MAKERERE UNIVERSITY

EFFECT OF PAWPAW JUICE EXTRACT ON THE MICROSCOPIC PARAMETERS OF BUCK EPIDIDYMAL SEMEN

BY

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DECLARATION

I **Daniel Baguma,** declare that the work presented in this report is mine and has never been presented to any academic institution of higher learning for the award of any academic qualification.

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DEDICATION

I dedicate this book to my family (father, mother and siblings) who have supported my dream both morally and financially.

I also dedicate this report to Dr. Mugizi Rwabita for the guidance and supervision throughout my writing. May God bless them abundantly

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I would like to thank the Almighty God for his protection over me that has enabled me progress in my academic journey successfully.

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LIST OF ABBREVIATIONS AND ACRONYMS

AI	Artificial Insemination
BBLT	Bachelor of Biomedical Laboratory Technology
CM	Centimeters
COVAB	College of Veterinary Medicine, animal resources and biosecurity
e.g.,	for example
etc.	and many more
HOST	Hypo-osmotic Swelling Test
Hrs	Hours
i.e.	that`s to say
IVF	Invitro fertilization
Lab	Laboratory
PPM	Percentage of progressive Motile.
ZIFT	Zygote Invitro fertilization

ABSTRACT

Antioxidants are key to sperm viability due to their protective effects against formation the formation of free oxygen radicals and cell damage of spermatozoa during preservation. In order to improve the life span of refrigerated buck semen, this study was carried out to determine the effect of pawpaw juice extract on spermatozoa viability of semen since it is rich in antioxidants. Pooled semen from three indigenous (Mubende) bucks, abuck per week diluted with Tris-egg yolk-based extender and supplemented with pawpaw fruit juice extract at different concentrations of 0, 2, 5 and 7/100ml respectively. Following dilution, the semen samples were assessed subjectively after invitro storage at 5^0 C for 0, 24, 48 and 72 hours as regard sperm motility, abnormality and integrity using a phase-contrast microscope. The results showed highest level of sperm abnormalities in extended semen supplemented with pawpaw juice extract 7/100ml in all time intervals and lowest levels of sperm abnormalities in extended semen supplemented with pawpaw juice extract at 5/100ml at 0hours but higher than the control. This indicated a protective measure in pawpaw juice extract at 5/100ml. The results also showed highest levels of sperm motility in control compared to other samples with time. However better progressive motility was recorded in extended semen supplemented with pawpaw juice extract at 2/100ml compared to all pawpaw juice extract extenders. It is also important to note a sharp decrease in progressive motility in extended semen supplemented with 5/100ml with time when compared with the rest of the samples. The results showed almost a similar level of acrosome integrity in all categories of the semen extenders with time. Highest levels of acrosome integrity were observed in the control sample at 0hours and the least levels of acrosome integrity was observed in extended semen supplemented with pawpaw juice extract at 7/100ml. The findings revealed that the antioxidants in pawpaw fruit have less protective ability to maintain sperm viability during storage of sperms at low temperatures.

CHAPTER ONE

INTRODUCTION

1.1 Background

The goat has a reduced food intake, its feeding is cheap and its being a small animal makes it easy to keep (Monteiro 2017). Therefore, artificial insemination has become a common method of breeding for domestically kept goats in developed countries in Europe and North America (Morell, 2011).

Al has been used in Uganda for over 60 years but only less than 10%, a small population of the country's herd has been bred that way (Eklundh 2013). The lower success rate of AI is mainly due to extender composition, depth of semen deposition, breed and hormone treatment (Mellado *et al.,* 2004 and 2006).

The survival of sperm after collection in seminal plasma for longer periods during preservation at low temperatures requires dilation with appropriate extender in order to maintain viability of spermatozoa. However, viability of spermatozoa detoriates at low temperatures during storage.

Sperm cells are subject to oxidative stress resulting to lipid peroxidation, which can lead to reduced sperm viability and fertility (Donghue and Donoghue, 1997). Although semen contains antioxidants that counteract the damaging effects of lipid peroxidation and prevents excessive peroxide formation (Lewis *et al.,* 1997), the endogenous antioxidative capacity of semen may be not enough during storage (Maxwell and Salamon, 1993).

Laboratory studies indicated that addition of exogenous antioxidants to semen extenders could improve the motility and survival\viability of spermatozoa (Sanchez-Partida *et al.,* 2002).

Fruits being the main source of various natural antioxidants components is a key additive (Cao *et al.,* 1996)

Pawpaw fruit contains antioxidants such as carotene, vitamin C, vitamin B, flavonoids, folate and Panthotenic and proved to function as singlet and triplet oxygen quenchers,

free radical scavengers and peroxide decomposers (Larson, 1988). However, there is limited data regarding freezing or lowering temperature of semen obtained from goat bucks with pawpaw juice extract during storage.

The aim of the study is therefore to determine the effects of adding different levels or concentrations of pawpaw juices to semen extenders on sperm viability of goat bucks during storage at low temperatures of 5^0C.

1.2 Problem Statement

Though many Ugandans are goat keepers, less AI is practiced due to its being costly and low success rate with cryopreserved semen after process. The low success rate of AI is due to low viability of sperms in cryopreserved semen resulting from damaged acrosome heads, poor progressive motility and high levels of abnormal spermatozoa leading to low conception rate in goats. This force farmer to stick on natural mating which is key route to disease spread and poor breed maintenance within the society. There is need to improve the success rate of AI in order to attract farmers to practice the technique. this can be achieved by supplementing semen extenders with antioxidants rich juices such as pawpaw juice that are known to prevent peroxide formation. Pawpaw fruit, a readily available fruit in the market and village homes, at a cheaper cost is a key source of antioxidants such as carotene and vitamins.

Therefore, the study is to determine the effect of supplementing tris-egg yolk extender with pawpaw juice extract on the microscopic parameters of bucks' semen.

1.3 Objective

1.3.1 General Objective

To examine the effect of pawpaw juice on the microscopic parameters of buck epididymal semen during storage at low temperatures.

1.3.2 Specific Objectives

i. To determine the effect of pawpaw juice extract on the morphology of goat sperm during storage at low temperatures.

ii. To evaluate the effect of pawpaw juice extract on the motility of sperms in the goats' semen during storage at low temperatures.

iii. To determine the effect of pawpaw juice extract on the membrane integrity of goat sperm during storage at low temperatures.

1.5 Significance\ Justification

Positive and successful result from the study will help minimize costs on cryopreservation materials and also improve the quality of sperms which will encourage many farmers to practice Al due to improved success rates and minimized losses.

1.6 Research Hypothesis

Supplementation of extender with pawpaw juice extract maintains the morphology of buck's semen stored at low temperatures. Supplementation of extender with pawpaw juice extract maintains the membrane integrity of sperms in goats semen stored at low temperatures.

CHAPTER TWO

LITERATURE REVIEW

2.1 Semen cryopreservation

Semen cryopreservation is also commonly known as sperm freezing which involves the process of lowering temperature (freezing sperm) and storing it for future use, such as AI, improvement of different genetic species and preservation of good quality rare breeds. Cryopreservation of sperms is a sequential process of reduction in temperature, dehydration of cells and freezing of structurally intact cells (Ugur, 2019). The lowering of temperature to 4^0C reduces cellular metabolic activity and increases the life span of sperm cells. Cellular activity resumes after thawing of the sperm cells. (Barbas and Mascarenhas, 2008). Cryopreservation of buck`s semen is a complex process which requires balance of a number of factors in order to achieve optimum results (Gangawar and Jindal, 2016).

Goat semen is reported to be prone to deleterious concussions that are as a result of freezing and cooling. the semen sample in medium includes buffer, a sugar, antibiotics, salt, penetrating and non-penetrating cryoprotectant (Purdy, 2006). The most commonly used penetrating CPA are glycerol, ethylene glycol, dimethyl sulfoxide DMSO and propylene glycol. (Sharma and Sharma, 2020).

2.2 Semen extenders

A semen extender is a liquid diluent that is added to increase volume, preservation and provide sperm cells as a source of energy, protection from biochemical and physical damage as well as maintenance of the immediate environment of a sperm that is suitable for its survival during cryopreservation. A number of extenders prepared both commercially and locally are available with components such as electrolytes, egg yolk, milk, sugars and buffers, used in cryopreservation. It has been noted that glycerol and milk were the first to be used for freezing of semen with glycerol still being used today as a major CPA (Anand *et al.*, 2015).

Semen extenders for buck semen contain buffering system to maintain PH of the medium (Tris, sodium phosphate, citric acid), cryoshock preservatives (glycerin, egg

yolk, soy-lecithin, milk), energy source (fructose) and antibiotics to provide a microbial free environment (Purdy, 2006). Desired skimmed milk of tris glucose based hypertonic diluents are reportedly to be frequently used in buck semen with PH ranges 6.75 to 7.0 as mammalian normal range is 7.2 to 7.8 with the inability to diffuse across cell membranes. Sucrose, lactose, trehalose, raffinos and dextons induce cell dehydration and lower incidences of intracellular ice formation. a greater protective effect has been reported in monosaccharides than in disaccharides. (Gangawar and Jindal, 2016).

2.3 Methods of Epididymal sperm collection

There are a variety of ways to collect epididymal sperm on both live and dead sperm donors. Collection from dead donor (testacles) is much easier because the entire testicle can be easily removed for processing (Shakir *et al.,* 2022). Live donors have to be restrained using a given method of collection i.e. either chemical or physical restrain. The aim is to acquire live and quality sperms to be used in Assisted reproductive technologies (ART) such as artificial insemination (AI), invitro fertilization (IVF), ZIFT, etc. for production of healthy offspring. Therefore, sperm from cauda epididymis can be collected by floating method i.e. cut cauda into pieces and float in plasma solution at 4c (Lima *et al.,* 2013). A method for microsurgical epididymal sperm aspiration (MESA) by Monseny *et al.,* (1994). A small incision was made through the scrotom to expose the epididymis and a single epididymal tubular loop was freed under the surgical microscope. A longitudinal incision was made into the epididymal tubule. TO aspirate sperms, 0.2mm diameter, modified glass capillary attached by a silicon tubes to a reservoir containing the culture medium is connected to another tube connected to a 50ml syringe to create negative pressure (Moni, 1992). The epididymis is a long, highly convoluted tubular duct that lies alongside the testes. It is made up of three parts i.e. head, body and tai. The epididymis acts as a storage area and site or final maturation for sperm cells prior to ejaculation (Bethany, 2019). The major source of energy for epididymal sperm is from fatty acid oxidation involving carnitine-dependants systems (Casillas, 1972).

2.4 Methods of sperm analysis to determine quality

There are various methods to assess sperm (semen) quality and each method attempts to show a correlation to fertility. The conventional measurements of sperm quality and

male fertility are inadequate and any assessment should involve several test of sperm cell function ie increase the fertility prognosis (Purvis and christronsen, 1992). Saacke (1983) classified sperm quality trails into two categories ie viability related traits and morphological traits. (saacke 1983). Viability traits are the parameters that are most affected by physiological or pathological condition and processing by the expert.

2.5 Sperm motility

Sperm motility has been considered as the major criterion of male fertility. Its main objective is to determine the motility of the sperm in the sample (Malmgreno, 1997).

In a study to compare rat caudal sperm and caput sperm motility in IVF success, Caput epididymal sperm moved in a circular motion while caudal sperm moved in a forward pecking motion and their respective fertilization rates were 8 and 93%. Therefore, progressive motility was correlated to IVF fertility (Blandau and Rumery, 1964).

Percentage of progressive motile (PPM) sperm is the percentage of sperm moving in a forward direction (and not a circular movement or stationery). These sperm are more likely to swim to the oocyte, whether *in nitro* or *in vivo*. A number of methods can be used to determine PPM. The traditional method of subjective microscope estimation (Malmgren, 1997). Is cheap and simple but the PPM estimates can vary greatly among and within experts (technician).

This method can be completed with a standard microscope slide and coverslip or with gridded slides marked to aid in the ease of counting. Gridded slides include the Neubauer cytotheter, the Hemocytometer R, disposable slides such as cell-Vu r, etc. using a specialized slide, the technician counts the number of sperm moving forward as well as sperm not moving progressively forward in a predetermined number of squares

2.6 Morphology

Sperm morphology refers to the size, shape and appearance of individual sperm. It's one of, but by no means the only contributing factor to male fertility. Not all of individual sperms look exactly alike. Abnormalities in sperm size and shape can occur in the head, mid piece or tail. (Jennifer Huizen, 2017) In a study to assess morphological

attributes of epididymal spermatozoa from indigenous goat breeds (Mekasha *et al.*, 2007). It was discovered that in caput and corpus the proportion of detached heads proximal droplets, abnormal mid piece and simple bent tails were greatest, simple bent tails were lowest in the cauda epididymis.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

The study was experimental since it involved an intervention which was pawpaw fruit juice extract that is supplemented to extended semen. The juice was added at different concentrations (0/100ml,2/100ml,5/100ml and 7/100ml) and its effectiveness on different sperm parameters were analyzed at different time intervals i.e. 0hrs, 24hrs, 48hrs and 72hrs of storage at low temperatures of 5^0C.

3.2 Description of the study subject

The study involved testes and pawpaw fruit. The testes were collected from Kalerwe market abattoir early in the morning from freshly slaughtered indigenous (Mubende goats) i.e. maximum of 2hrs. The bucks were within a range of 2-3years of age with scrotal circumference of about 25cm and the testes would be freely movable within the scrotal sac. These were much emphasized to the attendants.

The pawpaw fruits in the study were obtained from Kalerwe market from a freshly stocked bundle.

The pawpaw fruits were of medium to large size of fully-grown ripe Vega F1 fruit (reddish flesh) to provide the necessary nutritional requirements and only fresh fruits were collected. The fruits were ripe since ripe fruits are believed to have most antioxidant effect and glucose compared to unripe ones.

3.3 Study area

The goat testes and ripe pawpaw fruits were collected from Kalerwe abattoir and Kalerwe market respectively. This is because Kalerwe market is one of the largest markets along Gayaza road close to Northern by-pass about 5-6kilometers from Kampala city centre. The materials were collected here because the market and abattoir were easily accessible at a cheaper transport cost and the distance from the market to COVAB Assisted Reproductive Laboratory where collection and analysis took place, was short and therefore semen could be collected at a proper time. The COVAB

Assisted Reproductive Laboratory is located in Makerere University on Kampala hill 5km to the north of the city centre.

3.4 Sampling strategies

The buck testes and pawpaw fruit were selected by inclusion and exclusion.

3.5 Inclusion

Testes were collected within 2hours after slaughter in a cool box. Only animals with both and equally sized left and right testes that were in a healthy mood and appearance would be collected from the abattoir. The testes had scrotal circumference of about 25cm and a close range and fully movable within. Pawpaw fruits were collected only when fresh and ripe since they would contain high levels of antioxidants and glucose.

3.6 Exclusion

Testes from goats with signs of delay and not freshly slaughtered were not collected. Wilt pawpaw fruits with signs of stock lasting were not collected.

3.7 Sample size determination

One pair of the testes was collected once in a week for five weeks containing all the desired qualities i.e. scrotal circumference, same size of left and right testes from a freshly slaughtered animal.

Sperms were collected from each and this was extended together.

The extended sperms were supplemented with pawpaw juice extract at different concentrations i.e. 0%, 20%, 50% and 70%.

These were packed in straws with eight (8) straws for each concentration to be analyzed for all microscopic parameters at different time intervals of cryopreservation i.e. 0hrs, 24hrs, 48hrs and 72hrs.

A total of three animals were analyzed with an animal per week.

3.8 Pawpaw juice preparation

The juice extract from pawpaw fruits were produced basing on the procedure by Adenkule with a few modifications (Adekunle *et al.,* 2018). The pawpaw fruit were washed with clean water and then peeled, cut into pieces and remove all seeds. The pawpaw fruit flesh was introduced into the blender to remove the juice.

The blended contents were sieved to obtain clear juice which was put into a clean plastic tube (falcon tubes). The juice was centrifuged at 3000 revolutions per minute for 10 minutes.

The clear supernatant fluid was thereafter decanted into clean tube.

3.9 Sperm collection and processing

After immediate collection of a pair of testis, to the COVAB Assisted Reproduction Laboratory in a cool box, they were washed with clean water and the scrotal sac was cut to expose the testis. The epididymis was cut and separated from the testis (Lima *et al.,* 2013). The lumen of the ductus deferens was calculated with blunted needle loaded with warm normal saline. The cut tissue was suspended over a clean sterile petri-dish. Gently applied pressure to the syringe to allow sperms flow at the caudal (flush) due to pressure of the warm normal saline. (Ferrazm *et al* 2010). The collected sperms were then extended using Tris egg yolk based extender. This was divided into portions and then supplemented with pawpaw juice extract at different concentrations before being packed into straws.

3.10 Data collection methods

3.10.1 Sperm motility

Mass motility of the sperms was assessed after invitro storage by placing a drop of sperms (around 5mm diameter) onto a prewarmed glass slide. This was observed under a phase-contrast microscope under X10 objective lens without a cover slip. Motility was observed under three fields and the mean of the three successive evaluations was recorded as the final motility score (Umar *et al* 2015).

3.10.2 Sperm acrosome integrity

Sperm acrosome integrity was assessed with eosin-nigrosin smears. At the end of every 24hours of storage, 3ul of semen sample was placed on a microscopic slide followed by 2ul of eosin-nigrosin drop. A smear was made and allowed to dry on a slide warmer before evaluation. The proportion of the sperm cells with intact acrosome was estimated under a phase-contrast microscope. The sperms with intact acrosome membrane resisted to be penetrated by the die and therefore appeared white heads.

3.10.3 Morphology(abnormality)

Morphologywas assessed by the use of eosin-nigrosin staining. By preparation of a thin smear with the stain, the morphology of the buck sperm was evaluated under a light microscope using oil emulsion for any abnormality.

3.11 Packaging of sperms

The sperms sample collected were diluted or extended with Tris egg yolk extender and supplemented with pawpaw juice extract at 0, 2, 5 and 7/100ml concentrations.The semen was then packaged in 0.5ml labeled straws using a micro pipette (Lemma 2011).

3.10.5 Storage

The straw was clearly labelled and gently cooled from room temperature up to a temperature of 5^0C, at which they were maintained and only be removed during analysis.

3.11 Data quality control

All laboratory procedures were performed following standard processing procedures (SOPs) of the COVAB Assisted Reproductive Laboratory that were in place. Manufacture's guidelines were key in the use of various reagents and materials to minimize errors. Controls were used during performance of the different laboratory procedures to easily ditracte errors. Labeling was key during the different procedures and packaging to prevent sample loss.

3.12 Data Analysis

Data was analyzed both manually and also using designed technologies.

Manually, basing on the trend of flow of the different means of the parameters with time. Also, with the various software designs, such as SPSS was used in order to provide means, standard deviations error of mean, significant differences and p-values.

3.13 Ethical approval and consideration

Approval of the study was obtained from the institutional ethical review board of the college of Veterinary Medicine Animal Resources and Biosecurity of Makerere university.

3.14 Limitation

Limited and absence of clear data sources about specific items such as data about types of pawpaw fruits.

Lack of funding as the research is individual based.

CHAPTER FOUR

RESULTS

4.1 Effect of pawpaw juice extract on the morphology of goat sperm

The study was set to determine the effect of pawpaw juice extract on goat sperm morphology after cryopreservation (percentage abnormality)**.** The means of percentage abnormalities of different samples are presented in table 1 below. The results showed that spermatozoa extended with 5/100ml pawpaw juice extract had the lowest percentage abnormalities (p<0.05) compared to other pawpaw juice extract concentrations except the control at 0,24,48 and 72hours of storage.

Table 1: Percentage means of sperm abnormalities of different concentrations of pawpaw juice extract during storage at low temperatures

	Pawpaw juice concentration			
Time	**Control (%)**	**2/100ml (%)**	**5/100 ml (%)**	**7/100ml (%)**
0 hours	1.5	2.5	2.0	3.5
24 hours	2.5	5.5	3.5	9.5
48 hours	4.0	7.5	9.5	11.0
72 hours	10	13	12.0	13.5

Table 2: Analysed sperm abnormality means, standard deviations, and mean differences of different concentrations of pawpaw juice extract compared to control

Extract	**Mean ± SD**	**Mean difference**	***p*-value**
2/100 ml	7.1 ± 4.4	2.6	1.000
5/100 ml	6.75 ± 4.8	2.25	1.000
7/100 ml	9.4 ± 4.25	4.9	0.822
Control	4.5 ± 3.8	--	1

4.2 Effect of pawpaw juice extract on the motility of goat sperm

The study was also aimed at determining the effect of pawpaw juice extract on goat sperm motility after storage at different time intervals. The results showed highest

progressive motility in the control at 0hours and lowest progressive motility at 0hours was seen in bucks semen supplemented with pawpaw juice extract at 7/100ml. For each of the samples there was continuous decrease in progressive motility with time and the decrease was less in the control but highest in sample supplemented with pawpaw juice extract of 5/100ml. all samples supplemented with pawpaw juice extract had a lower progressive motility compared to the control. There is no significant difference between each of the samples supplemented with pawpaw juice extract and the control (p<0.05).

Table 3: Percentage means of sperm progressive motility of different concentrations of pawpaw juice extract during storage at low temperatures

	Pawpaw juice concentration			
Time	**Control**	**2/100ml**	**5/100 ml**	**7/100ml**
0 hours	90.5	74.0	69.0	67.5
24 hours	76.5	49.0	45.0	37.5
48 hours	65.5	32.5	10.0	7.5
72 hours	50	0	0	0

Table 4: Analysed sperm motility means, standard deviations, and mean differences of different concentrations of pawpaw juice extract compared to control

Extract	**Mean ± SD**	**Mean difference**	***p*-value**
2/100 ml	38.9 ± 31.0	-31.8	0.838
5/100 ml	31 ± 31.8	-39.6	0.431
7/100 ml	28.1 ± 30.8	-42.5	0.335
Control	70.6 ± 17.1	--	1

4.3 Effect of pawpaw juice extract on the integrity of goat sperm

The effect of different concentrations of pawpaw juice extract on acrosome integrity was also determined and the means are presented in table 5. The percentage of intact acrosome were almost similar among the different concentrations with time. However, it is important to note that the 2/100ml had the highest proportion (%) of sperms with intact acrosome compared to the rest of the pawpaw juice concentrations. This was

followed by 5/100ml at (86.9%) while 7/100ml had the least proportion (83.5%) of intact acrosome**.**

Table 5: Percentage means of sperm acrosome integrity of different concentrations of pawpaw juice extract during storage at low temperatures

	Pawpaw juice concentration			
Time	**Control**	**2/100ml**	**5/100 ml**	**7/100ml**
0 hours	97.0	93.0	92	90.5
24 hours	92.5	89.5	87.0	86.5
48 hours	87.5	86.0	85.0	81.5
72 hours	81.5	79.0	79.5	75.5

Table 6: Analysed sperm integrity means, standard deviations, and mean differences of different concentrations of pawpaw juice extract compared to control

Extract	**Mean ± SD**	**Mean difference**	***p*-value**
2/100 ml	86.9 ± 6.0	-2.7	1.000
5/100 ml	85.9 ± 5.1	-3.7	1.000
7/100 ml	83.5 ± 6.5	-6.1	1.000
Control	89.6 ± 6.7	--	1

CHAPHTER FIVE

DISCUSSION

Artificial insemination is one of the fastest emerging breeding methods. Despite its success elsewhere, it is rarely practiced in Uganda and where used it has shown low success rates probably caused by low sperm viability as a result of lipid peroxidation. In line with this, pawpaw juice has the capacity to counteract the accumulation of free oxygen radicals and lipid peroxides as it is known as one of the rich fruits in Vitamins A and E which are good antioxidants. Therefore, this research aimed at determining the effects of pawpaw juice extract on the microscopic parameters of goat sperm.

The present study revealed that there was a higher level of sperm abnormalities in the various concentrations of the extended semen supplemented with pawpaw juice extract in comparison to the control. The observed proportion of sperm abnormalities in this study were within the normal ranges of post-thawed goat semen studied by Brazillian College of Animal Reproduction (Henry and Neves,1998). A study by Revell, (2003) indicated that semen processing does not necessarily increase the proportion of sperm abnormalities, this contradicts the results of this study. The increasing level of sperm abnormalities might be due to the different processing procedures and transfers.

The study also showed a decrease in motility of sperms in the various levels of extenders compared to the control at 0, 24, 48, and 72hours. The reason for the decreasing motility (low sperm motility) as higher levels of pawpaw juice extract was being added to the extended semen sample is unclear. Fresh ripe pawpaw fruits were used in this present study, thus the activity of enzyme papain, a protein dissolving enzyme in pawpaw could not have been accounted for the reduced motility as enzyme papain is only found in unripe pawpaw fruits (Hewitt *et al.*, 2002). This is known to destroy cells in the first step of cell culture preparations, dissociate extracellular matrix molecules holding the cells together thus its activity leads to complete lysis of cells. (Lopes *et al*., 2007). However, the possible reason for the decreasing or low sperm motility might have been due to toxicity and environmental shock as the activity of the juice should have possibly reached the higher level than necessary to promote improvement in sperm motility. Despite having a protective effect, some compositions in semen diluents at higher concentrations can become harmful to spermatozoa due to

their potential toxicity (Futino *et al*., 2010). the reducing potential and radical scavenging ability are highest in ripe and lowest in over ripe pawpaw fruit (Harris, 2008), pawpaw used in this present study was fully ripe which could also account for the low sperm motility.

The present study also revealed a similar percentage of intact acrosome among the various concentrations of semen samples and the control. Appreciable values comparable to the control group were obtained for intact acrosome following supplementation of extended semen with pawpaw juice extract indicating reduced damage to sperm cells during storage. The results obtained are in line with the protective ability of Vitamin C, E and carotenes in pawpaw juice. This agreed with the work of (Thuwanut *et al*., 2008), who reported that vitamin E supplementation of tris-egg yolk extender improved the motility and integrity of the sperm membrane and DNA of frozen-thawed epididymal cat spermatozoa. Also, supplementation of freezing extender with antioxidants, especially vitamin E, has been reported to have beneficial effects on acrosome integrity (Andrabi *et al.*,2008). Low level pawpaw juice extraction probably 2/100ml has the highest proportions of spermatozoa integrity and the level of integrity declines with increasing levels of pawpaw juice extract which is in line with (Futino *et al.*,2010) i.e. some semen diluents at higher levels can become harmful.

CHAPHTER SIX

CONCLUSIONS AND RECOMMENDATION

6.1 Conclusion

The findings of the study revealed that supplementation of extended semen with pawpaw juice extract at a concentration of 5/100ml maintained sperm morphology i.e decreased sperm abnormality with storage and also maintained sperm integrity though with a high decrease in sperm motility**.**

However, the findings of this study showed that extension of spermatozoa with different concentrations of pawpaw juice extracts had high levels of sperm abnormalities, low sperm motility and low sperm integrity compared to the control. This is possibly because only one type of pawpaw fruit was used in this study.

6.2 Recommendations

From the study, it is therefore recommended that supplementation of semen with an external source of anti-oxidant during extension should be studied to come up with better solutions that can improve on sperm viability.

Also, since the study involved a single specie of the pawpaw fruit it is therefore recommended that studies on various species of pawpaw fruit be carried out to come up with the best solution to improve sperm viability.

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