group at spermatozoa production (a and b were used to indicate that the column which have different letters are significantly different (p <0.05)).

Figure 4.13 indicates the effect of season and temperature on major spermatozoa defects at semen collection. Semen collection in autumn presented major spermatozoa defects over all breeds were higher compared to all other seasons. The season when semen was collected significantly affected only pyriform heads in frozen-thawed semen, that was found to be significantly higher (P <0.05) in winter semen collection compared to spring.

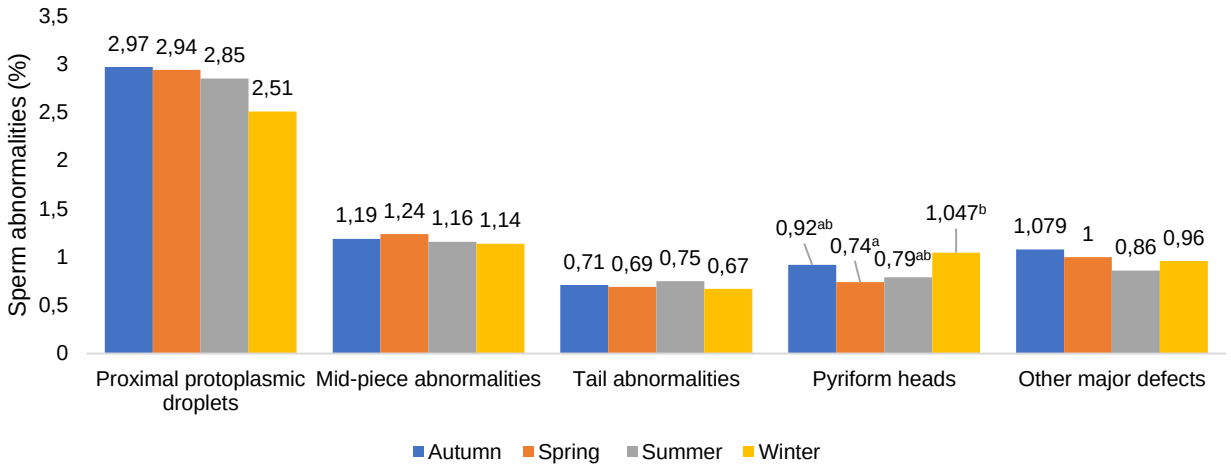

Figure 4.13 Major spermatozoa defects in frozen-thawed semen collected per season over seven years (a and b were used to indicate that the column which have different letters are significantly different (p <0.05)).

Figure 4.14 shows the effect of season and significant differences between major spermatozoa defects 60 days before semen collection. Spring had varied major spermatozoa defect values observed. The season when spermatozoa production occurred affected the percentage of mid-piece abnormalities that were observed to be significantly (P < 0.05) higher at spring production and pyriform heads being significantly (P <0.05) higher at winter spermatozoa production.

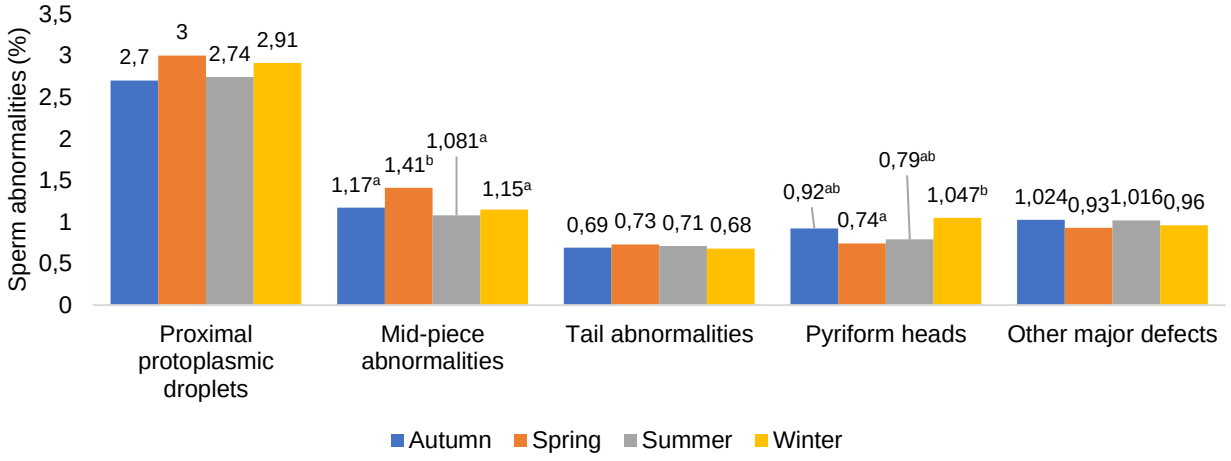

Figure 4.14 Major sperm defects in frozen-thawed semen per season when spermatozoa production occurred over a seven-year period (a and b were used to indicate that the column with different letters are significantly different (p <0.05)).

Table 4.24 indicates the significant differences between provinces and the major defects in a specific THI group during semen collection. It was observed that Eastern Cape had lower values for pyriform heads, that does not differ significantly from the other provinces, except Limpopo. The province with higher values was observed to be Limpopo for mid-piece abnormalities.

Table 4.24 Summary of major spermatozoa defects (%) with significant differences in frozen-thawed semen at THI 2 group during semen collection per province over seven years.

	Mid-piece abnormalities	Pyriform heads
	THI 2	**THI 2**
Eastern Cape	1.36 ± 0.10 ^{ab}	0.63 ± 0.12 ^{a}
Free State	1.017 ± 0.059 ^{b}	0.89 ± 0.073 ^{ab}
Gauteng	0.89 ± 0.13 ^{b}	1.016 ± 0.17 ^{ab}
Kwazulu-Natal	1.077 ± 0.15 ^{b}	0.73 ± 0.17 ^{ab}
Limpopo	1.95 ± 0.19 ^{a}	1.23 ± 0.25 ^{ab}
Mpumalanga	1.066 ± 0.074 ^{b}	1.033 ± 0.092 ^{ab}
North West	1.30 ± 0.089 ^{ab}	1.31 ± 0.11 ^{b}

All results are expressed as mean ± SD.
^{ab} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table 4.25 shows the effect of temperature on major sperm defects in different between provinces and major defects in THI group 60 days prior to semen collection. It was observed that Eastern Cape had the lowest major sperm defect values. The provinces; Limpopo, Mpumalanga and North West had similar values of major spermatozoa defects.

Table 4.25 Summary of major spermatozoa defects (%) with significant differences in frozen-thawed semen at THI groups during spermatozoa production per province over seven years.

	Mid-piece abnormalities	Pyriform heads
	THI 3	**THI 2**
Eastern Cape	1.36 ± 0.20 $^{\mathbf{ab}}$	0.63 ± 0.11 $^{\mathbf{a}}$
Free State	1.11 ± 0.068 $^{\mathbf{b}}$	0.89 ± 0.07 $^{\mathbf{ab}}$
Gauteng	1.24 ± 0.15 $^{\mathbf{ab}}$	1.016 ± 0.16 $^{\mathbf{ab}}$
Kwazulu-Natal	1.24 ± 0.17 $^{\mathbf{ab}}$	0.73 ± 0.16 $^{\mathbf{ab}}$
Limpopo	1.51 ± 0.085 $^{\mathbf{a}}$	1.23 ± 0.23 $^{\mathbf{ab}}$
Mpumalanga	1.25 ± 0.11 $^{\mathbf{ab}}$	1.033 ± 0.087 $^{\mathbf{ab}}$
North West	1.11 ± 0.069 $^{\mathbf{b}}$	1.31 ± 0.10 $^{\mathbf{b}}$

All results are expressed as mean ± SD.
^{ab} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table 4.26 display the comparison and significant difference between breeds concerning major spermatozoa defects in the specific THI group during semen collection. It was observed that Simbra semen had the highest values for major spermatozoa defects. Most of the major spermatozoa defects had higher values in the THI 3 group.

Table 4.26 Summary of major spermatozoa defects (%) in frozen-thawed semen per breed in different THI groups during semen collection over seven years.

	Mid-piece abnormalities		Pyriform heads		Other major defects
	THI 2	THI 3	THI 2	THI 3	THI 3
Beefmaster	0.97 ± 0.08 [b]	1.15 ± 0.10 [ab]	0.88 ± 0.096 [a]	0.51 ± 0.13 [a]	0.11 ± 0.066 [a]
Bonsmara	1.28 ± 0.070 [ab]	1.35 ± 0.068 [ab]	0.76 ± 0.086 [a]	0.69 ± 0.097 [ab]	0.096 ± 0.048 [a]
Boran	0.93 ± 0.089 [b]	0.95 ± 0.11 [a]	0.79 ± 0.11 [a]	0.65 ± 0.16 [ab]	0.23 ± 0.090 [ab]
Brahman	1.42 ± 0.073 [a]	1.40 ± 0.064 [b]	0.89 ± 0.10 [a]	0.81 ± 0.088 [ab]	0.19 ± 0.047 [a]
Holstein	0.71 ± 0.18 [b]	0.97 ± 0.12 [ab]	0.89 ± 0.18 [ab]	1.033 ± 0.14 [ab]	0.19 ± 0.09 [ab]
Simbra	0.98 ± 0.091 [b]	1.067 ± 0.079 [ab]	1.47 ± 0.097 [b]	1.13 ± 0.087 [b]	0.46 ± 0.055 [b]

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

The effect of breed on major spermatozoa defects at different THI 60 days before semen collection is displayed in Table 4.27. Boran semen was observed to have the lowest major spermatozoa defect values in the last column and Brahman semen had the highest major spermatozoa mid-piece abnormalities at a THI 3.

Table 4.27 Summary of major spermatozoa defects (%) with significant differences in frozen-thawed semen THI groups during spermatozoa production per breed over seven years.

	Proximal Protoplasmic droplets	Mid-piece abnormalities		Pyriform heads		Other major defects
	THI 3	THI 2	THI 3	THI 2	THI 3	THI 3
Beefmaster	3.92 ± 0.40 [a]	1.012 ± 0.079 [b]	1.19 ± 0.12 [ab]	0.88 ± 0.091 [a]	0.51 ± 0.13 [a]	1.094 ± 0.10 [ab]
Bonsmara	2.36 ± 0.24 [b]	1.39 ± 0.073 [a]	1.27 ± 0.072 [ab]	0.76 ± 0.082 [a]	0.69 ± 0.099 [ab]	1.25 ± 0.075 [a]
Boran	3.00 ± 0.34 [ab]	0.97 ± 0.10 [b]	0.92 ± 0.11 [b]	0.79 ± 0.11 [a]	0.65 ± 0.16 [ab]	0.75 ± 0.12 [b]
Brahman	2.94 ± 0.22 [ab]	1.26 ± 0.069 [ab]	1.50 ± 0.069 [a]	0.89 ± 0.095 [a]	0.81 ± 0.090 [ab]	0.94 ± 0.07 [ab]
Holstein	2.38 ± 0.43 [ab]	0.95 ± 0.15 [ab]	0.92 ± 0.14 [b]	0.89 ± 0.17 [ab]	1.033 ± 0.14 [ab]	0.75 ± 0.14 [ab]
Simbra	3.079 ± 0.28 [ab]	1.0032 ± 0.088 [b]	1.038 ± 0.086 [b]	1.47 ± 0.092 [b]	1.13 ± 0.089 [b]	0.93 ± 0.084 [ab]

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table 4.28 show the effect of season and temperature as co-variate on major spermatozoa defects during semen collection. Spring and summer semen collection had higher values for major spermatozoa defects. It was observed that winter collection had lower values of major spermatozoa defects. No value was displayed in summer at THI 2 for other major defects due to number of samples being too few to analyse. Pyriform heads were significantly ($P < 0.05$) lower when semen was collected in spring and other major defects that were significantly ($P < 0.05$) higher in spring than winter.

Table 4.28 Summary of major spermatozoa defects (%) in frozen-thawed semen in different THI groups at semen collection per season over seven years.

	Pyriform heads	Other major defects
	THI 2	**THI 2**
Autumn	0.94 ± 0.096 [b]	0.26 ± 0.071 [ab]
Spring	0.46 ± 0.13 [a]	0.37 ± 0.068 [a]
Summer	0.67 ± 0.77 [ab]	-
Winter	1.028 ± 0.05 [b]	0.16 ± 0.036 [b]

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table 4.29 indicates the effect of season and temperature as co-variate on major sperm defects at 60 days prior to semen collection. The season that was observed to have the lower values of major sperm defects was summer. The season with the highest percentage of major sperm defects was observed to be winter. All the major sperm defects values were higher in the THI 3 group.

Table 4.29 Summary of major spermatozoa defects (%) with significant differences in frozen-thawed semen in different THI group during sperm production per season over seven years.

	Mid-piece abnormalities		Pyriform heads
	THI 2	**THI 3**	**THI 2**
Autumn	1.046 ± 0.082 [b]	1.22 ± 0.062 [ab]	0.94 ± 0.091 [b]
Spring	1.76 ± 0.17 [a]	1.36 ± 0.069 [a]	0.46 ± 0.13 [a]
Summer	0.80 ± 0.98 [ab]	1.082 ± 0.067 [b]	0.67 ± 0.74 [ab]
Winter	1.14 ± 0.039 [b]	1.23 ± 0.13 [ab]	1.028 ± 0.048 [b]

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column with different letters are significantly different ($p < 0.05$).

Chapter 5: Discussion

5.1 Introduction

In this study the effect of South African temperature on semen characteristics of stud and commercial bulls was evaluated. The semen samples were collected over a seven-year period on farms in eight of SA provinces and the results are discussed in this chapter.

5.2 Fresh semen

Fresh semen characteristics recorded in this study included semen volume (ml), linear movement (%), non-linear movement (%), major defects (%) and minor defects (%). Ranges and standards for the various semen characteristic was confirmed by studying relevant literature. An acceptable semen volume should be within a range of 4 ml to 6 ml. In fresh semen volumes of lower than 3 ml is regarded as questionable (Entwistle & Fordyce, 2003; Bearden *et al.*, 2004; Mitchell & Doak, 2004). In this study semen volumes varied from 4.7 ml to 6 ml for the different breeds across provinces. Fresh semen, linear and non-linear sperm movements are regarded as satisfactory if above 60% and lower than 10 % respectively (Bearden *et al.*, 2004; Mitchell & Doak, 2004). The total number of major and minor defects in a fresh and frozen-thawed semen sample should be below 30% for a bull to be classified as fertile. Acceptable ranges for major defects should be between or lower than 10 - 20 % and minor defects to be between or lower than 8 -10% (Bearden *et al.*, 2004; Mitchell & Doak, 2004; Chhillar *et al.*, 2012; Tasdemir *et al.*, 2013). In this study the results for the fresh semen characteristics were comparable to ranges provided in literature.

In this study the electro-ejaculation semen collection method was used, and this method is known to result in larger volumes of semen, but with lower sperm concentration in semen (Sarsaifi *et al.*, 2013). Electro-ejaculated post-thawed semen have more acceptable semen characteristics, while frozen-thawed semen quality will always vary due to different inseminators (Sarsaifi *et al.*, 2013; Ntemka *et al.*, 2016). The semen is usually collected in a pre-warmed 15 – 25 mL tube, where the first step is to record volume and colour of semen. Immediately after volume and colour recording, a drop of sperm was placed on two pre-warmed microscope slides with one being covered with a cover slip and examined under a microscope for spermatozoa defects and the open slide examined for spermatozoa motility. An aliquot is then fixed in buffer solution followed by scanning with the CASA system to analyse the remaining spermatozoa characteristics (Nichi *et al.*, 2006; Microptic, 2014).

Variations between provinces for each semen characteristic over seven years were observed. Variations were expected due to the number of fresh semen records available for analyses. For the Eastern Cape (3.02 %), KwaZulu-Natal (3.74 %) and the Northern Cape (3.24 %) only a small number of records were available compared to the Free State and North West contributing 22.56 % and 27.85 % of the records. Variations among semen characteristics over the seven years and between provinces were relatively small.

In this study, semen characteristics were affected by breed with the Drakensberger and Boran having the highest semen volume and Limousin and Nguni the lowest semen volume. The highest spermatozoa linear movement were in Simmentaler semen with Nguni semen having the lowest non-linear spermatozoa movement. Beefmaster bulls had the lowest spermatozoa motility values. Despite differences for major defects in the semen of the various breeds, all fresh semen records were comparable to the ranges within different studies (Entwistle & Fordyce, 2003; Mitchell & Doak, 2004).

The indigenous, Drakensberger bulls had significantly ($P < 0.05$) higher semen volume, linear spermatozoa movement and lower ($P < 0.0001$) major and minor spermatozoa defects compared to composite, Bos indicus and Bos taurus breeds, demonstrating a higher overall semen quality. The semen of Bos indicus breeds had lower linear spermatozoa movement and lower major defects ($P < 0.0001$) compared to many of the Bos taurus and composite breeds; these results were across provinces. In contrast to this study it has been reported that Bos indicus bulls had higher major defects compared to Bos taurus bulls (Shukla *et al.*, 2010). A recent study by D'Andre *et al.* (2017) reported that breed does effect semen volume, spermatozoa concentration, mass motility and individual motility. According to Chenoweth (2005) some spermatozoa defects are directly linked to the bull genetics, namely some acrosome defects, spermatozoa head defects, mid-piece defects and spermatozoa tail defects. The number of fresh semen records per breed in this study were low for indigenous bulls (3.39 %), compared to the 13.07 % of Bos indicus bulls, 20.42 % Bos taurus bulls and 63.07 % composite bulls, therefore the results of breed effect in this study should be interpreted with caution.

The fresh semen characteristics tested for season adhered to the acceptable ranges for semen characteristics as discussed earlier. No significant differences for fresh semen volume and fresh semen major spermatozoa defects were observed between seasons at time of collection. There were however some differences for semen collected during summer when spermatozoa production occurred in spring. A similar observation was made by Snoja *et al.* (2013) where higher semen volume (3.98 – 7.53 ml) and total spermatozoa produced ($4.08 \times 10^9 – 9.90 \times 10^9$) were observed in summer. Snoja *et al.* (2013) explained that variation of semen volume is due to volume that adjust differentially to the amount of spermatozoa produced each month. In this study variation in spermatozoa movement was observed between seasons, where the higher spermatozoa linear movement was in spring and the worst in winter during collection and spermatogenesis. A study done by D'Andre *et al.* (2017) found that semen collection in spring had the best quality semen characteristics. D'Andre *et al.* (2017) based the conclusions on higher semen mass motility (> 70 %) and higher percentage normal spermatozoa observed during spring and they found that the poorest semen volume (4.61 ml) was in autumn with winter having the poorest semen morphology. In this study higher major defects ($P < 0.05$) were observed for spermatozoa produced during spring (13.79 ± 0.19 %) compared to spermatozoa produced in autumn (14.94 ± 0.20 %) and summer (14.98 ± 0.24 %). Therefore, it can be concluded that results of semen quality were best when semen was collected during summer when spermatogenesis occurred during spring. In this study, the season when semen was collected and the season when sperm were produced did significantly affect the proximal protoplasmic droplets, pyriform heads and

other major defects that was also observed in a study by Vilakazi & Webb (2004). The results of this study indicated higher major defects as expected for summer and autumn months, where Vilakazi & Webb (2004) indicated only negative effects for summer. Nichi *et al.* (2006) found in their study that primary defects and major defects were both higher during summer than in winter and suggested that there is an association between lipid peroxidase and sperm quality. Lower percentage of major sperm defects in semen could be linked to winter increasing to spring.

Despite the fresh semen characteristics values being within the satisfactory ranges, THI had an influence on bull semen characteristics during spermatogenesis and at time of semen collection. In this study THI was defined in three zones with THI 1 at temperatures > 27 - 31 °C, THI 2 at temperatures between 32 – 37 °C and THI 3 was at temperatures > 38 °C. Significantly lower ($P < 0.0001$) spermatozoa linear and non-linear spermatozoa movements were observed at THI 2 at both semen collection and spermatozoa production than at THI 3. Bull semen collected at a THI 2 had significantly lower spermatozoa movement compared to spermatozoa of bulls at a THI 3. In a study done by Al-Kanaan *et al.* (2015) it was reported that semen collection and spermatogenesis at THI > 60 showed a decrease in fresh semen volume (5.97 ml) and the number of spermatozoa produced (7.89×10^9), although spermatozoa motility decreased steadily from THI 65 - 75 resulting in the lowest spermatozoa motility at > THI 75. High temperatures during semen collection result in decreased spermatozoa motility and percentage viability and high temperatures one to 65 days before semen collection had a stronger effect, causing lower ($P < 0.05$) semen volume, spermatozoa concentration, number of sperm and spermatozoa motility (Fuerst-Waltl *et al.* 2006). Meyerhoeffer *et al.* (1985) also found lower spermatozoa motility when bulls experienced high temperatures two weeks before semen collection, concluding that heat stress affected spermatogenesis. Temperature at spermatozoa production affected minor defects as it was significantly ($P < 0.05$) lower compared to bull semen at a THI 2 than bull semen at a THI 3 in this study. THI 2 and THI 3 most likely affect the spermatids in the Sertoli cells reducing spermatozoa motility due to abnormal spermatozoa morphology (Hopper, 2014; Simoni & Huhtaniemi, 2017). Collection of semen from bulls experiencing a THI 3 had significantly lower abnormal loose heads, lower pyriform heads and significantly higher other major defects within their semen, which indicates that spermatozoa at collection was affected by the level of ROS present in the semen sample causing lipid peroxidation to damage sperm DNA and chromatin structure (Nichi *et al.*, 2006; Takahashi, 2012). Spermatozoa production at THI 2, resulted in significantly lower spermatozoa tail abnormalities, other spermatozoa major defects and significantly higher abnormal loose heads of spermatozoa. Hafez & Hafez (2013) stated that high ambient temperatures during spermatogenesis in the testis caused higher germ cell apoptosis and tailless spermatozoa. The authors also stated that high ambient temperatures during sperm maturation in the epididymis caused increased number of acrosomal damage and long-term exposure caused higher cytoplasmic droplets in semen (Hafez & Hafez, 2013). Literature supports the observations from this study of high temperatures (THI 3) affecting tail abnormalities and other major defects (Simoni & Huhtaniemi, 2017).

The semen from different breeds collected in THI 2 and THI 3 showed significant effects on major defects. It was observed that Bos Taurus bulls within THI 3 at sperm production had higher major spermatozoa defects. Similar results were reported by Nichi *et al.* (2006) where Bos taurus bulls in Brazil had significantly

higher percentage of major spermatozoa defects and Bos indicus had significantly higher minor defects when subjected to higher temperatures. Nichi *et al.* (2006) concluded that major defects were affected by breed and that GPx activity is higher in heat stressed (THI 2) Bos taurus bulls versus Bos indicus in summer. Therefore, lipid peroxidation is higher in Bos taurus bulls that is consistent with higher percentage defective spermatozoa. In this study lower major defects have been observed when semen was collected from Bos indicus bulls experiencing heat stress in THI3. It has been shown that Bos taurus bulls in Australia was less adapted compared to Bos indicus bulls, which were better adapted to continuous periods of high heat and humidity (Beatty *et al*. 2006). Bos indicus cattle had improved heat loss ability through anatomical and physiological features like their skin and shorter hair coats, that prevented a drop-in feed intake to maintain heat balance (Beatty *et al.*, 2006). The results from this study also indicated that Bos indicus bulls tended to have the desired semen quality in THI 2 and THI 3 environments which are associated with higher temperatures.

5.3 Frozen-thawed semen

For frozen-thawed semen the same methods are used for collection, followed by cryopreservation at 35 °C. A diluter with added antibiotics was added to a semen sample in a ratio of 3:1 and then cooled down to 5 °C and polyvinylchloride straws with semen are filled and sealed. Straws are placed in liquid nitrogen container 5.5 cm above liquid nitrogen level to be frozen until used (Bearden *et al.*, 2004). Thawing does affect sperm motility and acrosome integrity. The EE method provides a more acceptable post-thawed semen quality permitting a faster reproduction rate (Sarsaifi *et al.*, 2013), while the quality of frozen semen will always vary between different inseminators (Ntemka *et al.*, 2016).

Frozen-thawed semen characteristics included in this study were percentage live spermatozoa (%), sperm linear movement (%), spermatozoa non-linear movement (%), spermatozoa count/mL, viability (%), major and minor spermatozoa defects (%). The satisfactory range for the percentage live spermatozoa is 40 – 60 % and is questionable if it is below 40 %. Frozen-thawed semen linear and non-linear spermatozoa movements are acceptable when it is > 40 % and < 6 %, respectively. The acceptable thawed semen linear movement is lower than the acceptable ranges in fresh semen, as thawed semen is used for AI (Entwistle & Fordyce, 2003; Bearden *et al.*, 2004). Spermatozoa count above 60 is suitable, but between 50 – 60 is questionable. Spermatozoa viability should be > 58 % to be accepted for low motility but can range between 40 – 57 % if spermatozoa motility is high (Ayad, 2018). The sum of spermatozoa major and minor defects should be < 30 % to be recognized and used for AI (Bearden *et al.*, 2004).

The analyses for province showed an effect on frozen-thawed semen for the different characteristics that can be attributed to many factors such as time of collection, the semen collector and process of freezing. These variables could not be accounted for in this study and therefore no conclusion can be made for the effect of province.

Frozen-thawed semen characteristics records per breed were within acceptable ranges. Semen viability was lower than the acceptable range between 40 – 50 %. It was observed that Bos Indicus breeds with frozen-

thawed semen had higher percentage of live spermatozoa (54.27 ± 0.78 %), linear spermatozoa movement (50.08 ± 1.12 %) and lower total spermatozoa defects (15.35 %) compared to composite bull breeds which had significantly (P <0.05) higher total spermatozoa count (85.29 ± 1.07 %) and spermatozoa viability (50.36 ± 0.67 %). Similar to the results in this study it was reported that Bos Indicus bulls had higher percentage live spermatozoa (35.96 ± 0.5 %) but lower post-freezing spermatozoa motility (40.43 ± 0.5 %) in Rwanda, East Africa (D'Andre *et al*. 2017). According to a study done by Brito *et al.* (2002), Bos Indicus bulls in Brazil had significantly higher sperm production (10 – 12.5 x 10^9) and sperm concentration (1.5 – 2 x 10^9/ mL) versus Bos taurus bulls that had lower sperm defects (13.3 %, P < 0.10). In the current study the different breeds had varied results for specific major defects being either low or high thus no clear conclusion could be made for breed effect on individual major defects (Hopper, 2014). Brito *et al.* (2002) also found that Bos indicus bulls had a slower decrease in semen quality compared to Bos taurus and crossbred bulls but Bos taurus bulls had more abnormal spermatozoa heads and mid-piece abnormalities compared to Bos indicus semen defects. In this study composite breeds had more abnormal spermatozoa heads and mid-piece abnormalities.

The frozen-thawed semen displayed characteristics in acceptable ranges when analysed for season except for the percentage viability that were lower. No significant differences could be observed for % live spermatozoa, linear spermatozoa movement and major spermatozoa defects between seasons during sperm production and during semen collection. It was observed that the semen collected in spring had better quality frozen-thawed semen characteristics as it had a higher spermatozoa count/ml (81.79 ± 0.89), spermatozoa viability (50.13 ± 0.59 %) and lower minor defects (5.48 ± 0.16 %) (P <0.05). Autumn spermatogenesis had higher minor spermatozoa defects (6.31 ± 0.19 %). Sabés-Alsina *et al.* (2017) observed similar results in their study, indicating that semen collection in spring had higher spermatozoa motility (60.74 ± 14.25 %), viable spermatozoa (55.98 ± 8.07 %) and spermatozoa count (49.29 ± 5.54). Significant differences were found between individual major defects in frozen-thawed semen, that indicated winter had higher major defects during semen collection and spermatogenesis. Spermatogenesis occurring in spring and semen collected in summer produced better semen quality based on the characteristics analysed in this study, that is similar to what was observed for fresh semen. Therefore, the freeze-thawing process did not affect semen characteristics differently between seasons.

The values recorded for the frozen-thawed semen characteristics at a specific THI, were within the acceptable ranges, excluding spermatozoa viability (48.32 – 49.02 %) that was lower than the satisfactory percentage. Frozen-thawed semen had significantly (P <0.05) lower non-linear spermatozoa movement for bulls at a THI 2 than bulls at a THI 3 at semen collection and spermatogenesis, that differed to the fresh semen results. Spermatogenesis at a THI 3 produced significantly (P <0.05) lower spermatozoa count (78.26 – 80.82), higher major defects (12.14 – 13.03 %) and higher minor defects (5.59 – 6.16 %). Spermatozoa count is affected due to spermatocytes in stertoli cells that were affected by severe heat that kills spermatocytes in the meiotic phase as explained by Hopper (2014). Tail abnormalities in fresh and frozen-thawed semen were significantly higher at THI 3. Frozen-thawed semen have antioxidants added during the freezing process resulting in less significant difference between individual major defects than observed in fresh semen. Simon & Huhtaniemi (2017) explained the reason for different spermatozoa defects called the maturation defect, that

occurs when the concentration of ROS is overproduced compared to level of antioxidant level and leads to oxidative damage of spermatozoa membranes, spermatozoa proteins and spermatozoa DNA. When testis of bulls was insulated and influenced by high temperatures at day 1 – 21 of spermatogenesis produced higher tail abnormalities, pyriform heads and nuclear vacuoles, as concluded by Hopper (2014). Hafez & Hafez (2013) observed that semen collected 14 – 16 days after bulls experienced high temperatures had significantly higher morphologically abnormal spermatozoa, concluding that spermatozoa developed in testis during high temperatures produced higher dead and tailless spermatozoa.

It was observed that Bos indicus breeds had higher ($P< 0.05$) mid-piece abnormalities and pyriform heads, but lower other major defects at a THI 3 during spermatogenesis and semen collection. Composite breeds had higher other major defects at high temperatures affecting spermatozoa maturation in the epididymis and Bos indicus bull breeds spermatozoa major defects were more greatly affected by high temperatures that was observed in other studies (Brito *et al.*, 2002; Hopper, 2014; Simoni & Huhtaniemi, 2017). Nichi *et al.* (2006) observed higher levels of ROS and GPx activity with lower antioxidant enzymes present in Bos taurus bull semen in summer compared to Bos indicus bull semen. Nichi *et al.* (2006) then observed that lipid peroxidation of spermatozoa membranes was higher in Bos taurus semen, this was found to be consistent with higher percentage spermatozoa defects in Bos taurus bull semen. It was observed that composite breeds semen is more resistant to the freezing and thawing process than Bos indicus. This differed from the observations in fresh semen major defects per breed.

Bulls experiencing a THI 2 and THI 3 when spermatozoa were produced had significantly higher mid-piece abnormalities in spring and bulls experiencing THI 3 had significantly ($P <0.05$) higher pyriform heads in spring. As observed by Hopper (2014) THI 2 caused increase temperature in testis increasing pyriform heads, but THI 3 caused epididymis temperature to increase and damage maturation of spermatozoa, thus increasing mid-piece abnormalities in frozen-thawed semen.

In conclusion, the results of this study indicated that there are seasonal and breed differences among semen characteristics and temperature does affect spermatozoa quality. Further research is required for understanding the physiological mechanisms for improved adaptation to heat to ensure semen quality and subsequently herd fertility.

Chapter 6: Conclusion

The study conducted recognizes that the province, breed, season and temperature-humidity in South Africa are important sources of variation that should be considered in semen quality. Province did have an effect on semen characteristics, although no conclusion could be made as the number of records per province varied greatly. It was identified that South African indigenous bulls had superior quality fresh semen compared to Bos indicus, composite breed and Bos Taurus bulls. When bulls experienced a THI 3, it was identified that Bos indicus breeds should be used due to their higher quality semen during heat stress compared to Bos Taurus breeds. Semen characteristics of frozen-thawed semen for composite breed bulls were of superior quality. Concerning season, it was concluded that the highest quality semen is produced during spring therefore semen collection should occur in summer.

High temperatures induced heat stress in bulls increasing the temperature of the bull testis. Temperature during semen collection within a THI 2 (32 - 37 °C) produced decreased spermatozoa motility and THI 3 (> 38 °C) semen had increased percentage of minor defects and other major defects. Semen produced during a THI 2 had significantly higher abnormal loose heads and at a THI 3 significantly higher tail abnormalities and other major defects. Bulls that experience a THI 3 influenced semen quality negatively resulting in decreased spermatozoa count, increased major defects, minor defects and tail abnormalities. It can therefore be concluded that high temperatures affect semen quality negatively and it would be more advantageous using bulls during THI 2 that produce higher quality semen.

It is therefore recommended that the temperature, humidity, season and breed should be given urgent attention in any bull management system in SA in order to obtain the best semen quality. Sufficient evidence for climate change with increased average temperatures which necessitate the need for selection of hardy breeds. SA has access to a number of adapted breeds and genetics which could be exploited in crossbreeding systems for higher fertility and sustainable beef production. Further research focussing on the interactions, as well as the flexibility between different types of livestock production systems facing the above-mentioned stressors would be advantageous to the livestock industry.

Recommendations

In this study there were some received data challenges, which included the imbalance in the number of records among provinces and breeds. The time of spermatogenesis was estimated and require traits with more controlled data collection. Several records of measurements were biased and could not be used. The inclusion of more independent variables like age and nutrition could improve this study. There is insufficient research on the effect of temperature and humidity in SA beef cattle. This study serves as a benchmark for further research.

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Addendum A1 Fresh semen specific major and minor defects.

Major defects	Minor defects
Badly malformed	Loose heads
Double heads	Bended
Acrosome knobs	Single mitochondria
Peri-formed heads	Bended head
Narrow base	Curled tail
Abnormal base	Distal protoplasmic droplet
Abnormal head size	Abaxial implant
Kraters	Damaged acrosome
Coiled tails	Other minor defects
Other head defects	
Abnormal loose heads	
Mid-piece reflex	
Dag defects	
Pseudo drops	
Double mid-piece	
Cork screw tail	
Other mid-piece defects	
Broken flagellum	
Stomp tail	
Proximal protoplasmic droplet	
Other major defects	

Addendum A2 Frozen semen's specific major and minor defects.

Major defects	Minor defects
Severely deformed	Loose heads
Double heads	Bended
Acrosome knobs	Single mitochondria
Peri-formed heads	Bended head
Narrow base	Curled tail
Abnormal base	Distal protoplasmic droplet
Abnormal head size	Abaxial implant
Kraters	Damaged acrosome
Coiled tails	Other minor defects
Other head defects	
Abnormal loose heads	
Mid-piece reflex	
Dag defects	
Pseudo drops	
Multiple mid-piece	
Cork screw tail	
Other mid-piece defects	
Broken flagellum	
Stump tail	
Proximal protoplasmic droplet	
Other major defects	

Addendum B Summary of the towns linked to specific weather station.

Weather station	Town
WarmbadTowoomba - 0589594 1	Alma
	Bela Bela
	Groblersdal
	Marble Hall
	Modimole
	Nylstroom
	Pienaarsrivier
	Roedtan
	Settlers
	Thabazimbi
	Tuinplaas
	Warmbad
Potgietersrus - 0633882 7 (Mokopane)	Baltimore
	Mokopane
	Naboomspruit
	Potgietersrus
	Mookgophong
	Vaalwater
Pietersburg Wo - 0677802BX (Polokwane)	Dendron
	Ladanna
	Marnitz
	Pietersburg
	Polokwane
	Tombruke
Tzaneen-Westfalia Estate - 0679194 5	Duiwelskloof
	Dwaalboom
	Letsitele
	Tzaneen
Thohoyandou Wo - 0723664 6	Louis Trichardt
	Swartwater
	Tolwe
	Vivo
Graskop Aws - 0594626B9	Ohrigstad
Ellisras - 0674341 8	Ellisras
	Lephalale
Belfast - 0517041 2	Laersdrif
Lydenburg - 0554816A7	Roossenekal
Pilanesberg - 0548375A4	Swartklip
Ermelo Wo - 0479870 X	Amsterdam
	Bethal
	Ermelo
	Hendrina
	Kriel
	Morgenzon
	Trichardt
	Amersfoort
	Kinross
	Secunda
Newcastle - 0370856 3	Volksrust
	Wakkerstroom
	Perdekop
	Dundee
	Ingogo
	Newcastle
	Utrecht
Witbank - 0515320 8	Tasbetpark
	Witbank
	Stoffberg
	Ogies
	Laersdrif, Middelburg
	Warmbad
Springs - 0476762A3	Delmas

	Leslie
	Balfour
	Brakpan
	Springs
	Sundra
	Boksburg
Belfast - 0517041 2	Middelburg
	Belfast
	Highveld
Kruger Mpumalanga Int. Air. - 0556173 6	Komatiepoort
Rustenburg - 0511399 X	Boons
	Derby
	Koster
	Kroondal
	Magaliesburg
	Moedwil
	Mooi nooi
	Rustenburg
	Swartruggens
Tosca - 0504833 6	Bray
	Piet Plessis
	Tosca
Bloemhof - 0362189 7	Christiana
Lichtenburg - 0472278 0	Coligney
	Lichtenburg
	Ventersdorp
	Sannieshof
	Molopo
Vryburg - 0432237 3	Delareyville
	Schweizer Reneke
	Stella
	Vryburg
	Kameel
Klerksdorp - 0436204 1	Klerksdorp
	Orkney
	Ottosdal
	Wolmaranstad
	Flamwood
	Leeudoringstad
	Stilfontein
Potchefstroom - 0437104A4	Noordbrug
	Potchefstroom
	Rysmierbult
	Viljoenskroon
Pilanesberg - 0548375A4	Sun City
	Dwaalboom
Kimberley	Warrenton
Mafikeng Wo - 0508047 0	Buhrmansdrift
	Setlagoli
	Slurry
Taung - 0360453A0	Reivilo
	Hartswater
Pretoria Unisa - 0513346 0	Pta
	Silverlakes
	Brits
	Damdoryn
	Hartbeesfontein
	Riverwalk
Irene Wo - 0513385A2	Fonteinriet
Riverview - 0339327 3	Centurion
	Mildersdrift
Wonderboom Airport - 0513369A5	Silverton
	Onderstepoort
	Doornpoort

	Montanapark
	Wonderboom
	Derdepoortpark
	Koedoespoort
	Pyramid
	Sinoville
Jhb Bot Tuine - 0475879 0	Magaliesburg
	Eikenhof
	Mildersdrif
	City Deep
	Nigel
Johannesburg Int Wo - 0476399 0	Birchleigh
	Kempton park
	Bonaero park
	Halfway house
	Isando
	Benoni
Mooi River - 0268883 6	Town Bush Road
	Mooirivier
	Kwazulu-Natal
Ladysmith - 0300454 3	Bergville
	Ladysmith
	Wasbank
Vryheid - 0372527 1	Bloedrivier
	Kingsley
	Louwsburg
	Vryheid
	Paulpietersburg
Greytown - 0270155 9	Dalton
Durban Wo - 0240808A2	Durban
	Hillcrest
	Musgrave
Pongola - 0410175 X	Pongola
Margate - 0182591A4	Port Shepstone
Ixopo - 0210099A7	Underberg
Bethlehem Wo - 0331585 9	Arlington
	Bethlehem
	Fouriesburg
	Kestell
	Lindley
	Senekal
Bloemfontein Wo - 0261516B0	Bainsvlei
	Bloemfontein
	Brandfort
	Danhof
	Fitchardt Park
	Langenhoven Park
	Noordstad
Kimberley Wo - 0290468A9	Boshof
	Jacobsdal
Kroonstad - 0365398 8	Bothaville
	Koppies
	Kroonstad
	Steynsrus
Welkom - 0364300 1	Bultfontein
	Hoopstad
	Odendaalsrus
	Wesselsbron
Wepener - 0232654 4	Dewetsdorp
	Wepener
Excelsior Ceres - 0063807 2	Excelsior
	Marquard
Fauresmith - 0291570 1	Fauresmith
	Luckhoff
Ficksburg - 0296709AX	Ficksburg

	Ladybrand
Frankfort - Tnk - 0403886A0	Frankfort
	Heilbron
	Reitz
	Villiers
	Tweeling
	Petrus steyn
Ladysmith - 0300454 3	Harrismith
Vereeniging - 0438784 3	Oranjeville
	Parys
	Sasolburg
	Vanderbijlpark
	Vredefort
	Drie riviere
	Vereeniging
	Carletonville
	Heidelberg
	Vanderbijlpark
	Fochville
	Ennerdale
	Three rivers
	Vaal Marina
Vrede - 0405326A9	Vrede
	Warden
	Greylingstad
	Standerton
Virginia - 0241076 6	Winburg
Noupoort - 0144791 2	Colesberg
Postmasburg - 0321110 7	Danielskuil
	Griekwastad
Kathu - 0356880 4	Deben
	Kuruman
De Aar Wo - 0169880 1	Petrusville
Prieska - 0224400 8	Prieska
Fort Beaufort - 0078227A3	Adelaide
	Fort Beaufort
Aliwal-North Plaatkop - 0175678A0	Aliwal-North
	Burgersdorp
	Lady grey
Barkly-Oos (Caerleon) - 0177176 4	Barkly-Oos
Somerset East - 0055363 1	Bedfort
	Cookhouse
Addo Elephant Park - 0055447A7	Colchester
Uitenhage - 0034763 X	Colleen Glen
	Lovemore Park
Jamestown - 0148517A9	Dordrecht
Elliot - 0150620AX	Elliot
East London Wo - 0059572B8	Haga-Haga
Port Elizabeth AWOS – 0035209B1	Humansdorp
	Jeffreys bay
Joubertina Aws - 0031650A4	Joubertina
Grahamstown - 0056917 8	Kirkwood
Queenstown - 0123685 X	Komani
East London Wo - 0059572B8	Komga
Port Alfred - Airport - 0037574 1	Port Alfred
Tsitsikamma - 0015692A4	Tarkastad
Mosselbay - 0012221 5	Hartenbos
George Witfontein - 0028776 9	George
Cape Town Slangkop - 0004549 2	Killarney Gardens
Mosselbay - 0012221 5	Mosselbaai
	Hartenbos
Plettenbergbaai - 0014545 4	Plettenbergbaai
Strand - 0005609 8	Sanlamhof
	Stellenbosch
Knysna - 0014123 3	Sedgefield
Langebaanweg Aws - 0061298 8	Yzerfontein

Addendum C1 The summary tables with significant differences between year and semen characteristics or individual major defects and year.

Table C 1.1 Summary of the number of fresh semen observations per breed per year.

	Beef-master	Bons-mara	Boran	Brahman	Drakens-berger	Limousin	Nguni	Simbra	Simmen-taler	Total	Frequency
2010	142	404	7	90	16	80	3	115	122	979	**6.48**
2011		1071	82	340	113	135	68	260	574	3066	**20.29**
2012	480	576	48	343	99	105	69	129	425	2301	**15.23**
2013	476	686	99	293	62	53	27	247	318	2261	**14.96**
2014	412	652	99	478	91	33	15	202	358	2340	**15.48**
2015	461	739	28	187	39	20	9	145	186	1814	**12.00**
2016	328	624	36	208	93	65	6	178	267	1805	**11.94**
2017	155	189	6	37	0	0	0	39	121	547	**3.62**
Total	2877	4941	405	1976	513	491	197	1315	2398	15113	**100**
Frequency	19.04	32.69	2.68	13.07	3.39	3.25	1.30	8.70	15.87	100	

Table C 1.2 Summary of the number of frozen bull semen observations per breed per year.

	Beefmaster	Bonsmara	Boran	Brahman	Holsteins-Fries	Simbra	Total	Frequency
2010	24	47	21	41	17	30	180	**8.87**
2011	40	94	81	78	26	68	387	**19.07**
2012	55	73	59	79	16	43	325	**16.02**
2013	55	74	49	92	15	60	345	**17.00**
2014	33	68	28	107	9	59	304	**14.98**
2015	26	57	13	64	24	52	236	**11.63**
2016	50	75	14	60	16	37	252	**12.42**
Total	283	488	265	521	123	349	2029	**100**
	13.95	24.05	13.06	25.68	6.06	17.20	100	

Table C 1.3 The mean (± SE) between years for each fresh semen characteristics when collected.

	Volume (ml)	Linear Movement (%)	Non-Linear Movement (%)	Major Defects (%)	Minor Defects (%)
2010	4.86 ± 0.073^{a}	83.20 ± 0.55^{a}	5.29 ± 0.071^{b}	11.61 ± 0.31^{a}	7.17 ± 0.17^{ac}
2011	5.013 ± 0.041^{ab}	81.20 ± 0.31^{ab}	5.42 ± 0.040^{bc}	12.85 ± 0.18^{a}	7.34 ± 0.098^{c}
2012	5.24 ± 0.047^{bc}	80.55 ± 0.35^{bc}	5.62 ± 0.046^{ac}	14.019 ± 0.21^{b}	7.43 ± 0.11^{c}
2013	5.30 ± 0.047^{c}	81.39 ± 0.36^{ab}	5.55 ± 0.046^{bc}	13.91 ± 0.20^{b}	6.0074 ± 0.11^{b}
2014	5.37 ± 0.046^{c}	81.23 ± 0.35^{ab}	5.85 ± 0.045^{a}	14.25 ± 0.20^{b}	6.55 ± 0.11^{ab}
2015	5.66 ± 0.052^{d}	82.20 ± 0.40^{ab}	5.57 ± 0.051^{bc}	16.30 ± 0.23^{c}	7.76 ± 0.12^{cd}
2016	5.69 ± 0.053^{d}	82.41 ± 0.40^{ab}	5.50 ± 0.052^{bc}	17.46 ± 0.23^{c}	7.48 ± 0.13^{c}
2017	5.44 ± 0.096^{cd}	77.84 ± 0.72^{c}	5.63 ± 0.094^{abc}	16.93 ± 0.41^{c}	8.65 ± 0.23^{d}

All results are expressed as mean ± SD.
^{abc} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 1.4 The mean (± SE) between years for each fresh semen characteristics during spermatogenesis.

	Volume (ml)	Linear Movement (%)	Non-Linear Movement (%)	Major Defects (%)	Minor Defects (%)
2010	4.90 ± 0.073^{a}	83.013 ± 0.55^{a}	5.29 ± 0.071^{b}	11.33 ± 0.45^{a}	7.076 ± 0.17^{ab}
2011	5.07 ± 0.041^{ab}	81.45 ± 0.31^{ab}	5.40 ± 0.041^{bc}	13.24 ± 0.25^{ab}	7.31 ± 0.10^{bc}
2012	5.20 ± 0.048^{abc}	80.46 ± 0.36^{b}	5.62 ± 0.047^{ac}	12.98 ± 0.34^{ab}	7.36 ± 0.12^{bc}
2013	5.36 ± 0.049^{cd}	82.18 ± 0.37^{ab}	5.50 ± 0.049^{bc}	13.80 ± 0.30^{b}	5.91 ± 0.12
2014	5.36 ± 0.049^{cd}	81.0079 ± 0.37^{ab}	5.86 ± 0.048^{a}	13.97 ± 0.30^{b}	6.69 ± 0.12^{a}
2015	5.57 ± 0.052^{de}	82.18 ± 0.39^{ab}	5.56 ± 0.052^{bc}	16.13 ± 0.27^{c}	7.90 ± 0.13^{cd}
2016	5.69 ± 0.056^{e}	81.87 ± 0.42^{ab}	5.49 ± 0.056^{bc}	18.095 ± 0.33^{d}	7.54 ± 0.14^{bcd}
2017	5.48 ± 0.14^{bcde}	76.84 ± 1.027x	5.66 ± 0.13^{abc}	17.74 ± 0.62^{cd}	8.68 ± 0.33^{d}

All results are expressed as mean ± SD.
^{abc} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 1.5 Summary of fresh semen major defects (%) in different year when collection occurred.

	Proximal Protoplasmic droplets	Mid-piece Abnormalities	Tail abnormalities	Loose heads	Pyriform heads	Other major defects
2010	4.22 ± 0.16^{b}	1.066 ± 0.043^{a}	0.87 ± 0.024^{b}	1.50 ± 0.38^{ab}	0.70 ± 0.081^{abc}	0.0094 ± 0.037^{a}
2011	3.45 ± 0.10^{a}	1.30 ± 0.024	0.65 ± 0.013^{a}	1.36 ± 0.057^{b}	0.82 ± 0.025^{b}	0.15 ± 0.019^{a}
2012	4.28 ± 0.11^{b}	1.30 ± 0.027	0.72 ± 0.015^{ac}	1.71 ± 0.05^{a}	0.61 ± 0.034^{a}	0.41 ± 0.022
2013	3.79 ± 0.11^{ab}	1.31 ± 0.027	0.69 ± 0.014^{ac}	1.10 ± 0.094^{b}	0.63 ± 0.037^{a}	0.86 ± 0.021
2014	3.90 ± 0.11^{ab}	1.28 ± 0.026	0.74 ± 0.014^{c}	1.64 ± 0.07^{a}	0.82 ± 0.029^{b}	0.72 ± 0.022^{b}
2015	3.92 ± 0.12^{ab}	1.32 ± 0.03	0.76 ± 0.015^{bc}	1.23 ± 0.087^{b}	1.0072 ± 0.026^{c}	0.69 ± 0.023^{b}
2016	4.06 ± 0.12^{b}	1.23 ± 0.031^{a}	0.75 ± 0.016^{c}	1.46 ± 0.073^{ab}	1.54 ± 0.025	0.73 ± 0.023^{b}

All results are expressed as mean ± SD.
^{abc} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 1.6 Summary of fresh semen major defects (%) in different years during spermatogenesis.

	Proximal Protoplasmic droplets	Mid-piece Abnormalities	Tail abnormalities	Loose heads	Pyriform heads	Other major defects
2010	4.29 ± 0.17^{ab}	1.079 ± 0.044^{a}	0.83 ± 0.025^{b}	1.73 ± 0.36^{abc}	0.67 ± 0.076^{ab}	0.90 ± 0.32^{abcd}
2011	3.57 ± 0.11^{a}	1.28 ± 0.024^{b}	0.65 ± 0.013^{a}	1.40 ± 0.056^{abc}	0.80 ± 0.024^{b}	0.80 ± 0.043^{ab}
2012	4.17 ± 0.12^{b}	1.31 ± 0.028^{b}	0.69 ± 0.015^{ac}	1.69 ± 0.054^{a}	0.60 ± 0.035^{a}	0.82 ± 0.035^{a}
2013	3.81 ± 0.12^{ab}	1.29 ± 0.028^{b}	0.69 ± 0.015^{ac}	1.11 ± 0.10^{b}	0.66 ± 0.036^{ab}	1.0084 ± 0.026^{d}
2014	3.89 ± 0.12^{ab}	1.29 ± 0.028^{b}	0.74 ± 0.015^{bc}	1.68 ± 0.075^{ac}	0.82 ± 0.03^{b}	1.19 ± 0.031^{c}
2015	3.93 ± 0.12^{ab}	1.31 ± 0.030^{b}	0.77 ± 0.015^{bc}	1.25 ± 0.09^{bc}	1.011 ± 0.026	1.00 ± 0.031^{bd}
2016	4.22 ± 0.13^{b}	1.23 ± 0.033^{ab}	0.76 ± 0.016^{bc}	1.55 ± 0.073^{abc}	1.52 ± 0.025	1.11 ± 0.032^{cd}

All results are expressed as mean ± SD.
^{abcd} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 1.7 Summary of fresh semen individual major defects (%) per THI group in different years when collected.

	Proximal Protoplasmic droplets	Mid-piece Abnormalities	Tail abnormalities		Loose heads		Pyriform heads		Other major defects	
	THI 2	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**
2010	4.40 ± 0.39^{ab}	1.089 ± 0.048^{a}	0.74 ± 0.061^{ab}	0.89 ± 0.027	1.14 ± 0.43^{ab}	2.33 ± 0.68^{ab}	0.70 ± 0.22^{ab}	0.72 ± 0.080^{ab}	0.043 ± 0.096^{ab}	0.0031 ± 0.039
2011	3.027 ± 0.18^{a}	1.31 ± 0.03^{b}	0.64 ± 0.021^{a}	0.66 ± 0.016^{a}	1.45 ± 0.095^{b}	1.31 ± 0.071^{b}	1.00045 ± 0.038^{b}	0.60 ± 0.032^{a}	0.019 ± 0.033^{a}	0.23 ± 0.024
2012	4.43 ± 0.17^{b}	1.32 ± 0.035^{b}	0.66 ± 0.027^{ab}	0.74 ± 0.018^{ab}	1.59 ± 0.092^{ab}	1.75 ± 0.059^{a}	0.56 ± 0.068^{a}	0.63 ± 0.037^{a}	0.32 ± 0.042^{b}	0.45 ± 0.026
2013	3.81 ± 0.16^{ab}	1.33 ± 0.037^{b}	0.72 ± 0.021^{ab}	0.67 ± 0.019^{ab}	1.048 ± 0.14^{b}	1.13 ± 0.12^{b}	0.58 ± 0.071^{a}	0.67 ± 0.042^{ab}	0.87 ± 0.033^{c}	0.85 ± 0.028^{a}
2014	3.99 ± 0.20^{ab}	1.27 ± 0.032^{ab}	0.80 ± 0.027^{b}	0.71 ± 0.017^{ab}	1.95 ± 0.098^{a}	1.39 ± 0.096^{ab}	0.77 ± 0.067^{ab}	0.84 ± 0.030^{b}	0.65 ± 0.042^{d}	0.74 ± 0.025^{a}
2015	3.75 ± 0.20^{ab}	1.31 ± 0.037^{ab}	0.76 ± 0.026^{ab}	0.76 ± 0.019^{b}	1.15 ± 0.14^{b}	1.27 ± 0.11^{b}	0.96 ± 0.051^{b}	1.037 ± 0.028^{c}	0.64 ± 0.041^{d}	0.71 ± 0.027^{a}
2016	3.89 ± 0.18^{ab}	1.25 ± 0.04^{ab}	0.78 ± 0.025^{b}	0.74 ± 0.02^{ab}	1.53 ± 0.099^{ab}	1.40 ± 0.10^{ab}	2.097 ± 0.042	1.041 ± 0.031^{c}	0.75 ± 0.039^{cd}	0.72 ± 0.03^{a}

All results are expressed as mean ± SD.
^{abc} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 1.8 Summary of fresh semen individual major defects (%) per THI group in different years during spermatogenesis.

	Proximal Protoplasmic droplets	Mid-piece Abnormalities	Tail abnormalities		Loose heads	Pyriform heads		Other major defects	
	THI 2	**THI 3**	**THI 2**	**THI 3**	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**
2010	4.52 ± 0.23^{b}	1.058 ± 0.062^{a}	0.72 ± 0.039^{ab}	0.93 ± 0.31	3.00 ± 0.58^{ab}	0.59 ± 0.10^{ab}	0.76 ± 0.11^{ab}	0.93 ± 0.38^{abc}	0.83 ± 0.60^{abc}
2011	3.11 ± 0.16^{a}	1.27 ± 0.033^{ab}	0.60 ± 0.021^{a}	0.68 ± 0.015^{a}	1.40 ± 0.057^{ab}	0.88 ± 0.033^{bc}	0.71 ± 0.035^{a}	0.76 ± 0.10^{ab}	0.81 ± 0.048^{a}
2012	4.11 ± 0.14^{b}	1.32 ± 0.046^{ab}	0.71 ± 0.02^{b}	0.66 ± 0.024^{a}	1.59 ± 0.10^{ab}	0.58 ± 0.043^{a}	0.64 ± 0.06^{a}	0.81 ± 0.042^{a}	0.83 ± 0.062^{ab}
2013	3.84 ± 0.17^{ab}	1.27 ± 0.039^{ab}	0.69 ± 0.023^{ab}	0.68 ± 0.018^{a}	1.077 ± 0.11^{a}	0.71 ± 0.051^{ab}	0.61 ± 0.052^{a}	0.97 ± 0.038^{ab}	1.04 ± 0.034^{bc}
2014	3.96 ± 0.16^{ab}	1.31 ± 0.04^{ab}	0.74 ± 0.023^{b}	0.75 ± 0.02^{ab}	1.73 ± 0.083^{b}	0.89 ± 0.041^{bc}	0.74 ± 0.044^{a}	1.20 ± 0.042^{c}	1.18 ± 0.046^{bc}
2015	3.76 ± 0.24^{ab}	1.33 ± 0.035^{b}	0.72 ± 0.033^{ab}	0.78 ± 0.016^{b}	1.30 ± 0.086^{ab}	1.093 ± 0.051^{c}	0.98 ± 0.03^{b}	1.019 ± 0.063^{abc}	0.99 ± 0.036^{abc}
2016	3.95 ± 0.19^{ab}	1.22 ± 0.044^{ab}	0.76 ± 0.027^{b}	0.76 ± 0.02^{ab}	1.50 ± 0.077^{ab}	1.58 ± 0.038	1.48 ± 0.034	1.097 ± 0.049^{bc}	1.12 ± 0.043^{c}

All results are expressed as mean ± SD.
^{abc} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Addendum C2 Results of individual minor defects compared to province, breed, THI, season and year.

Table C 2.1 Summary of fresh semen minor defects (%) per province at collection.

	Distal protoplasmic droplet	Mid-piece abnormalities	Tail abnormalities (Curled Distal)	Head abnormalities	Loose heads
Frequency	7110	5864	4145	3689	6815
Eastern Cape	3.69 ± 0.21^{ab}	0.78 ± 0.042^{ab}	1.47 ± 0.24	2.084 ± 0.071^{b}	2.21 ± 0.22^{b}
Free State	3.50 ± 0.079^{b}	0.80 ± 0.016^{ab}	1.95 ± 0.07	1.62 ± 0.035^{a}	3.62 ± 0.076^{ac}
Gauteng	3.93 ± 0.14^{ab}	0.82 ± 0.027^{ab}	1.77 ± 0.13	1.49 ± 0.063^{a}	3.82 ± 0.14^{ac}
Kwazulu-Natal	4.39 ± 0.19^{a}	0.80 ± 0.043^{ab}	2.43 ± 0.17	1.55 ± 0.11^{a}	4.13 ± 0.19^{a}
Limpopo	3.88 ± 0.093^{ab}	0.89 ± 0.019^{a}	2.11 ± 0.087	1.62 ± 0.041^{a}	3.37 ± 0.093^{acd}
Mpumalanga	3.52 ± 0.099^{b}	0.82 ± 0.02^{ab}	1.79 ± 0.096	1.59 ± 0.046^{a}	3.62 ± 0.093^{acd}
North West	3.46 ± 0.07^{b}	0.78 ± 0.014^{b}	1.93 ± 0.068	1.58 ± 0.031^{a}	3.23 ± 0.068^{d}
Northern Cape	3.55 ± 0.21^{ab}	0.71 ± 0.043^{b}	1.89 ± 0.2	1.47 ± 0.087^{a}	2.95 ± 0.19^{bcd}

All results are expressed as mean ± SD.
^{abcd} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.2 Summary of fresh semen minor defects (%) per breed at collection.

	Distal protoplasmic droplet	Mid-piece abnormalities	Tail abnormalities (Curled Distal)	Head abnormalities	Loose heads
BEEFMASTER	3.86 ± 0.08 ^{c}	0.80 ± 0.018^{ab}	1.99 ± 0.082^{ab}	1.68 ± 0.039^{abc}	3.68 ± 0.084
BONSMARA	3.41 ± 0.067^{ab}	0.89 ± 0.013^{a}	2.17 ± 0.058^{a}	1.56 ± 0.029^{ac}	3.54 ± 0.063
BORAN	3.57 ± 0.22^{abc}	0.82 ± 0.046^{ab}	1.84 ± 0.21^{ab}	1.86 ± 0.087^{ab}	3.022 ± 0.23
BRAHMAN	3.94 ± 0.10^{c}	0.77 ± 0.021^{b}	1.86 ± 0.11^{ab}	1.80 ± 0.045^{b}	3.30 ± 0.10
DRAKENSBERGER	2.67 ± 0.21^{a}	0.71 ± 0.043^{b}	1.95 ± 0.21^{ab}	1.64 ± 0.087^{abc}	3.003 ± 0.20
LIMOUSIN	3.36 ± 0.23^{abc}	0.88 ± 0.052^{ab}	1.67 ± 0.24^{ab}	1.74 ± 0.12^{abc}	3.16 ± 0.23
NGUNI	2.86 ± 0.37^{abc}	0.65 ± 0.067^{ab}	2.00 ± 0.30^{ab}	1.28 ± 0.15^{abc}	3.64 ± 0.29
SIMBRA	3.85 ± 0.13^{bc}	0.72 ± 0.028^{b}	1.67 ± 0.12^{ab}	1.56 ± 0.059^{abc}	3.50 ± 0.13
SIMMENTALER	3.66 ± 0.093^{bc}	0.70 ± 0.02^{b}	1.59 ± 0.094^{b}	1.46 ± 0.042^{c}	3.23 ± 0.089

All results are expressed as mean ± SD.
^{abc} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.3 Summary of fresh semen minor defects (%) per THI group at collection.

			Production rate	Distal protoplasmic droplet	Mid-piece abnormalities	Tail abnormalities (Curled Distal)	Head abnormalities	Loose heads
THI 1	**Fertile**	< 70 - 72	High	3.55 ± 0.33	0.95 ± 0.063	1.84 ± 0.3	1.44 ± 0.14 **ab**	3.39 ± 0.31
THI 2	**Decrease fertility**	73 - 78	Decreased	3.60 ± 0.064	0.79 ± 0.013	1.99 ± 0.059	1.69 ± 0.029 **a**	3.51 ± 0.061
THI 3	**No fertility**	79 - > 80	Zero	3.64 ± 0.047	0.81 ± 0.01	1.92 ± 0.045	1.58 ± 0.021 **b**	3.40 ± 0.046

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.4 Summary of fresh semen minor defects (%) per THI group at spermatogenesis.

			Production rate	Distal protoplasmic droplet	Mid-piece abnormalities	Tail abnormalities (Curled Distal)	Head abnormalities	Loose heads
THI 1	**Fertile**	< 70 - 72	High		0.95 ± 0.063			
THI 2	**Decrease fertility**	73 - 78	Decreased	3.63 ± 0.061	0.79 ± 0.013	1.89 ± 0.057	1.67 ± 0.027 **a**	3.32 ± 0.059 **a**
THI 3	**No fertility**	79 - > 80	Zero	3.64 ± 0.052	0.81 ± 0.01	1.99 ± 0.05	1.59 ± 0.023 **b**	3.50 ± 0.050 **b**

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.5 Summary of fresh semen minor defects (%) per season at collection.

	Distal protoplasmic droplet	Mid-piece Abnormalities	Tail abnormalities (Curled distal)	Head abnormalities	Loose heads
Autumn	3.61 ± 0.092 **ab**	0.87 ± 0.019 **a**	1.89 ± 0.087	1.54 ± 0.039 **a**	4.24 ± 0.084 **b**
Spring	3.52 ± 0.059 **a**	0.78 ± 0.012 **b**	1.93 ± 0.055	1.60 ± 0.027 **ab**	3.22 ± 0.059 **a**
Summer	3.93 ± 0.12 **b**	0.89 ± 0.025 **a**	1.71 ± 0.13	1.55 ± 0.053 **ab**	3.057 ± 0.12 **a**
Winter	3.67 ± 0.067 **ab**	0.78 ± 0.013 **b**	2.044 ± 0.06	1.70 ± 0.030 **b**	3.35 ± 0.064 **a**

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.6 Summary of fresh semen minor defects (%) per season at spermatogenesis.

	Distal protoplasmic droplet	Mid-piece Abnormalities	Tail abnormalities (Curled distal)	Head abnormalities	Loose heads
Autumn	3.69 ± 0.077^{ab}	0.82 ± 0.016^{ab}	2.079 ± 0.07	1.61 ± 0.037^{ab}	3.85 ± 0.07^{a}
Spring	3.47 ± 0.086^{a}	0.81 ± 0.018^{ab}	1.97 ± 0.082	1.58 ± 0.038^{ab}	3.068 ± 0.08^{b}
Summer	3.89 ± 0.11^{b}	0.85 ± 0.023^{a}	1.85 ± 0.11	1.50 ± 0.047^{a}	3.83 ± 0.11^{a}
Winter	3.62 ± 0.064^{ab}	0.78 ± 0.013^{b}	1.86 ± 0.061	1.69 ± 0.028^{b}	3.16 ± 0.064^{b}

All results are expressed as mean ± SD.
^{ab} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.7 Summary of fresh semen minor defects (%) per year at collection.

	Distal protoplasmic droplet	Mid-piece Abnormalities	Tail abnormalities (Curled distal)	Head abnormalities	Loose heads
2010	4.046 ± 0.14^{bc}	0.88 ± 0.05^{abc}	2.63 ± 0.14^{a}	1.95 ± 0.094^{a}	3.033 ± 0.15^{ac}
2011	3.10 ± 0.093^{a}	0.76 ± 0.017^{ab}	1.93 ± 0.071^{b}	1.27 ± 0.049^{b}	3.94 ± 0.075^{b}
2012	3.61 ± 0.092^{cd}	0.70 ± 0.023^{a}	1.91 ± 0.091^{b}	1.44 ± 0.06^{b}	3.68 ± 0.091^{bc}
2013	3.31 ± 0.10^{ad}	0.79 ± 0.018^{ab}	1.83 ± 0.089^{b}	1.83 ± 0.031^{a}	2.11 ± 0.10 ^{d}
2014	3.64 ± 0.094^{cd}	0.80 ± 0.02^{abc}	1.95 ± 0.096^{b}	1.73 ± 0.037^{a}	3.0013 ± 0.097^{a}
2015	4.24 ± 0.10^{b}	0.85 ± 0.02^{bc}	2.06 ± 0.10^{ab}	1.51 ± 0.045^{b}	3.38 ± 0.11^{ac}
2016	3.65 ± 0.10^{cd}	0.90 ± 0.02^{c}	1.64 ± 0.12^{b}	1.42 ± 0.052^{b}	3.71 ± 0.11^{bc}

All results are expressed as mean ± SD.
^{abcd} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.8 Summary of fresh semen minor defects (%) per year at spermatogenesis.

	Distal protoplasmic droplet	Mid-piece Abnormalities	Tail abnormalities (Curled distal)	Head abnormalities	Loose heads
2010	4.063 ± 0.14^{cd}	0.88 ± 0.05^{abc}	2.60 ± 0.14^{a}	1.91 ± 0.093^{ab}	3.026 ± 0.15^{b}
2011	3.042 ± 0.098^{a}	0.74 ± 0.017^{ab}	1.90 ± 0.075^{b}	1.26 ± 0.049^{c}	3.96 ± 0.076^{a}
2012	3.63 ± 0.096^{bc}	0.71 ± 0.024^{a}	1.94 ± 0.094^{b}	1.50 ± 0.06^{bcd}	3.57 ± 0.094^{ab}
2013	3.31 ± 0.11^{ab}	0.81 ± 0.018^{abc}	1.77 ± 0.096^{b}	1.85 ± 0.032^{a}	1.99 ± 0.11^{c}
2014	3.62 ± 0.10^{bc}	0.81 ± 0.022^{abc}	2.0082 ± 0.11^{ab}	1.74 ± 0.042^{ab}	3.13 ± 0.1^{b}
2015	4.23 ± 0.10^{d}	0.84 ± 0.02^{bc}	2.056 ± 0.11^{ab}	1.53 ± 0.047^{bd}	3.54 ± 0.11^{ab}
2016	3.92 ± 0.11^{cd}	0.91 ± 0.022^{c}	1.64 ± 0.13^{b}	1.40 ± 0.054^{cd}	3.57 ± 0.12^{ab}

All results are expressed as mean ± SD.
^{abcd} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.9 Summary of fresh semen individual minor defects (%) per THI group in different provinces.

	Distal protoplasmic droplet		Mid-piece abnormalities		Tail abnormalities (Curled Distal)		Head abnormalities		Abnormal Loose heads	
Frequency	3728	7110	2106	3670	1500	2588	1207	2432	3922	6815
	THI 2	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**
Eastern Cape	3.67 ± 0.21 ^{ab}	4.00 ± 0.74	0.74 ± 0.043 ^{ab}	1.25 ± 0.15 ^{ab}	1.47 ± 0.30	1.44 ± 0.53	2.11 ± 0.081 ^{a}	1.80 ± 0.22	1.86 ± 0.22 ^{b}	6.86 ± 0.84 ^{a}
Free State	3.50 ± 0.12 ^{b}	3.49 ± 0.11	0.74 ± 0.023 ^{a}	0.86 ± 0.023 ^{ab}	1.95 ± 0.11	1.96 ± 0.092	1.61 ± 0.058 ^{b}	1.62 ± 0.045	3.52 ± 0.11 ^{a}	3.72 ± 0.11 ^{ab}
Gauteng	3.76 ± 0.27 ^{ab}	4.003 ± 0.16	0.83 ± 0.05 ^{ab}	0.79 ± 0.032 ^{ab}	1.90 ± 0.27	1.68 ± 0.15	1.63 ± 0.13 ^{ab}	1.44 ± 0.071	4.25 ± 0.24 ^{a}	3.56 ± 0.17 ^{bcd}
Kwazulu-Natal	4.66 ± 0.25 ^{a}	4.10 ± 0.31	0.88 ± 0.055 ^{ab}	0.69 ± 0.072 ^{ab}	2.65 ± 0.26	2.24 ± 0.27	1.62 ± 0.18 ^{ab}	1.47 ± 0.18	4.41 ± 0.25 ^{a}	3.83 ± 0.29 ^{abcd}
Limpopo	3.82 ± 0.26 ^{ab}	3.89 ± 0.10	0.99 ± 0.057 ^{b}	0.88 ± 0.021 ^{a}	2.81 ± 0.25	1.98 ± 0.088	1.62 ± 0.15 ^{ab}	1.62 ± 0.042	3.72 ± 0.26 ^{a}	3.32 ± 0.10 ^{bcd}
Mpumalanga	3.46 ± 0.13 ^{b}	3.61 ± 0.15	0.82 ± 0.025 ^{ab}	0.84 ± 0.034 ^{ab}	1.84 ± 0.14	1.73 ± 0.14	1.59 ± 0.064 ^{b}	1.60 ± 0.07	3.53 ± 0.12 ^{a}	3.73 ± 0.15 ^{abc}
North West	3.56 ± 0.16 ^{ab}	3.43 ± 0.079	0.81 ± 0.031 ^{ab}	0.76 ± 0.016 ^{b}	1.84 ± 0.18	1.95 ± 0.072	1.64 ± 0.083 ^{b}	1.58 ± 0.033	3.45 ± 0.15 ^{a}	3.19 ± 0.078 ^{cd}
Northern Cape	2.74 ± 0.39 ^{b}	3.87 ± 0.25	0.70 ± 0.075 ^{ab}	0.71 ± 0.053 ^{ab}	1.91 ± 0.35	1.88 ± 0.25	1.58 ± 0.18 ^{ab}	1.43 ± 0.099	3.77 ± 0.32 ^{a}	2.52 ± 0.24 ^{d}

All results are expressed as mean ± SD.

^{abcd} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.10 Summary of fresh semen individual minor defects (%) per THI group for each breed.

	Distal protoplasmic droplet		Mid-piece abnormalities		Tail abnormalities (Curled Distal)		Head abnormalities		Abnormal Loose heads	
	THI 2	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**
BEEFMASTER	3.93 ± 0.12 ^{a}	3.79 ± 0.12 ^{ab}	0.78 ± 0.024 ^{b}	0.83 ± 0.026 ^{ab}	1.91 ± 0.13	2.094 ± 0.11 ^{ab}	1.65 ± 0.06	1.71 ± 0.055 ^{a}	3.67 ± 0.12 ^{abc}	3.65 ± 0.12
BONSMARA	3.52 ± 0.14 ^{ab}	3.38 ± 0.077 ^{a}	0.95 ± 0.025 ^{a}	0.87 ± 0.015 ^{a}	2.33 ± 0.12	2.11 ± 0.064 ^{a}	1.56 ± 0.068	1.56 ± 0.032 ^{ab}	4.043 ± 0.12 ^{ab}	3.38 ± 0.075
BORAN	3.55 ± 0.38 ^{ab}	3.58 ± 0.28 ^{ab}	0.82 ± 0.076 ^{ab}	0.82 ± 0.057 ^{ab}	1.94 ± 0.38	1.77 ± 0.26 ^{ab}	1.98 ± 0.16	1.80 ± 0.10 ^{ab}	2.84 ± 0.37 ^{ac}	3.15 ± 0.30
BRAHMAN	3.81 ± 0.15 ^{ab}	4.038 ± 0.14 ^{b}	0.72 ± 0.03 ^{b}	0.82 ± 0.03 ^{ab}	1.85 ± 0.19	1.85 ± 0.13 ^{ab}	1.91 ± 0.07	1.70 ± 0.06 ^{ab}	3.015 ± 0.14 ^{c}	3.54 ± 0.14
DRAKENSBERGER	2.53 ± 0.29 ^{b}	2.83 ± 0.31 ^{ab}	0.68 ± 0.058 ^{b}	0.75 ± 0.063 ^{ab}	2.094 ± 0.32	1.79 ± 0.28 ^{ab}	1.77 ± 0.13	1.47 ± 0.13 ^{ab}	3.10 ± 0.25 ^{ac}	2.85 ± 0.32
LIMOUSIN	3.14 ± 0.41 ^{ab}	3.50 ± 0.28 ^{ab}	0.78 ± 0.099 ^{ab}	0.92 ± 0.061 ^{ab}	1.89 ± 0.49	1.56 ± 0.28 ^{ab}	1.29 ± 0.27	1.87 ± 0.13 ^{ab}	3.38 ± 0.41 ^{abc}	3.053 ± 0.28
NGUNI	3.22 ± 0.82 ^{ab}	2.77 ± 0.41 ^{ab}	0.63 ± 0.2 ^{ab}	0.66 ± 0.071 ^{ab}	3.93 ± 0.67	1.31 ± 0.33 ^{ab}	1.00 ± 0.42	1.33 ± 0.15 ^{ab}	6.42 ± 0.76 ^{b}	3.19 ± 0.31
SIMBRA	3.70 ± 0.21 ^{ab}	3.93 ± 0.16 ^{ab}	0.72 ± 0.046 ^{b}	0.73 ± 0.035 ^{ab}	1.71 ± 0.20	1.64 ± 0.14 ^{ab}	1.65 ± 0.11	1.53 ± 0.069 ^{ab}	3.50 ± 0.19 ^{abc}	3.49 ± 0.17
SIMMENTALER	3.14 ± 0.18 ^{ab}	3.83 ± 0.11 ^{ab}	0.70 ± 0.035 ^{b}	0.70 ± 0.024 ^{b}	1.51 ± 0.20	1.62 ± 0.10 ^{b}	1.58 ± 0.10	1.43 ± 0.045 ^{b}	3.071 ± 0.16 ^{c}	3.30 ± 0.11

All results are expressed as mean ± SD.
^{abc} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.11 Summary of fresh semen individual minor defects (%) per THI group in different seasons when collected.

	Distal protoplasmic droplet		Mid-piece Abnormalities		Tail abnormalities (Curled Distal)		Head abnormalities		Loose heads	
	THI 2	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**
Autumn	3.48 ± 0.20	3.65 ± 0.10 ^{ab}	0.89 ± 0.037 ^{a}	0.87 ± 0.022 ^{a}	1.82 ± 0.21	1.90 ± 0.092 ^{ab}	1.52 ± 0.095	1.56 ± 0.042	4.53 ± 0.17 ^{a}	4.13 ± 0.099 ^{b}
Spring	3.24 ± 0.26	3.54 ± 0.06 ^{a}	0.73 ± 0.055 ^{ab}	0.79 ± 0.013 ^{b}	1.93 ± 0.27	1.93 ± 0.052 ^{ab}	1.67 ± 0.14	1.59 ± 0.026	3.52 ± 0.25 ^{b}	3.20 ± 0.061 ^{a}
Summer	4.00 ± 3.92	3.95 ± 0.12 ^{b}		0.87 ± 0.027 ^{ab}		1.70 ± 0.12 ^{a}		1.56 ± 0.053	3.00 ± 3.76 ^{ab}	3.088 ± 0.13 ^{a}
Winter	3.65 ± 0.07	3.94 ± 0.21 ^{ab}	0.78 ± 0.014 ^{b}	0.74 ± 0.046 ^{ab}	2.010 ± 0.072	2.40 ± 0.18 ^{b}	1.71 ± 0.034	1.64 ± 0.081	3.35 ± 0.066 ^{b}	3.39 ± 0.22 ^{a}

All results are expressed as mean ± SD.
^{ab} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.12 Summary of fresh semen individual minor defects (%) per THI group in different seasons during spermatogenesis.

	Distal protoplasmic droplet		Mid-piece Abnormalities		Tail abnormalities (Curled Distal)		Head abnormalities		Loose heads	
	THI 2	THI 3	THI 2	THI 3	THI 2	THI 3	THI 2	THI 3	THI 2	THI 3
Autumn	3.52 ± 0.14	3.76 ± 0.092 ^{ab}	0.74 ± 0.023 ^{a}	0.86 ± 0.021	2.15 ± 0.11 ^{a}	2.034 ± 0.09	1.50 ± 0.066 ^{a}	1.66 ± 0.044	3.77 ± 0.11 ^{a}	3.90 ± 0.09 ^{a}
Spring	3.71 ± 0.29	3.44 ± 0.089 ^{a}	0.92 ± 0.052 ^{b}	0.80 ± 0.02	1.77 ± 0.30 ^{ab}	1.99 ± 0.085	1.71 ± 0.12 ^{ab}	1.57 ± 0.04	3.27 ± 0.26 ^{ab}	3.051 ± 0.088 ^{b}
Summer	2.00 ± 4.00	3.90 ± 0.11 ^{b}		0.85 ± 0.025		1.85 ± 0.11		1.50 ± 0.047	1.00 ± 3.47 ^{ab}	3.83 ± 0.11 ^{a}
Winter	3.66 ± 0.069	3.39 ± 0.16 ^{ab}	0.77 ± 0.012 ^{a}	0.81 ± 0.032	1.81 ± 0.067 ^{b}	2.12 ± 0.15	1.70 ± 0.03 ^{b}	1.65 ± 0.066	3.18 ± 0.064 ^{b}	3.077 ± 0.17 ^{b}

All results are expressed as mean ± SD.
^{ab} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.13 Summary of fresh semen individual minor defects (%) per THI group at each year when collected.

	Distal protoplasmic droplet		Mid-piece Abnormalities		Tail abnormalities (Curled Distal)	Head abnormalities		Loose heads	
	THI 2	THI 3	THI 2	THI 3	THI 3	THI 2	THI 3	THI 2	THI 3
2010	3.58 ± 0.34 ^{abc}	4.15 ± 0.16 ^{ac}	0.90 ± 0.13 ^{ab}	0.88 ± 0.056 ^{abc}	2.76 ± 0.14 ^{b}	2.10 ± 0.24 ^{ab}	1.92 ± 0.10 ^{ab}	3.027 ± 0.36 ^{ab}	3.042 ± 0.16 ^{ac}
2011	2.89 ± 0.17 ^{a}	3.20 ± 0.11 ^{b}	0.81 ± 0.025 ^{ab}	0.72 ± 0.024 ^{a}	1.95 ± 0.090 ^{a}	1.22 ± 0.099 ^{b}	1.29 ± 0.056 ^{c}	4.074 ± 0.12 ^{b}	3.87 ± 0.097 ^{d}
2012	3.81 ± 0.15 ^{bc}	3.49 ± 0.12 ^{ab}	0.65 ± 0.04 ^{a}	0.72 ± 0.029 ^{a}	1.72 ± 0.10 ^{a}	1.12 ± 0.16 ^{b}	1.50 ± 0.063 ^{acd}	3.49 ± 0.16 ^{b}	3.77 ± 0.11 ^{cd}
2013	3.29 ± 0.15 ^{ab}	3.33 ± 0.14 ^{b}	0.79 ± 0.025 ^{ab}	0.80 ± 0.025 ^{ab}	1.84 ± 0.11 ^{a}	1.88 ± 0.049 ^{a}	1.79 ± 0.042 ^{b}	2.085 ± 0.14 ^{a}	2.14 ± 0.15 ^{b}
2014	3.66 ± 0.17 ^{abc}	3.64 ± 0.11 ^{abc}	0.82 ± 0.045 ^{ab}	0.80 ± 0.023 ^{ab}	1.99 ± 0.10 ^{a}	1.97 ± 0.083 ^{a}	1.66 ± 0.041 ^{abd}	3.78 ± 0.16 ^{b}	2.63 ± 0.12 ^{ab}
2015	4.36 ± 0.17 ^{c}	4.14 ± 0.13 ^{c}	0.80 ± 0.035 ^{ab}	0.87 ± 0.025 ^{bc}	1.86 ± 0.11 ^{a}	1.61 ± 0.082 ^{ab}	1.46 ± 0.054 ^{cd}	3.34 ± 0.19 ^{b}	3.40 ± 0.14 ^{cd}
2016	3.67 ± 0.17 ^{abc}	3.66 ± 0.14 ^{abc}	0.85 ± 0.032 ^{b}	0.94 ± 0.026 ^{c}	1.48 ± 0.14 ^{a}	1.39 ± 0.091 ^{b}	1.45 ± 0.063 ^{cd}	4.00 ± 0.16 ^{b}	3.50 ± 0.15 ^{cd}

All results are expressed as mean ± SD.
^{abcd} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.14 Summary of fresh semen individual minor defects (%) per THI group at each year during spermatogenesis.

	Distal protoplasmic droplet		Mid-piece Abnormalities		Tail abnormalities (Curled Distal)		Head abnormalities		Loose heads	
	THI 2	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**
2010	4.031 ± 0.21 ^{b}	4.092 ± 0.20 ^{acd}	0.83 ± 0.057 ^{abc}	0.96 ± 0.084 ^{ab}	2.64 ± 0.19 ^{a}	2.55 ± 0.21 ^{a}	2.04 ± 0.12 ^{a}	1.70 ± 0.15 ^{ab}	2.85 ± 0.21 ^{c}	3.17 ± 0.22 ^{a}
2011	3.00 ± 0.16 ^{a}	3.069 ± 0.12 ^{b}	0.72 ± 0.023 ^{ab}	0.77 ± 0.025 ^{a}	1.84 ± 0.11 ^{ab}	1.96 ± 0.10 ^{ab}	1.21 ± 0.079 ^{b}	1.29 ± 0.062 ^{b}	4.14 ± 0.11 ^{a}	3.83 ± 0.10 ^{a}
2012	3.71 ± 0.12 ^{ab}	3.50 ± 0.15 ^{abcd}	0.68 ± 0.026 ^{a}	0.77 ± 0.044 ^{ab}	1.67 ± 0.13 ^{b}	2.28 ± 0.14 ^{ab}	1.29 ± 0.096 ^{b}	1.62 ± 0.076 ^{ab}	3.82 ± 0.11 ^{ab}	3.12 ± 0.17 ^{a}
2013	3.42 ± 0.16 ^{ab}	3.22 ± 0.15 ^{ab}	0.81 ± 0.024 ^{bc}	0.80 ± 0.027 ^{ab}	1.66 ± 0.14 ^{b}	1.86 ± 0.13 ^{ab}	1.92 ± 0.044 ^{a}	1.78 ± 0.047 ^{a}	1.76 ± 0.16 ^{d}	2.13 ± 0.15 ^{b}
2014	3.73 ± 0.15 ^{ab}	3.53 ± 0.14 ^{abc}	0.77 ± 0.027 ^{abc}	0.85 ± 0.035 ^{ab}	1.89 ± 0.14 ^{ab}	2.14 ± 0.15 ^{ab}	1.73 ± 0.057 ^{ac}	1.76 ± 0.061 ^{a}	2.73 ± 0.14 ^{c}	3.44 ± 0.14 ^{a}
2015	4.28 ± 0.21 ^{b}	4.22 ± 0.12 ^{d}	0.77 ± 0.038 ^{abc}	0.86 ± 0.025 ^{ab}	2.44 ± 0.23 ^{ab}	1.95 ± 0.12 ^{ab}	1.44 ± 0.094 ^{bc}	1.56 ± 0.054 ^{ab}	3.066 ± 0.22 ^{bc}	3.66 ± 0.13 ^{a}
2016	3.72 ± 0.17 ^{ab}	4.057 ± 0.15 ^{cd}	0.89 ± 0.029 ^{c}	0.92 ± 0.031 ^{b}	1.84 ± 0.21 ^{ab}	1.53 ± 0.16 ^{b}	1.44 ± 0.082 ^{bc}	1.37 ± 0.071 ^{b}	3.11 ± 0.18 ^{bc}	3.83 ± 0.15 ^{a}

All results are expressed as mean ± SD.
^{abcd} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.15 Summary of frozen semen minor defects (%) per province.

	Distal protoplasmic droplet	Mid-piece abnormalities	Loose heads
Frequency	1260	1120	1506
Eastern Cape	2.29 ± 0.28	1.20 ± 0.093	3.011 ± 0.39
Free State	2.63 ± 0.11	0.92 ± 0.040	3.14 ± 0.17
Gauteng	2.66 ± 0.22	0.83 ± 0.080	3.095 ± 0.34
Kwazulu-Natal	3.015 ± 0.28	0.76 ± 0.10	3.23 ± 0.42
Limpopo	2.58 ± 0.18	1.036 ± 0.060	3.20 ± 0.27
Mpumalanga	2.66 ± 0.16	0.84 ± 0.058	3.59 ± 0.24
North West	2.81 ± 0.13	0.91 ± 0.046	3.31 ± 0.21

All results are expressed as mean ± SD.

Table C 2.16 Summary of frozen semen minor defects (%) per breed.

	Distal protoplasmic droplet	Mid-piece abnormalities	Loose heads
BEEFMASTER	2.46 ± 0.17 ^{a}	0.82 ± 0.058	3.20 ± 0.25
BONSMARA	2.53 ± 0.13 ^{a}	1.047 ± 0.044	3.51 ± 0.19
BORAN	2.38 ± 0.19 ^{a}	0.91 ± 0.066	3.42 ± 0.27
BRAHMAN	2.69 ± 0.12 ^{ab}	0.96 ± 0.043	3.45 ± 0.19
HOLSTEINS-FRIES	2.63 ± 0.26 ^{ab}	0.75 ± 0.10	2.27 ± 0.39
SIMBRA	3.22 ± 0.15 ^{b}	0.84 ± 0.053	2.87 ± 0.22

All results are expressed as mean ± SD.
^{ab} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.17 Summary of frozen semen minor defects (%) per THI group at collection.

			Production rate	Distal protoplasmic droplet	Mid-piece abnormalities	Loose heads
THI 1	**Fertile**	< 70 - 72	High			
THI 2	**Decrease fertility**	73 - 78	Decreased	2.54 ± 0.10	0.93 ± 0.035	3.08 ± 0.15
THI 3	**No fertility**	79 - > 80	Zero	2.75 ± 0.08	0.92 ± 0.029	3.36 ± 0.12

All results are expressed as mean ± SD.

Table C 2.18 Summary of frozen semen minor defects (%) per THI group at spermatogenesis.

			Production rate	Distal protoplasmic droplet	Mid-piece abnormalities	Loose heads
THI 1	**Fertile**	< 70 - 72	High			
THI 2	**Decrease fertility**	73 - 78	Decreased	2.65 ± 0.099	0.93 ± 0.033	2.81 ± 0.15 [a]
THI 3	**No fertility**	79 - > 80	Zero	2.75 ± 0.086	0.92 ± 0.030	3.49 ± 0.13 [b]

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.19 Summary of frozen semen minor defects (%) per season at collection.

	Distal protoplasmic droplet	Mid-piece abnormalities	Loose heads
Autumn	2.92 ± 0.14	0.98 ± 0.047	3.71 ± 0.20 [a]
Spring	2.67 ± 0.11	0.92 ± 0.037	3.00 ± 0.17 [b]
Summer	2.61 ± 0.16	0.85 ± 0.059	3.44 ± 0.25 [ab]
Winter	2.57 ± 0.12	0.92 ± 0.04	3.044 ± 0.18 [ab]

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.20 Summary of frozen semen minor defects (%) per season at spermatogenesis.

	Distal protoplasmic droplet	Mid-piece abnormalities	Loose heads
Autumn	2.81 ± 0.13	0.96 ± 0.043	3.41 ± 0.19
Spring	2.66 ± 0.15	0.94 ± 0.054	3.29 ± 0.23
Summer	2.79 ± 0.16	0.93 ± 0.055	3.55 ± 0.25
Winter	2.62 ± 0.11	0.90 ± 0.036	2.86 ± 0.16

All results are expressed as mean ± SD.

Table C 2.21 Summary of frozen semen minor defects (%) per year at collection.

	Distal protoplasmic droplet	Mid-piece abnormalities	Loose heads
2010	2.69 ± 0.22 ^{abc}	1.00 ± 0.081	3.32 ± 0.32 ^{ab}
2011	2.038 ± 0.18 ^{a}	0.81 ± 0.058	3.68 ± 0.21 ^{a}
2012	2.16 ± 0.16 ^{ab}	0.79 ± 0.06	3.62 ± 0.22 ^{a}
2013	3.096 ± 0.15 ^{c}	0.97 ± 0.048	2.018 ± 0.29 ^{b}
2014	3.062 ± 0.16 ^{c}	0.93 ± 0.059	2.55 ± 0.27 ^{ab}
2015	3.00 ± 0.17 ^{bc}	1.00 ± 0.059	2.71 ± 0.30 ^{ab}
2016	2.66 ± 0.18 ^{abc}	0.94 ± 0.058	3.49 ± 0.27 ^{ab}

All results are expressed as mean ± SD.
^{abc} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.22 Summary of frozen semen minor defects (%) per year at spermatogenesis.

	Distal protoplasmic droplet	Mid-piece abnormalities	Loose heads
2010	2.78 ± 0.20 ^{ab}	0.97 ± 0.078	3.20 ± 0.30 ^{ab}
2011	1.74 ± 0.19 ^{a}	0.81 ± 0.059	3.77 ± 0.21 ^{a}
2012	2.37 ± 0.17 ^{ab}	0.84 ± 0.061	3.33 ± 0.23 ^{a}
2013	3.19 ± 0.15 ^{b}	0.98 ± 0.05	1.85 ± 0.32 ^{b}
2014	2.94 ± 0.16 ^{b}	0.96 ±0.064	2.55 ± 0.28 ^{ab}
2015	3.041 ± 0.17 ^{b}	0.98 ± 0.061	3.00 ± 0.30 ^{ab}
2016	2.70 ± 0.18 ^{ab}	0.95 ± 0.058	3.32 ± 0.27 ^{ab}

All results are expressed as mean ± SD.
^{ab} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.23 Summary of frozen semen individual minor defects (%) per THI group in different provinces.

	Distal protoplasmic droplets		Mid-piece abnormalities		Abnormal Loose heads	
	THI 2	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**
Frequency	465	795	443	677	600	906
Eastern Cape	2.33 ± 0.29	2.091 ± 0.70	1.22 ± 0.10	1.063 ± 0.26	3.13 ± 0.37	2.31 ± 1.11
Free State	2.57 ± 0.17	2.67 ± 0.15	0.93 ± 0.063	0.91 ± 0.054	2.89 ± 0.23	3.33 ± 0.24
Gauteng	2.23 ± 0.38	2.85 ± 0.28	0.73 ± 0.14	0.89 ± 0.099	2.98 ± 0.49	3.17 ± 0.47
Kwazulu-Natal	3.063 ± 0.37	2.97 ± 0.41	0.77 ± 0.16	0.76 ± 0.13	2.97 ± 0.56	3.43 ± 0.60
Limpopo	1.73 ± 0.54	2.67 ± 0.19	1.056 ± 0.18	1.033 ± 0.063	3.48 ± 0.71	3.17 ± 0.31
Mpumalanga	2.49 ± 0.20	2.89 ± 0.26	0.77 ± 0.081	0.92 ± 0.084	3.11 ± 0.29	4.19 ± 0.39
North West	2.78 ± 0.24	2.82 ± 0.16	1.00 ± 0.089	0.87 ± 0.054	3.44 ± 0.35	3.26 ± 0.27

All results are expressed as mean ± SD.

Table C 2.24 Summary of frozen semen individual minor defects (%) per THI group for each breed.

	Distal protoplasmic droplets		Mid-piece abnormalities		Abnormal Loose heads	
	THI 2	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**
BEEFMASTER	2.48 ± 0.22 [ab]	2.43 ± 0.25	0.77 ± 0.082	0.88 ± 0.084	3.027 ± 0.31	3.40 ± 0.40
BONSMARA	2.43 ± 0.20 [ab]	2.59 ± 0.17	1.061 ± 0.078	1.039 ± 0.055	3.44 ± 0.26	3.56 ± 0.27
BORAN	1.93 ± 0.25 [a]	2.84 ± 0.28	0.91 ± 0.092	0.91 ± 0.099	3.45 ± 0.33	3.39 ± 0.42
BRAHMAN	2.58 ± 0.20 [ab]	2.75 ± 0.15	1.00 ± 0.075	0.94 ± 0.053	3.046 ± 0.28	3.67 ± 0.26
HOLSTEINS-FRIES	3.29 ± 0.51 [ab]	2.44 ± 0.30	0.71 ± 0.22	0.77 ± 0.11	1.85 ± 0.73	2.39 ± 0.48
SIMBRA	3.28 ± 0.27 [b]	3.20 ± 0.18	0.90 ± 0.094	0.80 ± 0.065	2.44 ± 0.35	3.07 ± 0.29

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.25 Summary of frozen semen individual minor defects (%) per THI group in different seasons when collected.

	Distal protoplasmic droplets		Mid-piece abnormalities		Abnormal Loose heads	
	THI 2	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**
Autumn	2.61 ± 0.23	3.045 ± 0.17	0.86 ± 0.083	1.05 ± 0.058	3.14 ± 0.29	4.035 ± 0.27 [a]
Spring	2.51 ± 0.26	2.71 ± 0.12	1.039 ± 0.11	0.90 ± 0.039	3.063 ± 0.37	2.98 ± 0.20 [b]
Summer	1.00 ± 2.15	2.62 ± 0.17	1.00 ± 0.54	0.85 ± 0.058	1.50 ± 2.34	3.45 ± 0.27 [ab]
Winter	2.54 ± 0.12	2.82 ± 0.41	0.93 ± 0.043	0.75 ± 0.15	3.044 ± 0.16	3.032 ± 0.73 [ab]

All results are expressed as mean ± SD.
[ab] Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.26 Summary of frozen semen individual minor defects (%) per THI group in different seasons during spermatogenesis.

	Distal protoplasmic droplets		Mid-piece abnormalities		Abnormal Loose heads	
	THI 2	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**
Autumn	2.96 ± 0.23	2.75 ± 0.16	0.95 ± 0.073	0.96 ± 0.054	3.00 ± 0.22	3.56 ± 0.27
Spring	2.27 ± 0.48	2.70 ± 0.16	1.16 ± 0.17	0.92 ± 0.058	2.68 ± 0.43	3.36 ± 0.31
Summer	8.00 ± 2.24	2.77 ± 0.16	0.50 ± 0.73	0.93 ± 0.057	1.00 ± 2.26	3.57 ± 0.31
Winter	2.58 ± 0.11	2.91 ± 0.31	0.92 ± 0.037	0.75 ± 0.11	2.78 ± 0.10	3.40 ± 0.57

All results are expressed as mean ± SD.

Table C 2.27 Summary of frozen semen individual minor defects (%) per THI group at each year when collected.

	Distal protoplasmic droplets		Mid-piece abnormalities		Abnormal Loose heads	
	THI 2	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**
2010	2.21 ± 0.44 ^{ab}	2.84 ± 0.26	1.17 ± 0.18	0.95 ± 0.091	3.23 ± 0.56 ^{ab}	3.35 ± 0.40
2011	1.70 ± 0.28 ^{a}	2.25 ± 0.24	0.88 ± 0.082	0.72 ± 0.085	3.79 ± 0.28 ^{a}	3.58 ± 0.30
2012	2.093 ± 0.22 ^{ab}	2.23 ± 0.24	0.75 ± 0.098	0.81 ± 0.077	3.36 ± 0.30 ^{a}	3.83 ± 0.32
2013	2.96 ± 0.23 ^{ab}	3.19 ± 0.20	0.93 ± 0.075	1.011 ± 0.063	1.41 ± 0.39 ^{b}	2.48 ± 0.42
2014	3.18 ± 0.25 ^{b}	3.00 ± 0.20	1.088 ± 0.11	0.86 ± 0.072	3.27 ± 0.39 ^{ab}	2.12 ± 0.37
2015	2.66 ± 0.30 ^{ab}	3.15 ± 0.21	0.96 ± 0.10	1.02 ± 0.073	2.12 ± 0.44 ^{ab}	3.051 ± 0.41
2016	2.86 ± 0.26 ^{ab}	2.52 ± 0.24	0.98 ± 0.094	0.91 ± 0.075	2.70 ± 0.36 ^{ab}	4.092 ± 0.39

All results are expressed as mean ± SD.
^{ab} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).

Table C 2.28 Summary of frozen semen individual minor defects (%) per THI group at each year during spermatogenesis.

	Distal protoplasmic droplets		Mid-piece abnormalities		Abnormal Loose heads	
	THI 2	**THI 3**	**THI 2**	**THI 3**	**THI 2**	**THI 3**
2010	2.85 ± 0.29 ^{ab}	2.72 ± 0.28 ^{ab}	1.073 ± 0.099	0.81 ± 0.13	3.26 ± 0.24 ^{ac}	3.14 ± 0.54
2011	1.62 ± 0.29 ^{a}	1.82 ± 0.25 ^{a}	0.68 ± 0.088	0.90 ± 0.079	3.54 ± 0.19 ^{a}	3.97 ± 0.36
2012	1.92 ± 0.27 ^{a}	2.65 ± 0.22 ^{ab}	0.74 ± 0.091	0.91 ± 0.082	3.063 ± 0.21 ^{ac}	3.52 ± 0.38
2013	2.86 ± 0.21 ^{ab}	3.52 ± 0.22 ^{b}	0.96 ± 0.067	1.00 ± 0.073	1.49 ± 0.26 ^{b}	2.26 ± 0.57
2014	3.34 ± 0.24 ^{b}	2.60 ± 0.23 ^{ab}	1.09 ± 0.094	0.85 ± 0.088	2.83 ± 0.26 ^{ac}	2.35 ± 0.45
2015	2.94 ± 0.28 ^{ab}	3.10 ± 0.22 ^{b}	1.069 ± 0.096	0.93 ± 0.08	1.96 ± 0.31 ^{bc}	3.55 ± 0.46
2016	2.60 ± 0.25 ^{ab}	2.79 ± 0.24 ^{ab}	0.97 ± 0.085	0.95 ± 0.08	2.41 ± 0.25 ^{bc}	3.98 ± 0.44

All results are expressed as mean ± SD.
^{abc} Subscripts are used to indicate that the values in each column which have different letters are significantly different ($p < 0.05$).