PROSTAGLANDIN VERSUS PROGESTAGEN PROTOCOLS IN OESTRUS SYNCHRONIZATION IN THE YANKASA EWE

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN THERIOGENOLOGY

DEPARTMENT OF THERIOGENOLOGY AND PRODUCTION
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NIGERIA

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DECLARATION

I declare that the work in this thesis entitled " **Prostaglandin versus Progestagen Protocols in Oestrus Synchronization in the Yankasa Ewe**'' was carried out by me in the Department of Theriogenology and Production, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria under the supervision of Professor E. K. Bawa, Dr. A. I. Nwannenna and Dr. T. Aluwong**.** The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been previously presented for another degree or diploma at this or other Tertiary institution.

Friday Audu DANJUMA

Name of student	Signature	Date

CERTIFICATION

This thesis entitled '**PROSTAGLANDIN VERSUS PROGESTAGEN PROTOCOLS IN OESTRUS SYNCHRONIZATION IN THE YANKASA EWE**' by Friday Audu DANJUMA meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to Almighty God for His abundant grace, blessings and gift of life towards the achievement of this work.

To my loved ones, SP (Rtd) Frank Danjuma Audu, Mrs Hajaratu Audu, Anthony Danjuma, Joseph Danjuma, Kurutsi Audu Danjuma, and Rose Yusuf, for their encouragement, support, and prayers.

To the entire staff and post graduate students of the Department of Theriogenology and Production, Ahmadu Bello University, Zaria.

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ABSTRACT

This study was carried out to compare the effects of prostaglandins (Lutalyse® and estroPLAN®) and progestagen (CIDR® and FGA-45®) in the synchronization of oestrus in Yankasa ewes. Cycling Yankasa ewes (n = 26), aged 1 to 4 years and weighing 14-28 kg with body condition scores of 2.5-3.5 were used in this study. They were randomly allocated into four groups: Group I ([YK-LT], n = 7) ewes were treated with Lutalyse®, Group II ([YK-ET], n = 7) with estroPLAN®, Group III ([YK-CD], n = 5) with CIDR® and Group IV ([YK-FG], n = 7) with Fluorogestone acetate-45®. The prostaglandin treatments were 2 intramuscular injections given 12 days apart while the progestagens were a-12-day intravaginal implants. Natural breeding was carried out using matured, sexually active proven rams for 5 days following the second prostaglandin injection in groups I and II and progestagen withdrawal in groups III and IV. Breeding was used to evaluate the effect of treatment in each group of ewes on parameters of oestrus (oestrus response rate, time-to-onset of oestrus, duration of oestrus, mean-mount per oestrus-detection-time and tightness of oestrus synchrony), conception rate (non-return to oetrus), pregnancy rate or progesterone profile. The results showed that oestrus response was 85.7 %, 100 %, 100%, 100 %, in the YK-LT, YK-ET, YK-CD and YK-FG groups, respectively. Time to onset of oestrus was 68.23 ± 17.28 h, 49.29 ± 4.48 h, 33.48 ± 8.58 h and 53.27 ± 12.33 h in the YK-LT, YK-ET, YK-CD and YK-FG, respectively. Duration of oestrus was 51.35 ± 13.9 h, 31.19 ± 7.9 h, 34.85 ± 8.3 h and 24.90 ± 6.3 h in the YK-LT, YK-ET, YK-CD and YK-FG groups, respectively. Mounts per oestrus period were 24.6 ± 5.9, 14.3 ± 4.3, 23.2 ± 6.8 and 14.7 ± 4.6 in the YK-LT, YK-ET, YK-CD and YK-FG groups, respectively. Conception rate equaled pregnancy rate and was 100 %, 100 %, 100 % and 86% in the YK-LT, YK-ET, YK-CD and YK-FG groups, respectively. The average serum progesterone (P_4) concentrations 21 days post breeding were, 1.5 ± 0.54 ng/ml vs 2.7 ±0.35 ng/ml, 1.1 ± 0.28 ng/ml vs 3.1 ± 0.81

ng/ml, 1.1 ± 0.47 ng/ml vs 3.2 ± 0.44 ng/ml and 0.87 ± 0.38 ng/ml vs 2.3 ± 0.98 ng/ml in the YK-LT, YK-ET, YK-CD and YK-FG groups, respectively. Both protocols were efficient in oestrus synchronization. However, estroPLAN® and CIDR® offered the best results in synchronizing oestrus in Yankasa ewes and may be used by farmers to enhance productivity.

TABLE OF CONTENTS

TITLE **PAGES**

LIST OF TABLES

TITLE **PAGE**

LIST OF FIGURES

TITLE **PAGE**

LIST OF APPENDICES

TITLE **PAGE**

LIST OF ABBREVIATIONS

AI	Artificial insemination
BCS	Body Condition Score
CIDR	Controlled Internal Drug Release
CL	*Corpus Luteum*
CR	Conception Rate
d	Day
eCG	Equine Chorionic Gonadotrophin
FGA	Flourogestone Acetate
FSH	Follicle Stimulating Hormone
GnRH	Gonadotrophin Releasing Hormone
h	Hour
i.m.	Intramuscular
IU	International Unit
Kg	Kilogram
LH	Luteinizing Hormone
MAP	Methyl Acetoxy progesterone
µ	Microgram
Mg	Milligram
MGA	Melengestrol Acetate
Min.	Minute
ml.	Millilitre
n	Number
NAPRI	National Animal Production Research Institute
ng.	Nanogram
P4	Progesterone

$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PMSG	Pregnant Mare Serum Gonadotrophin
PR	Pregnancy Rate
PRID	Progesterone-Releasing Intravaginal Device
RIA	Radioimmunoassay
rpm	Rotation per minute
SEM	Standard Error of the Mean
WAD	West African Dwarf

CHAPTER ONE

INTRODUCTION

1.1 Background

Oestrous synchronization is a process aimed at bringing animals to oestrus (heat) at a desired time by use of exogenous hormones to manipulate the life span of the corpus luteum (CL) and (or) to modulate follicular development (Siriwat, 2011). Oestrous synchronization increases the number of females pregnant early in the breeding season, which results in a shorter lambing season and a more uniform lamb crop at weaning (Siriwat, 2011). Oestrus synchronization is a valuable management tool that has been successfully employed to enhance reproductive efficiency in ruminants (Kusina *et al.,* 2000). In small ruminants, it has been practiced for the last few decades. Many protocols have been developed to either shorten the luteal phase of the oestrus cycle with prostaglandins $F_{2\alpha}$ (Akusu and Egbunike, 1984; Ungerfeld and Rubianes, 2011) or prolong the luteal phase with exogenous progesterone (Gordon, 1975; Kruip and Brand, 1975; Oyedipe *et al.,* 1989; Kusina *et al.,* 2000; Omontese *et al.,* 2010). Several protocols using a combination of progesterone, gonadotrophins and prostaglandins have also been applied in sheep (Oyedipe *et al.,* 1989; Beck *et al.,* 1996; Zarkawi, 2001; Husein and Kridi, 2002; Jafar *et al.,* 2011).

Sheep is one of the principal domesticated small ruminant animals and they provide food and fibre products (Winrock International, 1983). They have lower feed requirements, compared to cattle due to their body size (Okunlola *et al*., 2010), which allows their integration into different farming systems (Hirpa and Abebe, 2008). Sheep play a significant role in the food chain and overall livelihoods of rural households,

where they are largely the property of women and children (Lebbie, 2004). The world population of sheep has been estimated as 1,078,948,201 (Food and Agricultural Organization, 2010). In Nigeria, sheep population is currently estimated at 33.9 million, making up 3.1% of the world's total (Food and Agriculture Organization of the United Nations, 2011). In Africa, majority of the sheep population is owned by individuals or families in rural areas and the number per group is small (Okunlola *et al.,* 2010). Traditionally, sheep serve as means of ready cash and as reserve against economic and agricultural production hardship (Hamito, 2008). Yankasa sheep is the most numerous breed of sheep in Nigeria, and having the widest distribution, it is found throughout the sub-humid and semi-arid zones (Federal Department of Livestock and Pest Control Services, 1991). It is estimated to constitute about 60% of the sheep population in Nigeria (Osinowo, 1992). Other breeds of sheep are Uda, Balami and West African Dwarf (WAD) sheep (Adebambo *et al.,* 2000; Ozung *et al.,* 2011). Fertility rates following oestrus synchronization have been attributed to factors such as heredity, breed, seasonality, age, environment, nutrition, diseases, semen quality, female reproductive status and hormonal treatment (Oyedipe *et al.,* 1989; Beck *et al.,* 1996; Lewis *et al.,* 1996; Husein *et al.,* 1998; Webb *et al.,* 2004; Yavuzer, 2005).

1.2 Statement of the Research Problem

In Nigeria, human consumption of animal protein is below the recommended 28 g/person/day for a balance diet due to under-production (Ibe, 2004). A deliberate and conscious effort is needed to improve livestock production so as to increase protein supply. To control reproduction in ewes, it is important to adopt advanced pre-induced ovulation at a specific time and to achieve artificial insemination or natural service with satisfactory results (Cognie, 1988).

A wide variety of protocols have been used for synchronization of oestrus in sheep (Ungerfeld and Rubianes, 2011). This ranges from intravaginal progestagen sponges inserted for 12 to 14 days followed by equine chorionic gonadotrophin (eCG) at sponge removal to the use of prostaglandins (Gordon, 1975; Oyedipe *et al.,* 1989; Zarkawi, 2001; Ungerfeld and Rubianes, 2011). Treatment of ewes with intravaginal sponges impregnated with progestagen (flurogestone acetate, FGA) or intravaginal device-containing progesterone, controlled internal drug release, (CIDR) for a period of 10 -16 days, with or without intramuscular injection of pregnant mare serum gonadotrophin (PMSG) at removal of intravaginal device, has been successfully used to improve reproductive performance (Kohno *et al.,* 2005; Omontese *et al.,* 2010; Jafar *et al.,* 2011).

Treatment of ewes with intravaginal sponges impregnated with methyl acetoxy progesterone (MAP) for 14 days, intravaginal device containing progesterone (Controlled Internal Drug Release, CIDR) for a period of 12 days and intramuscular administration of pregnant mare's serum gonadotrophin (PMSG) at device removal as

well as the use of prostaglandins in two intramuscular administrations 11 days apart have been successfully used to improve the reproductive performance (Hackett *et al.*, 1981; Jafar *et al.,* 2011). Comparative effect of different prostaglandins (estroPLAN® and Lutalyse®) and progestagens (CIDR® and FGA-45®) on oestrus response and reproductive performance in Yankasa sheep is yet to be evaluated in Nigeria.

1.3 Justification of the Study

Yankasa sheep constitute the largest sheep population in the northern part of Nigeria (Blench, 1999). Efforts to improve their reproductive efficiency through oestrus synchronization may improve the herd size as well as value for farmers. The application of assisted reproductive technologies (ARTs) such as oestrus synchronization will help improve reproductive performance in Yankasa sheep.

Significant increase in litter size has been reported following treatment with progestagens and prostaglandin $F_{2\alpha}$ or their analogues in other breeds of sheep (Ungerfeld and Rubianes, 2011; Jafar *et al.,* 2011). However, information regarding comparative oestrus synchronization efficiency and fertility in Yankasa breed of sheep induced by different hormone treatments (progestagen or prostaglandin) is sketchy in Nigeria. Combination protocols of oestrus synchronization in most cases are expensive for farmers. More so, data on the efficiency of single oestrus synchronization treatment protocols in the Yankasa breed of sheep is scarce. This means that information regarding comparative oestrus synchronization efficiency using single hormone treatment (prostaglandins or progestagens) in Yankasa reed will be useful in the design of an intensive and cost- effective breeding programme. This study will also provide

base-line data on reproductive performance in Yankasa ewes treated with different prostaglandins and progestagens.

1.4 Aim of the Study

The aim of this study was to compare the effectiveness of different prostaglandin (Lutalyse® and estroPLAN®) and progestagen (CIDR® and FGA-45®) treatment protocols in oestrus synchronization in Yankasa breed of sheep.

1.5 Objectives of the Study

To compare:

1. Effects of two different prostaglandin (Lutalyse® and estroPLAN®) and progestagen (CIDR® and FGA-45®) treatment protocols on oestrus parameters of Yankasa sheep.
2. Effects of two different prostaglandin (Lutalyse® and estroPLAN®) and progestagen (CIDR® and FGA-45®) treatment protocols on conception and pregnancy rates of Yankasa sheep.
3. Progesterone profile of Yankasa sheep following treatment with two different prostaglandin (Lutalyse® and estroPLAN®) and progestagen (CIDR® and FGA-45®).

CHAPTER TWO

LITERATURE REVIEW

2.1 Sheep

The origin of domestic sheep is not well known, but it is generally believed that sheep domestication occurred shortly after goat domestication (probably 7000 to 10000 years ago) in the Fertile Crescent of the Middle East (Groves and Leslie, 2011). Genetic studies have not yet provided a clear indication of the wild ancestors of modern domestic sheep, although progress has been made in addressing this question. The Anatolian Sheep or Asiatic Mouflon (*Ovis gmelini*) is likely the ancestor of domestic sheep and the European Mouflon (*O. musimon* or *O. orientalis musimon*), the feral descendants of the first domestic sheep brought to Europe (Rezaei *et al*., 2010; Groves and Leslie, 2011).

However, sheep is one of the principal domesticated small ruminant animal and they provide food and fibre products (Winrock International, 1983). Domestic sheep (*Ovis aries*) have long been important to humans for their milk, meat and wool (Payne, 1990). It has been estimated that there are about 1,495 breeds of sheep in the world (Food and Agricultural Organization, 2000). Meat producing ewes typically lactate for 90-150 days, while dairy breed ewes will lactate for 120-240 days, thereby increasing milk yield per ewe per year (Ricketts *et al.,* 1993).

2.2 Nigerian Breeds of Sheep

Nigeria has basically four definitive breeds of sheep: Yankasa, Balami, Uda, and the WAD (Adebambo *et al.,* 2000; Ozung *et al.,* 2011). The domestic sheep vary in size, shape, nature of colour coat, productivity and adaptability to different environmental conditions. The size and shape of appendages such as horn, tail, and ear also form some special characteristics possessed by sheep for adaptation to their environment (Devendra and Mcleory, 1982).

2.2.1 Yankasa

The breed is a medium-sized breed of sheep which has a fairly long thin tail and moderately long ears that are somewhat droopy. Yankasa rams have curved horns and a hairy white mane while ewes are polled (Pagot, 1992). They have white coat colour with black patches around the eyes, ears and muzzle. Yankasa rams stand 70 to 80 cm at the withers with the mature females weighing 25 to 40 kg and males weigh between 35 and 60 kg .They can be bred at 7 to 8 months of age (Oyedipe *et al.,* 1989; Pagot, 1992). The milk yield (kg) per lactation is between 30 and 56 kg and has a lactation length average of about 91 days. The peak milk yield per day is 960 grammes (Mason, 1996). The Yankasa sheep is the most numerous breed of sheep in Nigeria and also has the widest distribution, being found throughout the sub-humid and semi-arid zones (Federal Department of Livestock and Pest Control Services, 1991). It is estimated to constitute about 60% of the national sheep population (Osinowo, 1992).

2.2.2 Balami

Balami is the largest bodied native sheep in Nigeria. As a pastoral animal, it is confined to the semi-arid north and it is of great significance for religious ceremonies throughout the middle belt in Nigerian. It is white and hairy with pendulous ears, long-leg and a long thin tail. Rams are horned but ewes are normally polled (Roger, 1999). Another feature that makes the Balami distinctly recognizable is its Roman, bulbous nose that distinguishes it from the Yankasa. It has good potential as a meat producer. The weight of mature males ranges from 40 to 80 kg while that of female lies between 30 and 40 kg. The milk yield per lactation lies between 28 and 33 kg in 70 days (Roger, 1999).

2.2.3 Uda

The Uda is one of the most numerous and widespread sheep breeds in the Sahel region. They are large with straight and long face, long-legged and long tail. They have two distinctive coat colour of brown or black anterior and white posterior. The rams of the Uda are horned and the ewes are usually polled. The Uda is slightly small bodied than the Balami, although their size ranges overlap. They are raised for milk and meat production (Payne, 1990). The Uda is found in northern Nigeria, southern Niger, central Chad, northern Cameroon and western Sudan. The weight of mature females could be 30 to 40 kg while mature rams measure 79 to 97 cm at the withers and weigh 30 to 60 kg. Milk yield per lactation lies between 32 and 36 kg for an average lactation length of 91 days (Mason, 1996).

2.2.4 West african dwarf sheep

This breed is characterized by small body size, dwarf in genetic makeup and are highly trypano-tolerant. They have notable physical and sexual vigour as well as robustness that enable them to withstand the stress of climate, diseases and irregular feeding (Charray *et al.,* 1992). They also have flat and large head as well as thick muscle (Pagot, 1992). The ears are short and dropping. Only the males have horn except in the case of aberration. The coat colour is usually white or mixture of both white and black; their legs are long in proportion to the body size and posses a long tail (Payne, 1990).

Adult males weigh approximately 37 kg, while ewes have mature weight of 25 kg. The females have a short lambing interval and can be bred at the age of 7 to 8 months. The prolificacy of adult ewes is low to moderate ranging from 1.15 to 1.50 lambs per lambing. At less than 100 g per day under good feeding conditions, their growth rate is low and lamb mortality is high (Payne, 1990). West African Dwarf breed of sheep is the most numerous in the humid south western part of Nigeria, a large proportion of which is under extensive management system. The breed is primarily reared for meat (Sowande, 2007).

2.3 Management Systems of Sheep in Nigeria

Sheep management systems in Nigeria have been described to range from free range to tethering in subsistence production to confinement in semi intensive and intensive systems (Weaver, 2005).

2.3.1 Extensive system

This system is a traditional means of keeping sheep where individuals keep a few numbers of sheep with minimal investment but with potentially high returns. This system is characterized by free roaming animals scavenging for food (forages), exposure to high ambient temperatures and loss of body weight. There is also high incidence of diseases and parasitism, losses to stealing, motor accidents, poisoning by crop farmers and conflicts between livestock owners and crop farmers, losses to predators and indiscriminate mating (Weaver, 2005).

2.3.2 Intensive system

This system of management entails complete confinement of the animals either in pastures or in pens where feed and water are provided. Crop residues such as rice straw and bran, cassava peels, and brewer's dry grain are provided. Nutrition is further improved upon by the use of cut – and – carry grasses, legumes or browse supplemented with salt slicks. Under this situation, adequate nutrition is ensured and the welfare of the animals is constantly monitored with full veterinary care being provided. The advantage of this system is effective feed conversion to products of high value such as milk and meat. There is control of reproduction, improved performance and hygienic conditions, collection and use of faecal materials as farm yard manures, reduction in cases of

parasitic diseases, control of sheep against auto-knock downs and little or no damage to the environment (Weaver, 2005; Ozung, *et al*., 2011).

2.3.3 Semi-intensive system

The semi-intensive management system is intermediate between the intensive and the extensive management system. The system involves grazing of the animals on any available herbage during the day and housing them during the night. In this management system however, the sheep is fed in the morning and in the evening. Veterinary service is engaged when animals are observed to be diseased (Weaver, 2005; Ozung, *et al*., 2011).

2.4 Sheep Breeding Methods

Breeding in sheep involves two widely practiced methods namely, natural mating and artificial insemination (Smith *et al.,* 1995; Amoah *et al*., 1996). The natural breeding requires ewe in heat to be mated by a ram. Artificial insemination on the other hand involves collection of semen from a ram to be introduced into the ewe reproductive tract at oestrus.

2.4.1 Natural breeding

This is a practice commonly applied by purebred breeders in meat and fibre sheep production system. In meat production system, productivity is largely a function of the number of offspring born, weaned and the frequency with which they are produced (Boyazoglu *et al.,* 2005). Rams are kept separate from the ewes until at breeding when they are put together in the ratio of 1 ram to 30 ewes. Ewes often will be on heat for 12 to 36 hours and semen can live for about 24 hours in the reproductive tract of the ewe post breeding. The disadvantage of natural breeding is the high cost of buying and

maintaining the rams which is usually higher than the ewes (Boyazoglu *et al.*, 2005). Hand mating technique involves taking females in oestrus to a male for mating at a designated period. This was considered expensive, time consuming, labour intensive and less effective in getting females pregnant than paddock mating. Hand mating can achieve conception rates equal to that achieved in paddock mating only when males are kept away from the females until 48 hours after oestrus synchronization (D'Silver and Peter, 1995).

2.4.2 Artificial insemination

Artificial insemination (AI) is probably the most important single technique which facilitates the genetic improvement of animals. The availability of an efficient sheep AI service would yield similar benefits as in cattle and would greatly enhance the scope for pedigree and commercial breeders to respond positively and effectively to consumer demands. The widespread use of AI and the realisation of its full potential depend essentially on the use of frozen semen and thus, on the availability of techniques that result in acceptable fertility rates (Donovan and Hanrahan, 1999).

The advantage of AI is to maximize the use of outstanding rams thereby eliminating the need for rams on the farm. This will relatively cut down cost, reduce the risk of venereal disease transmission and improve herd management (Donovan and Hanrahan, 1999). The disadvantage of the use of AI includes the high cost of AI equipment, and increased labour in oestrus detection and insemination (Donovan and Hanrahan, 1999).

Four methods of AI have been developed in the sheep: Vaginal, cervical, transcervical and intrauterine (laparoscopic) methods. However, the intrauterine method usually at the end of oestrus is the most preferred (Halbert, *et al.*, 1990; Donovan, *et al.*, 1999).

Enumerated factors at AI known to affect fertility include number of spermatozoa per insemination, time of insemination, method and technique of insemination, experience of inseminator, type of oestrus induction (natural or artificial), female stress, climate and hygiene of the equipment (Donovan and Hanrahan, 1999).

2.5 Seasonality and Ovarian Activity

Reproduction in the sheep is characterized by seasonality in temperate regions of the world. However, most breeds of sheep in the tropics will breed throughout the year. Therefore, reproduction in temperate sheep follows a seasonal pattern by alternating periods of anoestrus and periods of sexual activity (Notter, 2002). During the periods of sexual activity they exhibit multiple oestrus cycles until the end of season or become pregnant. Therefore, they are intermittently polyoestrus or seasonally polyoestrus animals (Notter, 2002).

In temperate regions, initiation and termination of breeding season is predominantly controlled by the day length. The breeding season is initiated as the ratio of daylight to darkness decreases and end when increasing day lengths reach a ratio of nearly equal daylight and darkness. Shorter days induce oestrus in sheep so they are referred to as short day breeders, in contrast to long-day breeders such as the mare (Robinson and Karsch, 1987).

In the tropical zones, sheep tend to breed throughout the year due to less variation in day length. Therefore, there is a gradual loss of seasonality and adaptation to new environment following introduction of temperate breeds into the tropics (Rodrigues *et al.*, 2007). Even though photoperiod is the main determinant of the seasonality, other factors can influence reproductive pattern such as genetic (some breeds are more

resistant to light variation), management practices (male effect) and social causes (Deviche and Small, 2001). Genotype influences the seasonality and sexual activity in the ewe. Seasonality does not only affects mature animals, but also influence the age of attaining puberty. Although genetics plays a major role in the age of attainment of puberty, the season of birth and photoperiod can also allow the animal to come into oestrus at an early age, or delay puberty for several months (Foster, 1981).

2.6 The Oestrous Cycle

Oestrous cycles or sexual activities in non pregnant ewes occur at regular intervals during their breeding season. The oestrus cycle length is 16-17 days with a range of 14-19 days (Hafez, 1966). However, during the transition period between anoestrus and breeding season, shorter cycles of less than 12 days are particularly common and generally the first ovulations of the breeding season are silent, that is, not accompanied by oestrus behaviour (Jordan, 2005).

The oestrus cycle is divided into phases. The follicular phase lasts 3-4 days, while the luteal phase lasts about 14-15 days and is characterized by the maturation of the corpus luteum and high levels of progesterone reaching a maximum peak about 6 days after ovulation (Jordan, 2005).

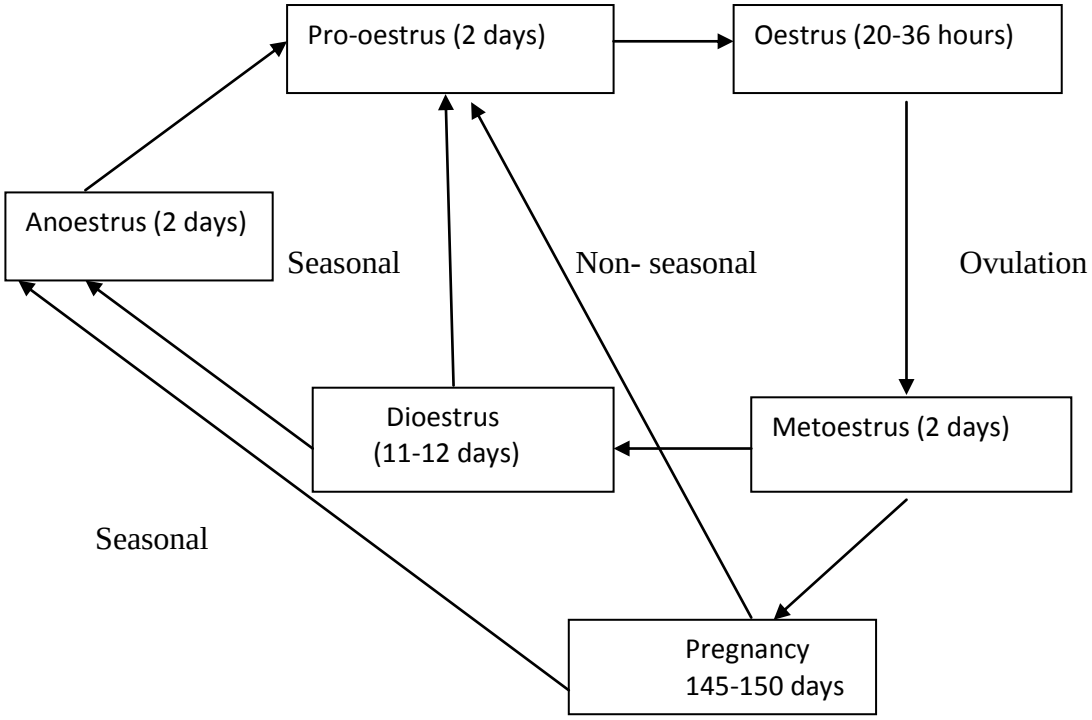


Figure 2.6: The oestrous and reproductive cycles of the ewe. (source; Pineda 1989).

2.6.1 Proestrus

This is the period immediately preceding behavioral oestrus lasting for about two (2) days and is characterized by regression of the *corpus luteum* (CL), decrease in serum progesterone and the emergence and growth of the ovulatory follicle (Goodman, 1994). There is also significant elevation in secretary function in the uterus, oedema in the endometrium and growth of the uterus. The vaginal epithelium is cornified with epithelial layers towards the lumen with increased blood flow to the mucosal layer. Pro-oestrus and oestrus are frequently referred to collectively as the follicular phase of the oestrous cycle (Goodman, 1994).

2.6.2 Oestrus

Oestrus is regarded as the period of sexual receptivity and mating (Goodman, 1994). This is characterized by softening of the cervical stroma, increased blood flow, further thickening of the epithelial layer of vagina and endometrium, increased secretion of watery mucus from the vaginal, cervical and uterine glands. Ovulation in sheep is a spontaneous process that does not require the act of coitus and it occurs 24 to 30 hours after the onset of oestrus behavior (Robertson, 1969).

Oestrus duration varies with age, breed, presence of the male and season ranging between 18 and 72 hours with an average around 36 hours (Hashemi *et al.,* 2006). This is shorter in temperate regions at the beginning and end of the breeding season, in the presence of the male and in the first breeding season of the young females (Hashemi *et al.,* 2006). Compared with other ruminant females, oestrus in the ewe is less apparent. Ewes in oestrus will seek a ram if present, may display tail-wagging and nuzzle his scrotum. If the ram tries to mount them they will stand to be mounted. However, if no

ram is available or if only an inexperienced ram is present oestrus can remain undetected (Bearden and Fuquay, 1984).

2.6.3 Metestrus

Metestrus is the period (2 days) during which the remnants of the ovulated follicle transform into an endocrine gland, the corpus luteum (CL). Luteal cells which make up the *corpus luteum* (CL) develop from the theca and granulose cells of the ovulated follicles. The luteal structure in this phase is also called the corpus haemorrhagicum (Keyes *et al.,* 1983).

2.6.4 Dioestrus

This is the period of oestrous cycle when there is a fully functional CL, secreting large amounts of progesterone and lasting from about 3 to 5 days onwards. Metoestrus and dioestrus are frequently referred to collectively as the luteal phase of the oestrous cycle. Regulation of cyclic activity is mainly under the control of the hypothalamic-pituitary-ovarian axis (Hashemi *et al.,* 2006).

2.7 Endocrinology of the Ovine Oestrous Cycle

2.7.1 Secretion of gonadotrophins

There are two distinct modes of gonadotrophin (follicle stimulating hormone and luteinizing hormone) discharge (Arthur *et al.,* 1989). The gonadotrophin surge lasts 8-12 h and is primarily triggered and sustained by decreased progesterone and increased oestradiol secretion during the final stage of the oestrous cycle (Joseph *et al.,* 1992).

The pulsatile or tonic LH release is generated in response to pulsatile GnRH release from the hypothalamus (Levine and Ramirez, 1982). Pulsatile LH release occurs

throughout the reproductive states in ewes, including the period before, during and after the preovulatory gonadotrophin surge and it is also present in ovariectomized ewes (Rawlings and Cook, 1993). The increase in tonic LH secretion during the pro-oestrous period results from an increase in pulse frequency. Follicle stimulating hormone (FSH) surge has been shown to coincide with the preovulatory LH surge (Baird *et al.,* 1991). The secondary FSH surge, which is lower in amplitude but longer in duration, occurs between 20–36 hours after the preovulatory gonadotrophin surge (Findlay *et al.,* 1990).

Tonic FSH secretion during the ovine oestrous cycle is non-pulsatile as in the case with LH (Wallace and McNeilly, 1985). These rhythmic fluctuations in serum FSH concentrations were previously suggested to be coincidental with waves of follicular growth (Smeaton and Robertson, 1971). This concept of temporal association between FSH peaks and follicular wave emergence was confirmed unequivocally in the experiments in which antral follicular development was monitored by ultrasonography (Bartlewski *et al.,* 1999a; Evans *et al.,* 2000; Evans *et al.,* 2001). During the oestrous cycle in the ewe, about 50% of the pituitary content of FSH is released every day, while only 1 to 5% of LH is secreted in the form of pulses on a daily basis (Taragnat *et al.,* 1998). Luteinizing hormone is stored in secretory granules which are emptied in response to GnRH. While on the other hand, FSH appears to be secreted constitutively, where the rate of secretion closely relates to the rate of synthesis (McNeilly *et al.,* 2003). The mechanisms that control these divergent patterns of gonadotrophin release are not fully understood (Padmanabhan *et al.,* 2003).

2.7.2 Secretion of oestradiol

Oestradiol secretion pattern in the ewe is pulsatile (Baird, 1978). Each pulse of LH is followed by a rise in the secretion of oestradiol-17β in cyclic and anoestrous ewes with

utero-ovarian autotransplants. The main sources of oestradiol are the largest non-atretic follicles (Evans *et al.*, 2000). During the ovine oestrous cycle, there are periods of increased circulating concentrations of oestradiol (Campbell *et al.*, 1995). During antral follicular growth monitoring by ultrasonography, it has been shown that 3 to 4 peaks in serum oestradiol concentrations occur in a cycle, each coinciding with the end of the growth phase of the largest follicle of a follicular wave (Bartlewski *et al.*, 1999b; Souza *et al.*, 1998). On the day of ovulation, peripheral concentrations of oestradiol fall to non-detectable levels, which coincide with the secondary peak of FSH secretion (Baird *et al.*, 1991).

In sheep, oestradiol provides positive feedback during the follicular phase of the oestrous cycle by enhancing GnRH secretion at the hypothalamic level and by increasing the responsiveness of the gonadotrophs to GnRH at the pituitary level (Herman and Adams, 1990). During the luteal phase of the ovine oestrous cycle, pulsatile release of LH is inversely related to circulating levels of luteal progesterone (Wheaton *et al.*, 1992). Oestradiol enhances the inhibitory effects of progesterone on LH secretion by acting primarily at the level of the hypothalamus (Martin *et al.*, 1988).

2.7.3 Secretion of progesterone

Despite the pulsatile secretion pattern of progesterone in the ewe during the luteal phase, progesterone pulses are not temporally associated with LH pulses. Following ovulation, serum progesterone concentrations increase from day 0 to day 11 and then reach a nadir by day 15 after ovulation (Bartlewski *et al.*, 1999b).

Progesterone serum concentrations seem to vary between breeds and some authors have shown that prolific ewes have higher serum concentrations of progesterone compared to

non-prolific breeds, while others have shown that prolific ewes have lower serum progesterone concentrations compared to non-prolific ewes (Bartlewski *et al.,* 1999b). However, the latter observation has been supported by subsequent study, where treatment with $PGF_{2\alpha}$ and an intra-vaginal progestagen sponge, during the mid-luteal phase of the cycle, to create a sub-luteal or low progesterone environment, increased ovulation rate in non-prolific ewes (Bartlewski *et al.,* 2003).

The inhibitory effect of progesterone is all the more pronounced in seasonally anoestrous ewes (Karsch *et al.,* 1987). In ovariectomized ewes, progesterone blocks the oestradiol-induced LH surge by preventing the increase in pulsatile GnRH release and perhaps also by decreasing the sensitivity of pituitary gonadotrophs to oestradiol (Koligian and Stormshak, 1977).

2.8 Folliculogenesis

Primordial follicles in most mammals have been known to be formed just before or soon after birth, as primary oocytes become surrounded by a squamous layer of somatic cells (pre-granulosa cells). The populations of primordial (resting pool) and primary (growing pool) ovarian follicles constitute the reserve pool of follicles that ewes utilize during their entire reproductive life and numbering about 40, 000 to 300, 000 primordial follicles in ewe lambs (Driancourt *et al.,* 1991).

Primordial follicles appear to continuously leave the non-growing pool of follicles by being converted into primary follicles (Van Wezel and Rodgers, 1996). When primary follicles become secondary follicles, they have two or three layers of granulosa cells (Fortune, 2003). Early antral or tertiary follicles are the next stage of follicular development followed by the formation of a complete antrum (Lundy *et al.,* 1999).

Antral follicles continue to grow under the influence of gonadotrophins and acquire steroidogenic capability to form mature Graafian follicles. The period of follicular growth from the primordial to the preovulatory stage exceeds 6 months. Growth from the primordial to the early preantral stage (0.2 mm in diameter) takes an average of 130 days (Cahill and Mauleon, 1981). It takes 24 to 35 more days to reach 0.5 mm in diameter, 5 days to reach 2.2 mm in size and about 4 days to reach a preovulatory size of 4.5 to 5 mm in diameter (Turnbull *et al.,* 1977).

2.8.1 Follicular waves in sheep

Sheep and cattle, as other domestic species, show two stages of ovarian antral follicle development (Mihm and Bleach, 2003). First, a 'slow growth phase' which is believed to be independent of gonadotrophins and the second, a 'fast growth phase' that requires gonadotrophin support and is usually described as a follicle wave (Sunderland *et al.,* 1994). In domestic ruminants, the growing phase is defined as the time taken by the individual antral follicle to grow from emergence, as recorded by transrectal ultrasonography, to its maximum size. The regressing phase is the time taken by this follicle to regress to the minimal recordable size (2 or 3 mm in diameter using ovarian ultrasonography) and the time period between the end of the growing phase and the onset of regression is defined as the static phase (Schrick *et al.,* 1993).

Recruitment refers to the uniform growth of a group of ovarian antral follicles that eventually gain the ability to fully respond to endocrine (gonadotrophic) stimuli. Selection is the process by which only a limited numbers of these cohorts of follicles are rescued from atresia and continue to grow to an ovulatory size (Ginther *et al.,* 1996). Dominance is a characteristic of a large selected ovarian antral follicle (dominant follicle) of a wave or cohort of follicles, that permits its survival and further

development in an endocrine environment suppressive to other co-existing follicles (subordinate follicles). Follicle emergence or follicular wave emergence is the beginning of the growth of a group of antral follicles from the minimum recordable size that subsequently ovulate or undergo atresia (Ginther *et al.,* 1996).

There has been a large increase in the understanding of follicular waves in both cyclic and anestrous ewes with use of transrectal ultrasonography (Bartlewski *et al.,* 1999a; Evans *et al.,* 2000). Follicular waves have also been documented, based on ultrasonographic observations, in pregnant ewes up to day 26 of pregnancy. It is noteworthy that no follicle ≥ 3 mm was observed on the CL bearing ovaries in sheep. This local inhibition of the CL on follicular dynamics is sustained, in the ovary that bore the CL during pregnancy, for up to 4 weeks after parturition (Bartlewski *et al.,* 2000a). Many of the studies above did not closely examine changes in the number of follicles among days of the cycle, as per the original definition of waves. In one study in cyclic sheep, it was shown that there are fluctuations in the number of follicles in different size classes consistent with the definition of follicle waves (Evans *et al.,* 2000). However, in many other studies the demonstration of follicular waves during the ovine oestrous cycle had to be based on those small follicles (3 mm in diameter) that grew and reached an ovulatory diameter of ≥ 5 mm as there were no periodic increases and decreases in the numbers of small follicles with respect to wave emergence (Evans, 2003).

The wave-like pattern of antral follicle growth in sheep differs from the orderly emergence of follicular waves in cattle in that emergence of sequential waves in ewes occurs more frequently and the 1-4 follicles that constitute a wave, all grow to reach an ovulatory diameter (Ginther, 1995; Bartlewski *et al.,* 1999a). In both cyclic and

anestrous ewes, a follicular wave consists of 1 to 4 follicles growing from 2 to 3 mm in diameter to a maximum size of 4 to 12 mm in diameter before regression or ovulation (Evans *et al.,* 2000; Vinoles *et al.,* 2001). In different breeds of sheep, there are 2 to 4 waves per cycle (Bartlewski *et al.,* 1999a; Evans *et al.,* 2000). Follicular waves emerge every 4-5 days and emergence of each wave is preceded by a transient increase in serum concentration of FSH in both cyclic and anestrous ewes (Duggavathi *et al.,* 2003; Duggavathi *et al.,* 2004).

It has been suggested that the largest, apparently dominant, follicle during the early luteal phase (Vinoles *et al.,* 1999) and follicular phase (Ravindra *et al.,* 1994) of the ovine oestrous cycle may partially suppress the growth of small follicles. However, the fact that more than one follicle acquires the ability to reach an ovulatory diameter in a single wave in sheep suggests that deviation may not occur during the growth of follicles in a wave. There has been some suggestion that in the ewe, the largest follicles in the first wave of the cycle differ from those in subsequent waves (Bartlewski *et al.,* 1999b); the largest follicles in the first wave of the ovine oestrous cycle may have a longer lifespan. In many breeds of sheep, ovulatory follicles appear to be similar in maximum diameter to the largest follicles of anovulatory follicular waves of the cycle. Even though the ovulatory follicles in sheep come from the final follicular wave of the cycle, about 50% of all ovulatory sized follicles in the penultimate wave in prolific Finn sheep are maintained and added to ovulatory follicles of the final wave of the cycle (Bartlewski *et al.,* 1999a). The existence of 2 ovulatory waves has also been shown in Rambouille x Booroola ewes (Gibbons *et al.,* 1999). In a recent study, such a mechanism of ovulation of follicles in the penultimate wave along with the follicle in

the final wave of the cycle was experimentally demonstrated using a treatment with $PGF_{2\alpha}$ and intra-vaginal medroxyprogesterone acetate sponges (Bartlewski *et al.*, 2003).

2.8.2 Hormonal control of folliculogenesis

The endometrium synthesizes $PGF_{2\alpha}$, antral follicles produce inhibin and oestrogens, the *corpus luteum* (CL) produces progesterone, the hypophysis synthesizes follicle stimulating hormone (FSH), luteinizing hormone (LH), oxytocin and prolactin and the hypothalamus releases gonadotrophin- releasing hormone (GnRH) to control oestrous cycle (Scaramuzzi *et al.*, 1993).

The hypothalamic- hypophyseal- ovarian axis primarily directs the regulation of the cycle. Luteinizing hormone and follicle stimulating hormone regulate maturation and growth of follicles, synthesis of steroids, ovulation and CL development (Scaramuzzi *et al.*, 1993). Just before the onset of oestrus, one or more follicles grow rapidly increasing the concentration of 17-B oestradiol in the blood from about 10 to 20 pg\ml (Błaszczyk *et al.*, 2004). The increased estrogen causes behavioral oestrus, stimulates the cervix to secret small amount of mucus, increases vascularization in the vulva and some changes in the epithelial tissue of the vagina (Theodosiadou *et al.*, 2004). During oestrous, the interplay of oestrogen with Gonadotrophin Releasing Hormone (GnRH), stimulate release of luteinizing Hormone (LH) from anterior pituitary gland. The concentration of LH in the blood rises to a peak after the beginning of oestrus and then both LH and oestradiol concentration fall rapidly (Younes, 2008). Luteinizing Hormone stimulates occurrence of ovulation about 14 hours after LH peak, or 24 hours after the beginning of oestrus (Pierson *et al.*, 2001; Alvarez *et al.*, 2007).

Gonadotrophins are the most important promoters of ovarian antral follicular emergence and growth (Picton *et al.*, 1990). In sheep, FSH receptors on the granulosa cells can be

detected as early at the primary follicle stage and their numbers increase when follicles continue to grow to 2 mm in diameter (Tisdall *et al.,* 1995). Luteinizing hormone receptors can be detected in the theca cells of large preantral follicles in sheep. However, during the later stages of follicle growth, when follicles in ovaries of sheep are about 4 mm in diameter, LH receptors appear also in the granulosa cells (Logan *et al.,* 2002). During the terminal growth of ovulatory follicles, serum concentrations of FSH were found to be minimal while LH concentrations increased prior to the preovulatory LH surge (Baird *et al.,* 1981). With these observations, it was concluded that early antral follicles are predominantly dependent on FSH and the terminal phases of folliculogenesis are under the control of LH (Campbell *et al.,* 1995).

Follicle stimulating hormone alone, but not LH alone, can stimulate the growth of follicles to a preovulatory size in long-term GnRH agonist treated ewes (Picton *et al.,* 1990). Withdrawal of FSH in the presence of LH results in the maintenance of preovulatory follicles in 50 to 55 % of GnRH-antagonist treated ewes (Campbell *et al.,* 1999). Subsequent study (Bartlewski *et al.,* 2000b) using frequent blood sampling, the temporal relationship between gonadotrophin secretory patterns and the growth of follicular waves during the early luteal phase were investigated. On the day of follicular wave emergence, mean serum FSH concentrations were high and the end of the growth phase of the largest follicle of the first wave of the cycle was associated with an increase in the pulse amplitude of LH secretion. However, whether this increase in LH pulse amplitude was associated with the static phase of the follicle or whether it was associated with decreasing LH pulse frequency in the face of increasing progesterone concentrations was not clear (Wheaton *et al.,* 1988).

In sheep, as in cattle (Adams *et al.*, 1992), there is solid agreement that a transient peak in serum FSH concentrations precede emergence of each follicular wave in both cyclic (Evans *et al.,* 2001; Duggavathi *et al.,* 2004) and anestrous ewes (Bartlewski *et al.,* 1998; Evans *et al.,* 2001).

2.9 Ovulation

Ovulation rate (number of eggs released at ovulation) is influenced by a number of factors including breed, age, reproductive status (dry or lactation) , season of the year, nutritional status and body condition of the ewe. The ovulation rate increases with age and reaches a maximum at about 3 to 6 years and then gradually declines (Schoenian and Burfening, 1990). The rate of ovulation is higher early in the breeding season than later (Lamberson and Thomas, 1982). Flushing or increasing the levels of nutrition before breeding is commonly practiced in sheep to increase ovulation rate. However, other factors such as body size, weights, condition and genotype may also contribute to the increase in ovulation rate (AbuEl-ella, 2006).

2.10 Pregnancy

If no embryo is present in the uterus on the twelfth day of oestrous cycle, the corpus luteum then regresses, leading to reoccurrence of oestrus. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) concentration increases in the pregnant and non pregnant ewe on day twelve (AbuEl-ella, 2006). In the non pregnant ewe, $PGF_{2\alpha}$ reaches a peak on the day fifteen and reaches the corpus luteum by means of the close apposition of the uterine vein and ovarian artery to cause luteolysis. The corpus luteum starts to regress on the fifteenth day and by day 16 the concentration of progesterone is basal (< 0.2 ng\ml). The fall in progesterone concentration stimulates oestradiol secretion by the growing follicles and

the absence of progesterone permits stimulation of gonadotrophin release by oestradiol (AbuEl-ella, 2006).

Once mating and fertilization are successfully completed, the trophoblast must signal its presence to the maternal system to prevent luteolysis and maintain progesterone production essential for the establishment of pregnancy (Geisert and Malayer, 2000). The blastocyst, before attaching to the endometrium, secretes a protein known as ovine trophoblast protein-1 (oTP-1) (Imakawa *et al.*, 1993) but later classified as ovine interferon-tau (oIFN-t) (Bazer *et al.*, 1997) that directly or indirectly prolong the lifespan of the corpus luteum and prevent return to ovarian cyclicity (Jainudeen and Hafez, 2000). This phenomenon is known as maternal recognition of pregnancy. However, the CL of the pregnant ewe requires more $PGF_{2\alpha}$ to cause luteolysis, this relative resistance might be produced by prostaglandin E2 from the uterus (AbuEl-ella, 2006).

2.11 Pregnancy Diagnosis

The need for pregnancy diagnosis is of great economic importance in the management of sheep (Gearhart *et al*., 1988, Karen, 2003). Several methods have been used to diagnose pregnancy in sheep and this includes; management method which involves observation for non-return to oestrus, radiography (Karen, 2003), progesterone assay (Skemesh *et al*., 1973) and ultrasonography (Medan *et al*., 2004). The accuracy of pregnancy diagnosis and determinant of foetal number by using the radiography method are 90% to 100 % (West, 1986; Karen, 2003).

Ultrasonography for pregnancy diagnosis in sheep is of three types (Karen, 2003). The first being the A-mode ultrasound with transducer containing piezoelectric crystal emits

ultrasound waves which penetrate the tissue under the skin (Karen, 2003). Accuracy of 97% after day 50 of gestation has been reported. The system is fast, convenient and involves a simple technique but it cannot determine the number of fetuses (Watt *et al*., 1984).

Doppler ultrasound system, which is the second type can detect the foetal heartbeats and blood flow in the uterine and foetal vessels (Karen, 2003). This system has an accuracy of a 100% in pregnancy diagnosis 60 days post breeding (Ishwar, 1994). The B-mode ultasonography system is the third type and is highly accurate, fast in determining foetal number and also foetal age. This system has an accuracy of 80% in pregnancy diagnosis 21 days post breeding (Medan *et al*., 2004).

Progesterone assay by enzyme immunoassay (EIA) and radioimmunoassay (RIA) to determine the concentration of progesterone in blood in ewes at day 18 after breeding (Karen, 2003) has an accuracy of 100% in pregnancy diagnosis 17 to 19 days post breeding (Wani *et al.*, 2010) and serum progesterone values higher than 1 ng/ml is considered positive for pregnancy (Boscos *et al*., 2003; Wani *et al.*, 2010).

2.12 Gestation

The gestation period of sheep lasts about 5 months, ranging from 145 to 152 days after conception. This length may vary with breed, parity and litter size (Timurkan and Yildiz, 2005). The first trimester of pregnancy in the ewe is luteodependent, but after about day 50- 55 of pregnancy progesterone is mainly produced by the placenta. Therefore, ovariectomy or administration of luteolytic doses of prostaglandin $F_{2\alpha}$ does not terminate pregnancy during the last two thirds of gestation (Timurkan and Yildiz, 2005).

Pregnancy leads to a state of sexual inactivity but is resumed few weeks after lambing, a period known as post–partum anoestrus. The post-partum anoestrus is mainly due to the antigonadotrophic effects exerted by the suckling lamb which normally disappears shortly after weaning (Bearden and Fuquay, 1984).

2.13 Oestrus Synchronization

Oestrus synchronization is a management practice that manipulates the luteal or follicular phase of the oestrous cycle (Wildeus, 2000; Patterson *et al.,* 2003), thus controlling oestrus and ovulation in cycling females to enable breeding to be conducted within a short period of time (Deutscher, 2010). This practice is applied to farm animals such as cattle (Deutscher, 2010), deer, sheep and goats (Freitas *et al.,* 1996; Wildeus, 2000). Various oestrous synchronization protocols have been studied in order to improve reproductive efficiency in ruminants (Kusina *et al.,* 2000).

2.13.1 Prostaglandin-$F_{2\alpha}$ and its analogue in reproduction

Prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) acts in oestrus synchronization by terminating the luteal phase through regression of the corpus luteum. Prostaglandins are used only during the breeding season as not all stages of the oestrus cycle are similarly receptive to prostaglandin treatment (Ataman and Akoz, 2006). Prostaglandins are not relevant in the control of sheep reproduction during the anoestrus period (i.e. shortage of CL) due to the fact that CL is only responsive to prostaglandins between days 5-14 of the oestrus cycle. Two injections, 10-14 days apart, are required for optimum synchronization (Gordon, 1999).

The most widely used protocols of $PGF_{2\alpha}$ in oestrus synchronization combines progesterone impregnated intravaginal sponges for 7 or 12 days and a subsequent single prostaglandin injection during the breeding and anoestrus seasons (Husein and Kridi, 2003). The other protocol is by two injections of prostaglandin at 11 days interval which can only be used during breeding season (Ataman and Aköz, 2006). Sozbülür *et al.* (2006) reported high lambing rates with double injection of 125 µg of Cloprostenol (a $PGF_{2\alpha}$ analogue) at 10 to 14 days interval in Tuj ewes during the breeding season. Other researchers used GnRH-$PGF_{2\alpha}$ combination which is found to be effective in the synchronization of the oestrus in ewes (Ataman and Aköz, 2006).

2.13.1.1 Lutalyse®

This product contains the naturally occurring prostaglandin F2 alpha (dinoprost) as the tromethamine salt. Each millilitre (ml) contains dinoprost tromethamine equivalent to 5 milligram (mg) dinoprost with 16.5 mg of benzyl alcohol added as preservative. Lutalyse is used to control the timing of oestrus and ovulation in cycling ewes that have functional corpus luteum. Injection of 5 ml lutalyse intramuscularly either once or twice at a 9 to 12 day interval will result in oestrus 1 to 5 days after injection (Salverson *et al.*, 2002; Fred and Doug, 2012).

2.13.1.2 estroPLAN®

This product contains the synthetic analogue of the naturally occurring PGF2α (dinoprost) as the sodium salt. Each ml contains 250 µg/ml of cloprostenol sodium. In the reproductive system, it plays a role in ovulation, luteolysis, gamete transport, uterine motility, expulsion of foetal membranes. In the ewe, oestrus occurs 2-5 days after

luteolysis following intramuscular injection of 250 μg either once or twice at a 5 to 14 day interval (Kusina *et al.*, 2000; Jainudeen and Hafez, 2000).

2.13.2 Progestagen and its analogue in reproduction

Oestrous synchronization in ewes can be achieved by the use of intravaginal devices impregnated with progesterone or synthetic progestagen such as controlled internal drug release (CIDR), progesterone releasing intravaginal device (PRID), fluorogestone acetate (FGA) or Methyl acetoxy progesterone (MAP) (Fukui *et al*., 1999; Karaca *et al*., 2009; Omontese *et al.,* 2010).

Progestagens can be administered by different methods (sponge, injection, implants, or as feed additives), by several routes (intravaginal, intramuscular, oral, or subcutaneous) and at different doses (Neils, 1997). The progestagen can be supplemented with follicle stimulating hormone (FSH), equine chorionic gonadotrophin (eCG) and pregnant mare serum gonadotrophin (PMSG) treatments to induce follicular growth, oestrus and ovulation (Neils, 1997). The dose of PMSG must be adapted to breed, season, flock, age and physiological status of the animals (Neils, 1997).

2.13.2.1 Progesterone impregnated intravaginal sponges

The Intravaginal sponges impregnated with progestagen have been widely used in sheep during the breeding and non-breeding season (Romano, 2004). There are two commercially available sponges, based on either flurogestone acetate (FGA), marketed as Chronogest (Intervet, Angers, France), or Methyl acetoxy progesterone, marketed as Veramix (Pharmacia & Upjohn, Orangeville, Canada). Sponges are impregnated with 30, 40, or 45 mg of flurogestone acetate (FGA) or 60 mg of Methyl acetoxy

progesterone. They are inserted into the vagina with the aid of an applicator (Romano, 2004).

Intravaginal sponges are usually inserted over periods of 9 to 19 days and can be used in conjunction with pregnant mare serum gonadotrophin (PMSG), particularly for out-of-season breeding, injected at time of sponge removal or 48 hours prior to sponge removal. Females usually exhibited oestrus within 24 to 48 hours after sponge removal (Wildeus, 2000). After withdrawal of the progestagen source, the increasing amounts of gonadotrophin released leads to oestrus and ovulation.

2.13.2.2 Controlled internal drug release (CIDR)

This is a progesterone-impregnated silicon elastomere intravaginal device developed in the 1980s in New Zealand for oestrus and ovulation control in sheep (Welch *et al*., 1984). The controlled internal drug release (CIDR) is an alternative device to progestagen sponges for oestrus synchronization in ruminants. The usage of CIDR provides advantages compared with the sponges such as elimination of foul-smelling mucus discharged upon removal of sponges, lower loss rates, higher percentage of animals coming into oestrus, earlier exhibited oestrus and more compact oestrus (Nathanielsz, 1988). Oestrus synchronization protocols using CIDR vary from insertion of the CIDR for 5 to 16 days with hormone co-treatment using 100 to 500 IU of equine chorionic gonadotrophin (eCG) or pregnant mare serum gonadotrophin (PMSG) and/or 0.05 mg of prostaglandin $F_{2\alpha}$ (Menchaca and Rubianes, 2001; Whitley and Jackson, 2004; Hashemi *et al.*, 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was carried out at the sheep farm of the Animal Reproduction Research Programme, National Animal Production Research Institute (NAPRI), Shika, Ahmadu Bello University, Zaria. NAPRI is located in the Northern Guinea Savannah of Nigeria between 11˚N and 12˚N and between 7˚E and 33˚E, 650 m above sea level with an average annual maximum and minimum temperature of 35˚C and 26˚C, respectively. Shika has an average rainfall of 1100 mm, usually lasting from May to October with a mean relative humidity of 27%. The dry season lasts from November to April, with mean daily temperatures ranging from 15 - 36˚C and mean relative humidity of 21% (Akpa, *et al.,* 2002).

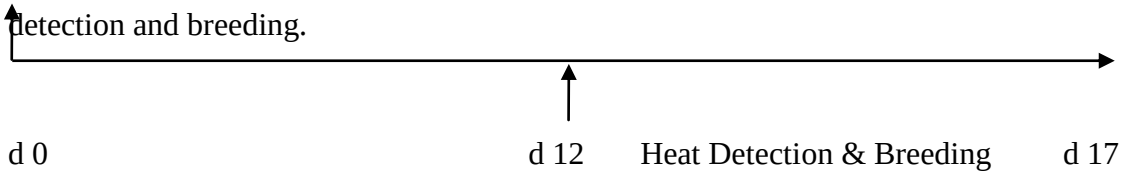
3.2 Experimental Animals, Herd Management and Housing

Twenty six (n= 26) Yankasa ewes, with mean age of 2.5 years using dentition, 21 kg mean body weight, with body condition score of 2.5-3.5 (Spahr, 2005) were used in this study. The ewes have shown at least two natural oestrous cycles, based on records (19 - 21 days). Individual ewes were identified by means of plastic ear tags. *Digitaria smutsii* (wooly finger grass) hay; concentrate supplement (cotton seed cake, groundnut cake, palm kannel cake, and soya bean cake at 0.5 kg day^{-1}) and water were given to ewes. Ewes were allowed to graze the pasture, and four rams were used for this study. This study was carried out between the months of January and February.

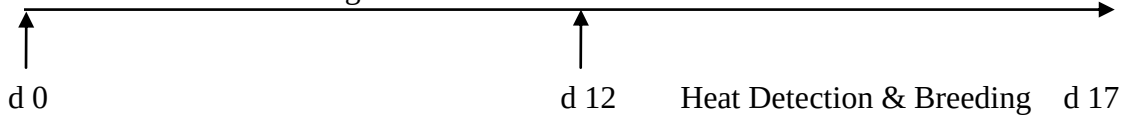
3.3 Treatment Protocols

Ewes were randomly allocated into four groups as follows;

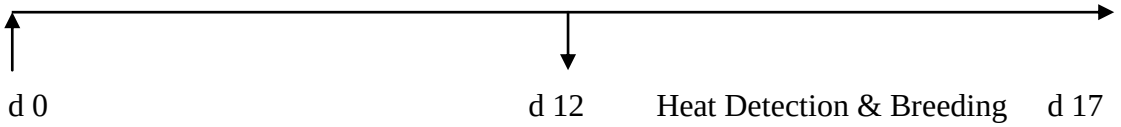
Group I (YK-LT): Yankasa ewes (n = 7) treated with two intramuscular administration of Lutalyse ® (Pharmacia & Upjohn) 12 days apart, followed by heat detection and breeding.



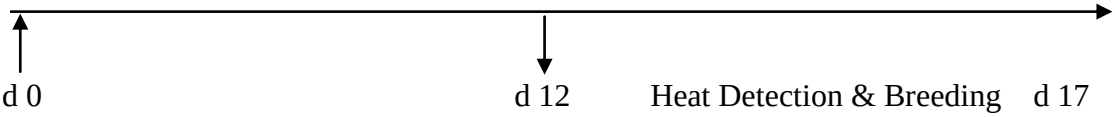
Group II (YK-ET): Yankasa ewes (n = 7) treated with two intramuscular administration of estroPLAN® (Parnell Australia Pty Ltd.) 12 days apart, followed by heat detection and breeding.



Group III (YK-CD): Yankasa ewes (n = 5) treated with EAZI- BREED™ CIDR® (Pharmacia & Upjohn Pty Limited, Rydalmere NSW) alone for 12 days.



Group IV (YK-FG): Yankasa ewes (n = 7) treated with FGA-45® Vaginal Sponge (Chronopgest, Intervet, Netherlands) alone for 12 days.



Key:

YK = Yankasa LT = Lutalyse® ET = estroPLAN® CD = EAZI- BREED® CIDR

FG = FGA-45® sponge

3.4 Treatment with Prostaglandins

Two injections of 10 mg Lutalyse® (Pharmacia & Upjohn) and 125 µg estroPLAN® (Parnell Australia Pty Ltd) were administered intramuscularly at 12 days intervals, to groups I and II respectively, using 5 ml syringes.

3.5 Treatment with Progestagens

3.5.1 Insertion

In group III, EAZI-BREED® CIDR (Appendix 6) was inserted into the vagina of ewes with the aid of an applicator as described by the manufacturer (Pharmacia & Upjohn Pty Limited, Rydalmere NSW). The applicator was disinfected with choloxylenol (Dettol) and lubricated using K-Y jelly. CIDR was loaded into the applicator so that the short legs were folded together with only the tips, protruding from the applicator. The tip of the loaded applicator was inserted into the anterior portion of the vagina. The applicator plunger was pressed to release the device, leaving the withdrawal cord protruding from the vulva. The applicator was wiped clean and disinfected after each insertion.

In group IV, FGA-45® vaginal sponge was inserted into the vagina of the ewes with the aid of an applicator as described by the manufacturer (Chronopgest CR, Intervet, Netherlands). The applicator was disinfected with choloxylenol (Dettol) and lubricator using K-Y jelly. The sponge was loaded into the end of the applicator so that the sponge was just behind the end of the applicator. The plunger was pushed gently to eject the sponge. The applicator was removed ensuring that the drawstring was still hanging outside the vagina. The applicator was wiped clean and disinfected after each insertion.

3.5.2 Removal

The progestagens (EAZI- BREED® CIDR and FGA-45® vaginal sponges) were allowed in place for 12 days after which they were removed by pulling the withdrawal cord or drawstring.

3.6 Oestrus Detection

Following the second injection of prostaglandins and removal of progestagen devices, the treated ewes were observed for behavioural oestrus manifestation twice daily (0700-1000 and 1500-1800 hours) for 5 days by exposing them to proven sexually active rams in the ratio of 1 male to 7 females (Abecia *et al.*, 2012). Ewes were considered to be in oestrus when they "stood to be mounted" by males. Other signs such as vigorous tail-wagging, reddened and swollen vulva, clear mucus discharge, restlessness, tail wagging, frequent bleating and frequent adoption of urination posture were observed (Zakari, 1981). Oestrus activity occurring within 120 hours after second injection of prostaglandins and withdrawal of progestagens were classified as synchronized. The following oestrus parameters were evaluated:

(i) Oestrus Response Rate (%): The number of ewes that manifested standing oestrus within 120 hours after treatments, over the number of ewes treated, expressed in percentage.

(ii) Time-to-onset of Oestrus: This was the time (hours) interval between second injection of prostaglandins or withdrawal of progestagens/exposure to ram and first expression of standing oestrus, expressed as Mean (± SEM) for the group.

(iii) Duration of Oestrus (Oestrus Period): This was the time (hours) interval between the first and last standing oestrus, expressed as Mean (± SEM) for the group.

(iv) Mounts per Oestrus-Detection-Time: This was the number of successful mounts in all ewes in the group during 120 hours of oestrus-detection-time expressed as Mean (± SEM).

(v) Rate of Oestrus Synchrony (%): This was the percentage of ewes observed to be in oestrus within 48 hours after the second injection of prostaglandin injection and withdrawal of progestagens.

3.7 Mating

Mating was allowed to continue in ewes that stood to be mounted by males during oestrus-detection exercise for as long as oestrus lasted.

3.8 Pregnancy Diagnosis

3.8.1 Blood Sampling

Blood samples (5 ml each) were collected via jugular veni-puncture from ewes weekly, starting on day 0 twice weekly, (day of first $PGF_{2\alpha}$ injections or insertion of progestagens), during oestrus period /natural mating every other day, and afterwards until day 44 again, twice weekly (21 days after mating). All blood samples collected were dispensed into sample bottles without anticoagulant and transported immediately to the laboratory. Serum was extracted by centrifugation at 2500 rpm for 15 minutes, placed in appropriately labeled serum vials and stored at -20˚C until analysis for progesterone concentration.

3.8.2 Enzyme Linked Immunosorbent Assay

Serum was analyzed for progesterone concentration by a commercially available ELISA (Monobind Inc. Lake Forest, CA 92630, USA.) that had been previously validated in our laboratory. Serum samples (0.025 ml) were assayed according to the manufacturer's instructions. The sensitivity of the assay was 0.105 ng/ml. Pregnancy was confirmed by persistent high levels of serum progesterone concentration above 1.5 ng/ml 21 days post-mating.

3.9 Pregnancy Outcome

1. Conception rate (%): This was the number of ewes that failed to return to oestrus 17- 21 days after breeding divided by the number of ewes bred in the treatment groups expressed as percentage.
2. Pregnancy rate (%): This was the number of ewes that presented serum progesterone concentrations of $\geq$ 1.5 ng/ml 21 days after breeding over the number of ewes treated, expressed in percentage.

3.10 Data Analysis

Oestrus response and pregnancy rates were expressed in percentages. GraphPad Prism 5 (2011) data package was employed for data analyses. Time to onset of oestrus, duration of oestrus, mounts per oestrus-detection-time and progesterone profile data were expressed as Mean ± SEM. Analysis of variance (Tukey's test) was used to compare means of time to onset of oestrus, duration of oestrus, mounts per oestrus detection time, within treatment groups. Values of $P < 0.05$ were considered significant.

CHAPTER FOUR

RESULTS

4.0 Oestrus Parameters

4.1 Oestrus Response Rate

Oestrus response rates of Yankasa ewes following treatment with prostaglandins or progestagens are shown in Table 4.1. Oestrus response rates were 85.7 %, in the YK-LT group and 100 % in the YK-ET, YK-CD and YK-FG groups. Synthetic prostaglandin (Cloprostenol sodium; ET) caused a 100 % oestrus response in Yankasa ewes and was better than the natural prostaglandin (Dinoprost tromethamine; LT 85.7 %). On the other hand, both progestagens caused oestrus (heat) in 100 % of ewes treated. Therefore, the progestagens proved to be better (100 % average) than the prostaglandins (93 % average) for oestrus induction in Yankasa ewes. Generally, the synthetic drugs (estroPLAN and Fluorogestone Acetate; 100 %) stimulated oestrus better than the natural products (Lutalyse and CIDR; 93 %).

Table 4.1: Oestrus response rate in Yankasa ewes treated with prostaglandin (Lutalyse® or estroPLAN®) or progestagen (CIDR® or FGA-45®).

Group ewes	Ewes Treated (n)	Ewes in oestrus (n)	Percentage of in oestrus
YK-LT	7	6	85.7
YK-ET	7	7	100
YK-CD	5	5	100
YK-FG	7	7	100

Key:
YK= Yankasa
LT= Lutalyse®
ET= estroPLAN®
CD= Controlled internal drug release (CIDR)
FG= Fluorogestone acetate (FGA-45 mg)

4.2 Time-to-Onset of Oestrus

Mean (± SEM) time interval between treatment and onset of oestrus in Yankasa ewes treated with prostaglandins or progestagens are shown in Table 4.2. Mean (± SEM) time-to-onset of oestrus was 68.23 ± 17.28 hours, 49.29 ± 4.48 hours, 33.48 ± 8.58 hours, and 53.27 ± 12.33 hours in the YK–LT, YK-ET, YK-CD and YK-FG group, respectively. In the prostaglandin ewes, estroPLAN stimulated oetrus faster (49.29 ± 4.48 hours) than lutalyse (68.23 ± 17.28 hours). While in the progestagen groups, CIDR was faster (33.48 ± 8.58 hours) than FGA (53.27 ± 12.33 hours) and both prostaglandin groups.

Table 4.2: Time to onset of oestrus (hours) in Yankasa ewes treated with prostaglandin (Lutalyse® or estroPLAN®) or progestagen (CIDR® or FGA-45®).

Group	Time to onset of oestrus Mean ± SEM (hours)
YK-LT	68.23 ± 17.3
YK-ET	49.29 ± 4.5
YK-CD	33.48 ± 8.6
YK-FG	53.27 ± 12.3

Key:
YK= Yankasa
LT= Lutalyse®
ET= estroPLAN®
CD= Controlled internal drug release (CIDR)
FG= Fluorogestone acetate (FGA-45 mg)

4.3 Duration of Oestrus (hours).

Mean (± SEM) duration of oestrus in Yankasa ewes following treatment with prostaglandins or progestagens are shown in Table 4.3. Mean (± SEM) duration of oestrus was 51.33 ± 13.9 hours, 31.19 ± 7.9 hours, 35.25 ± 8.3 hours and 25.30 ± 6.3 hours in the YK–LT, YK-ET, YK-CD and YK-FG groups, respectively.

In the prostaglandin groups, Lutalyse caused a longer (51.33 ± 13.9 hours) oestrus period than estroPLAN (31.19 ± 7.9 hours). In the progestagen ewes, CIDR group also had longer (35.25 ± 8.3 hours) than FGA (25.30 ± 6.3 hours). The data showed also that the prostaglandin treated ewes on the average, had longer duration of oestrus (51.33 ± 13.9 and 31.19 ± 7.9 hours) than the progestagen groups (35.25 ± 8.3 and 25.30 ± 6.3 hours) and that the natural products, all-together, stimulated longer oestrus period (51.33 ± 13.9 and 35.25 ± 8.3 hours) than the synthetic products (31.19 ± 7.9 and 25.30 ± 6.3 hours). There were no significant differences between the values in the prostaglandins and progestagen groups.

Table 4.3: Duration of oestrus in Yankasa ewes treated with prostaglandin (Lutalyse® or estroPLAN®) or progestagen (CIDR® or FGA-45®).

Group	Duration of oestrus Mean ± SEM (hours)
YK-LT	51.35 ± 13.9
YK-ET	31.19 ± 7.9
YK-CD	35.25 ± 8.3
YK-FG	25.30 ± 6.3

Key:
YK= Yankasa
LT= Lutalyse®
ET= estroPLAN®
CD= Controlled internal drug release (CIDR)
FG= Fluorogestone acetate (FGA-45 mg)
SEM= Standard Error of Mean

4.4 Mounts per Oestrus Detection Time

Mean (± SEM) mounts within the oestrus detection time (120 hours) in Yankasa ewes treated with prostaglandins or progestagens are shown in Figure 4.4. Mean (± SEM) mounts within 120 hours were 24.6 ± 5.9, 14.3 ± 4.3, 23.2 ± 6.8 and 14.7 ± 4.6 in the YK-LT, YK-ET, YK-CD and YK-FG groups, respectively. Generally, treatment with natural products (Lutalyse and CIDR) caused higher mounts (24.6 ± 5.9 and 23.2 ± 6.8) than synthetic products (estroPLAN and FGA) 14.3 ± 4.3 and 14.7 ± 4.6. There were no statistical differences across the treatment groups.

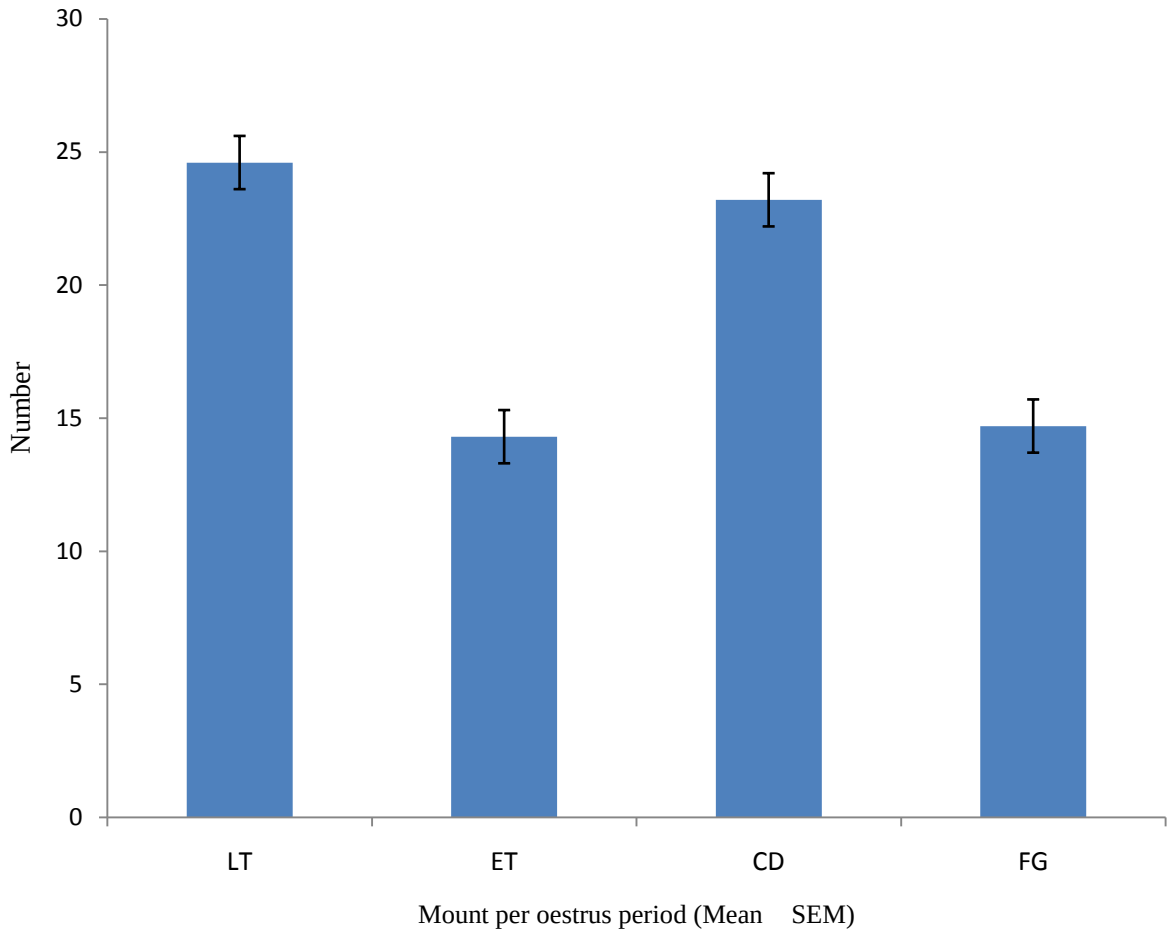


Figure 4.4: Mounts per oestrus detection time in Yankasa ewes treated with prostaglandin (Lutalyse® or estroPLAN®) or progestagen (CIDR® or FGA-45®).

Key:
YK= Yankasa
LT= Lutalyse®
ET= estroPLAN®
CD= Controlled internal drug release (CIDR)
FG= Fluorogestone acetate (FGA-45 mg)
SEM= Standard error of mean

4.5 Tightness of Synchrony

Tightness of synchrony in Yankasa ewes treated with prostaglandins or progestagens are shown in Table 4.5. Within 48 hours after second prostaglandin injection, 57 % of ewes treated with lutalyse (YK-LT) and all ewes (100 %) treated with estroPLAN (YK-ET) came on heat. The records for CIDR (YK-CD) and FGA-45 (YK-FG) were 100 % and 86 %, respectively. Correspondingly, the progestagens gave a better (93%) average synchrony than prostaglandins (78 %) while synthetic product groups were more tightly synchronized (93 %) than the groups treated with natural prostaglandins and progestagens (78 % average).

Table 4.5: Tightness of synchrony in Yankasa ewes treated with prostaglandin (Lutalyse® or estroPLAN®) or progestagen (CIDR® or FGA-45®).

Group (%)	Number of ewes treated (n)	Number of ewes in oestrus/ time (hour)					Total
		24	48	72	96	120	
YK-LT	7	1	3	0	1	1	57
YK-ET	7	1	6	0	0	0	100
YK-CD	5	3	2	0	0	0	100
YK-FG	7	3	3	0	0	1	86

Key:
YK= Yankasa
LT= Lutalyse®
ET= estroPLAN®
CD= Controlled internal drug release (CIDR)
FG= Fluorogestone acetate (FGA-45 mg)

4.6 Conception Rate

Conception rate (CR) using rate of non-return to oestrus, 17 – 21 days after breeding in Yankasa ewes treated with prostaglandins or progestagens are shown in Table 4.6. Conception rates were 100 % in the YK-LT, YK-ET, YK-CD and 86 % (6/7) in YK-FG group, respectively. Conception rates were all good, however, percentage average for conception rate was higher with the natural products (100 %) than with the synthetic products (93 %) and the prostaglandins (100 %) than progestagens (93 %). There were no statistical differences across the treatment groups.

Table 4.6: Non-return to oestrus or conception rate in Yankasa ewes bred after treatment with prostaglandin (Lutalyse or estroPLAN) or progestagen (CIDR® or FGA-45®).

Group	Ewes treated (n)	Ewes bred (n)	Non-return to oestrus ewes (n)	Conception rate (%)
YK-LT	7	7	7	100
YK-ET	7	7	7	100
YK-CD	5	5	5	100
YK-FG	7	7	6	86

Key:
n = Number
YK= Yankasa
LT= Lutalyse®
ET= estroPLAN®
CD= Controlled internal drug release (CIDR)
FG= Fluorogestone acetate (FGA-45 mg)

4.7 Pregnancy Rate

Pregnancy rate (PR) in groups of Yankasa ewes following treatment with prostaglandins or progestagens as determined by progesterone concentration of ≥ 1.5 ng/ml at day 21 after breeding are shown in Table 4.7. Pregnancy rates were 100 %, in the YK-LT, YK-ET, YK-CD and 71 % in YK-FG groups, respectively. Lowest pregnancy rate of 71 % was observed in ewes treated with progestagen pessaries containing Fluorogestone acetate 45 mg. However, percentage average for pregnancy rate was higher with the natural products (100 %) than with the synthetic products (93 %). There were no statistical differences across the treatment groups.

Table 4.7: Percentage of Yankasa ewes treated with prostaglandin (Lutalyse® or estroPLAN®) or progestagen (CIDR® or FGA-45®) showing pregnancy level (≥1.5 mg/ml) serum progesterone 21 days after breeding.

Group	Ewes treated (n)	Ewes with ≥1.5 mg/ml) serum progesterone(n)	Mean serum (±SEM) per group (ng/ml)	Pregnancy rate (n) (%)
YK-LT	7	7	2.7 ±0.35	7 (100)
YK-ET	7	7	3.1 ± 0.81	7 (100)
YK-CD	5	5	3.2 ± 0.44	5 (100)
YK-FG	7	5	2.3 ± 0.98	6 (71)

Key:
YK= Yankasa
LT= Lutalyse®
ET= estroPLAN®
CD= Controlled internal drug release (CIDR)
FG= Fluorogestone acetate (FGA-45 mg)

4.8 Progesterone Profile

Serum progesterone (P4) profile of Yankasa ewes monitored throughout this study is shown in Figure 4.2. Mean serum P_4 concentrations at the start of the study day zero were 1.5 ± 0.54 ng/ml, 1.1 ± 0.28 ng/ml, 1.1 ± 0.47 ng/ml and 0.87 ± 0.38 ng/ml in the YK-LT, YK-ET, YK-CD and YK-FG groups, respectively. The concentrations however rose again on day 38 (21 days after breeding) to 2.7 ± 0.35 ng /ml, 3.1 ± 0.81 ng /ml, 3.2 ± 0.44 ng /ml and 2.2 ± 0.37 ng /ml in the YK-LT, YK-ET, YK-CD and YK-FG groups, respectively.

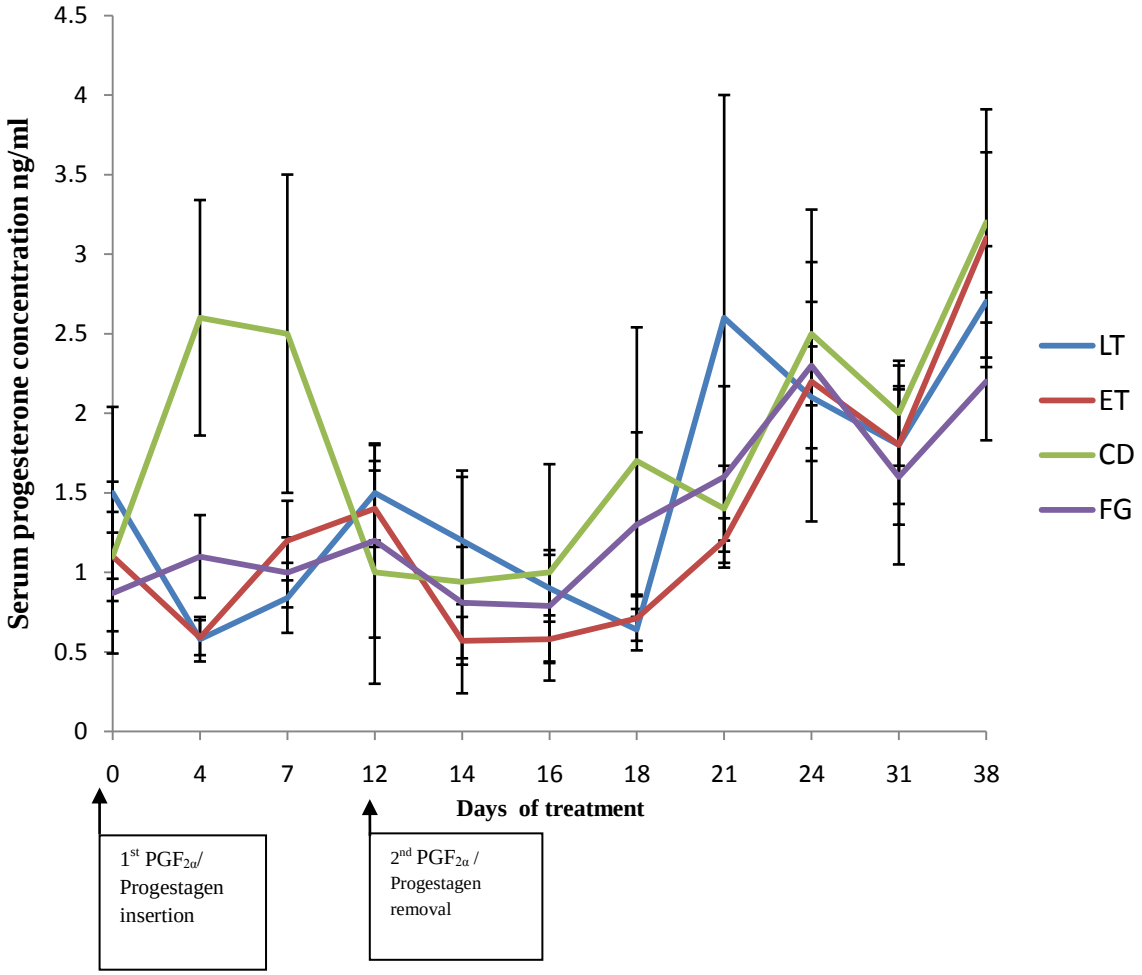


Figure 4.8: Progesterone profile of Yankasa ewes following oestrus synchronization with prostaglandin (Lutalyse® or estroPLAN) or progestagen (CIDR® or FGA-45®).

Key:
LT= Lutalyse®
ET= estroPLAN®
CD= Controlled internal drug release (CIDR)
FG= Fluorogestone acetate (FGA-45 mg)

CHAPTER FIVE

5.0 DISCUSSION

This study has shown that estroPLAN and CIDR offer the best results in synchronizing oestrus in Yankasa ewes. A hundred percent retention rate was observed in this study with CIDR and intravaginal sponge. This finding disagrees with the report of Knight and Hall (1998), who reported high CIDR loses in sheep. However, the 100 % retention observed in this study corroborates the report of Moeini *et al.* (2007) in Sanjabi and Lori sheep, as well as report of Omontese *et al.* (2010) in Yankasa ewes. The disagreement may be due to differences in management practices.

The 100 % oestrus response observed in Yankasa ewes treated with CIDR (YK-CD) and FGA-45 (YK-FG) corroborates the findings of Musa-Azara *et al.* (2011) where Yankasa ewes treated with intravaginal progestagen containing fluorogestone acetate, thus, indicating that both natural and synthetic progestagens were both efficient in inducing synchronized oestrus. This may be due to the high level negative feedback they exert on the hypothalamus to prevent gonadotrophin release. However, the 100 % oestrus response in this study with CIDR and FGA-45 were higher than the 70 % and 80 %, reported by Omontese *et al.* (2010) in Yankasa ewes treated with FGA-30 mg and CIDR, respectively. This may be due differences in the doses of progestagen treatments (Oyedipe *et al.,* 1989; Khono *et al.,* 2005; Moeini *et al.* 2007; Omontese *et al.,* 2010; Musa-Azara *et al.,* 2011). The oestrus response rate observed in the YK-ET group (100 %) was similar to the YK-FG group but higher than the YK-LT group (85.7 %) indicating the efficacy of the synthetics over natural products in synchronizing oestrus in Yankasa ewes. Failure of one ewe in the YK-LT group to show oestrus despite the

two injections of Lutalyse administered might be indicative of silent heat or presence of follicles at the time of treatment. The efficiency of the synthetic prostaglandin (estroPLAN) observed here is similar to report of Ozturkler *et al.* (2003), who observed 100 % oestrus response rate using estrumate 11 days apart in Tushin ewes. This however, differs from the 30 % oestrus response rate reported in Rahmani ewes (Tarek, 2012), 86 % in Akkaraman crossbreed sheep (Ataman and Akoz, 2006), the 81.25 % in Merino ewes (Greyling and Van der Westhuysen, 1979) and the 80 % in Naeimi ewes (Homeida *et al.,* 2009). These differences may be linked to variation in breed (Ataman and Akoz, 2006; Homeida *et al.,* 2009; Omontese *et al.*, 2010; Musa-Azara *et al.,* 2011; Tarek, 2012).

The average time-to-onset of oestrus in this present study ranged from 33 hours to 68 hours. This is similar to earlier reports (Zeleke *et al* 2005; Turk *et al.,* 2008; Martemucci and D'Alessandro, 2010; Özyurtlu *et al.,* 2010; Ungerfeld and Rubianes, 2011). In the prostaglandin treatments, lutalyse group had longer time-to-onset of oestrus (68.23 ± 17.3 hours) than estroPLAN (49.29 ± 4.48 hours). This finding is similar to that reported by Recai *et al.* (2013) in Merino sheep but longer than the 25.4 ± 1.12 and 30.6 ± 1.16 hours reported by Maurya *et al.* (2005) in Merino ewes also treated with lutalyse. Following this study, it was also observed that average mean time-to-onset of oestrus was approximately similar (50.86 ± 12.93 and 51.28 ± 8.4 hours) for the natural and synthetic products and clearly shorter for progestagens than for the prostaglandins (43.37 ± 10.46 versus 53.75 ± 10.38 hours). This implies that progestagens should be preferred when time is very important in synchronization of oestrus in sheep. Godfery *et al.* (1997) using CIDR, reported 33 hours onset which was similar to our findings in Yankasa ewes in this study. However, Mustafa *et al.* (2007) reported 42.6 ± 2.9 hours in

Awassi ewes. This study however, confirmed the apparent indication from other reports that time-to-onset of oestrus was shorter with natural progestagens than with synthetic progestagens (33.48 ± 8.58 versus 53.27 ± 12.33 hours). This corroborates the findings of Omontese *et al.* (2010) in Yankasa ewes treated with natural and synthetic progestagens. In addition, the time-to-onset of oestrus (53.27 ± 12.33) observed in Yankasa ewes treated with Fluorogestone acetate intravaginal sponges in this study, though longer than with CIDR (33.48 ± 8.58 hours) was shorter than the 77.6 ± 3.1 hours reported by Homeida *et al.* (2009) in Awassi ewes. These differences may be as a result of breed variation (Godfery *et al.* 1997; Mustafa *et al.,* 2007; Turk *et al.,* 2008; Homeida *et al.,* 2009; Martemucci and D'Alessandro 2010).

Duration of oestrus or oestrus period within the progestagens in this study, was shorter in the YK-FG (25.30 ± 6.3 hours) than in the YK-CD group (35.25 ± 8.3 hours). This corroborates the report of Omontese *et al.* (2010) in Yankasa ewes who observed shorter oestrus duration with FGA than the CIDR, respectively. Comparable values (25 hours) in duration of induced oestrus have been reported in Yankasa ewes (Oyedipe *et al.,* 1989; Musa-Azara *et al.,* 2011). This study revealed that the average mean duration of oestrus was longer with prostaglandins (41.26 ± 10.9 hours) than the progestagens (30.28 ± 7.3 hours) and long duration of oestrus is an advantage because it will increase the chances of breeding and consequently, conception. This is indicative of the higher conception rate with the CIDR group than the FGA. Based on this fact, the findings here that progestagens have shorter time to onset of oestrus, we can propound that the CIDR with the shortest time to oetrus (33.48 ± 8.58 hours) and an average duration (35.25 ± 8.3 hours) is the choice agent for synchronization of oestrus in the Yankasa ewe.

Statistical significant differences ($P > 0.05$) were not observed in the mount per oestrus in the different treatment groups. However, higher mounts were observed in the YK-LT than other groups. The 14.7 ± 4.6 mounts per oestrus in the group treated with FGA in this study was higher than earlier reports by Oyedipe *et al.* (1989). However, the average mean mount per oestrus period was higher with the natural products (23.9 ± 6.35 hours) than with the synthetic products (14.5 ± 4.45 hours) in this study. This may be suggesting that the natural products-induced oestrus are stimulating natural oestrus better than the synthetics-induced oestrus.

Highest oestrus synchrony was observed in ewes treated with CIDR and estroPLAN at 48 hours post treatment. This is consistent with the report of (Greyling and Van der Westhuysen 1979; Godfrey *et al.,* 1997) in Tropical haired. This study showed a tighter percentage synchrony average with the synthetic products (93 %) than with the natural products (78 %). However, with the good performance of CIDR (100 % synchrony), the progestagens are yet a better agent for tighter synchrony than the prostaglandins.

Conception rate was higher in the YK-CD group (100 %) than in the YK-FG group (86 %). Similar reports have been made in ewes (Godfrey *et al.,* 1997; Jafar *et al.,* 2011). The conception rate was also 100 % in both the YK-LT and YK-ET group. This was higher than the 86 % and 51 % reported by Godfrey *et al.* (1997) and Olivera- Muzante *et al.* (2011) in ewes. The percentage average for conception rate in this study was higher with the natural products (100 %), when compared with the synthetic products (93 %).

No significant statistical differences ($P > 0.05$) were observed in pregnancy across the treatment groups. However, the YK-CD had 100 % pregnancy rate and better than the YK-FG (85.5 %). Zonturlu *et al.* (2008) has reported higher pregnancy rates in Awassi ewes treated with CIDR (60 %) than FGA (52 %). The study also showed that ewes treated with prostaglandins had 100 % pregnancy rate as compared to 85.5 % in ewes treated with progestagens. This finding showed that $PGF_{2\alpha}$-treated ewes may result in higher pregnancy following breeding. This study further showed that percentage average for pregnancy rate was higher with the natural products (100 %) than with the synthetic products (85.5 %).

The progesterone values lower than 1.0 ng/ml in Yankasa ewes prior to treatment with prostaglandins or progestagens indicated that most of the ewes in the YK-FG group (0.87 ± 0.38 ng/ml) were in the follicular phase, while those in the YK-LT, YK-ET and YK-CD (1.5 ± 0.54 ng/ml, 1.1 ± 0.28 ng/ml, 1.1 ± 0.47 ng/ml) were in the luteal phase of the oestrus cycle. Mean progesterone concentration (1.1 ± 0.28 ng/ml, 1.1 ± 0.47 ng/ml) observed for Yankasa ewes at oestrus in this study is comparable to that reported by other researchers in different breeds of sheep (Oyedipe *et al.*, 1989; Homeida *et al.*, 2009).

Following insertion of CIDR® and FGA-45®, mean progesterone concentration increased from 1.1 ± 0.28 ng/ml and 1.1 ± 0.47 ng/ml to 3.2 ± 0.44 ng/ml and 2.3 ± 0.98 ng/ml respectively, on day 4. This was similar to earlier reports by Mustafa *et al.* (2007) in Awassi ewes. The rise in serum progesterone concentration following insertion of the progestagen (FGA-45® and CIDR® implant) is indicative of elevation in the endogenous progesterone levels induced by the exogenous progesterone sources. However, the level of rise in the CIDR treated ewes is far higher than that observed in the FGA treated

ewes at day 4 of treatment (Figure 4.8). This may be attributed to higher levels of progesterone released as well as negative feedback exerted on the hypothalamus by the CIDR compared to that by the FGA. This however did not affect the oestrus response rate but resulted in higher pregnancy rate in ewes treated with CIDR (100 %) than in ewes treated with FGA (71 %). This may be closely related to the high oestrus intensity in the CIDR treated ewes than in the FGA treated ewes. However, withdrawal of the implants resulted in sharp decline in serum progesterone concentration were observed, suggestive that the high levels observed during synchronization was exogenous. Progesterone levels dropped after day 12 to the lowest concentrations of 1.0 ± 0.70 ng /ml and 1.2 ± 0.61 ng /ml (day 14) in the YK-CD and YK-FG groups, respectively. The concentrations however rose again on day 38 (21 days after breeding) to 3.2 ± 0.44 ng /ml and 2.2 ± 0.37 ng /ml in the YK-CD and YK-FG groups, suggesting pregnancy.

The sharp decline in progesterone levels after the first and second injections of prostaglandin (Lutalyse® and estroPLAN®) indicate successful luteolysis of existing corpora lutea. Prostaglandins and analogues have been known to induce luteolysis of the corpus luteum in domestic animals (Ataman and Akoz, 2006; Homeida *et al.*, 2009). However, the effectiveness of $PGF_{2\alpha}$ in inducing luteolysis is reported to depend on the presence of a receptive or sensitive corpus luteum (CL). The CL of the ewe has been found to be receptive to $PGF_{2\alpha}$ or its analogues between days 5 and 14 of the estrous cycle (Gordon, 1999). At day 38 (21 days after mating), mean values of 2.7 ± 0.35 ng /ml, 3.1 ± 0.81 ng /ml, 3.2 ± 0.44 ng /ml and 2.2 ± 0.37 ng /ml in the YK-LT, YK-ET, YK-CD and YK-FG groups, respectively indicative of pregnancy. This is in agreement with the reports of Tarek *et al.* (2012) in Rahmani breed of sheep.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

i. Both protocols were efficient in oestrus synchronization. However, estroPLAN® and CIDR® offered the best results in synchronizing oestrus in Yankasa ewes.

ii. This study also confirmed that a 12-day treatment protocol using two doses of prostaglandin (estroPLAN® and Lutalyse®) and progestagen (CIDR® and FGA-45®) can be used in synchronizing oestrus in Yankasa ewes.

iii. Conception and pregnancy rates were better with synthetic prostaglandin (estroPLAN®) and Natural progestagen (CIDR®) than with the natural prostaglandin (Lutalyse®) and synthetic progestagen (FGA-45®) In Yankasa ewes.

iv. Progesterone profile is a reliable tool in pregnancy diagnosis in the Yankasa ewes following treatment with prostaglandins or progestagen treatments.

6.2 Recommendations

Following this study, it is therefore recommended that:

i. Further studies be done to evaluate ovulation time, gestation length and lambing rate in Yankasa ewes treated with prostaglandins and progestagens.

ii. Farmers should be encouraged to adopt the use of estroPLAN® or CIDR® synchronization protocol in Yankasa ewes to enhance productivity.

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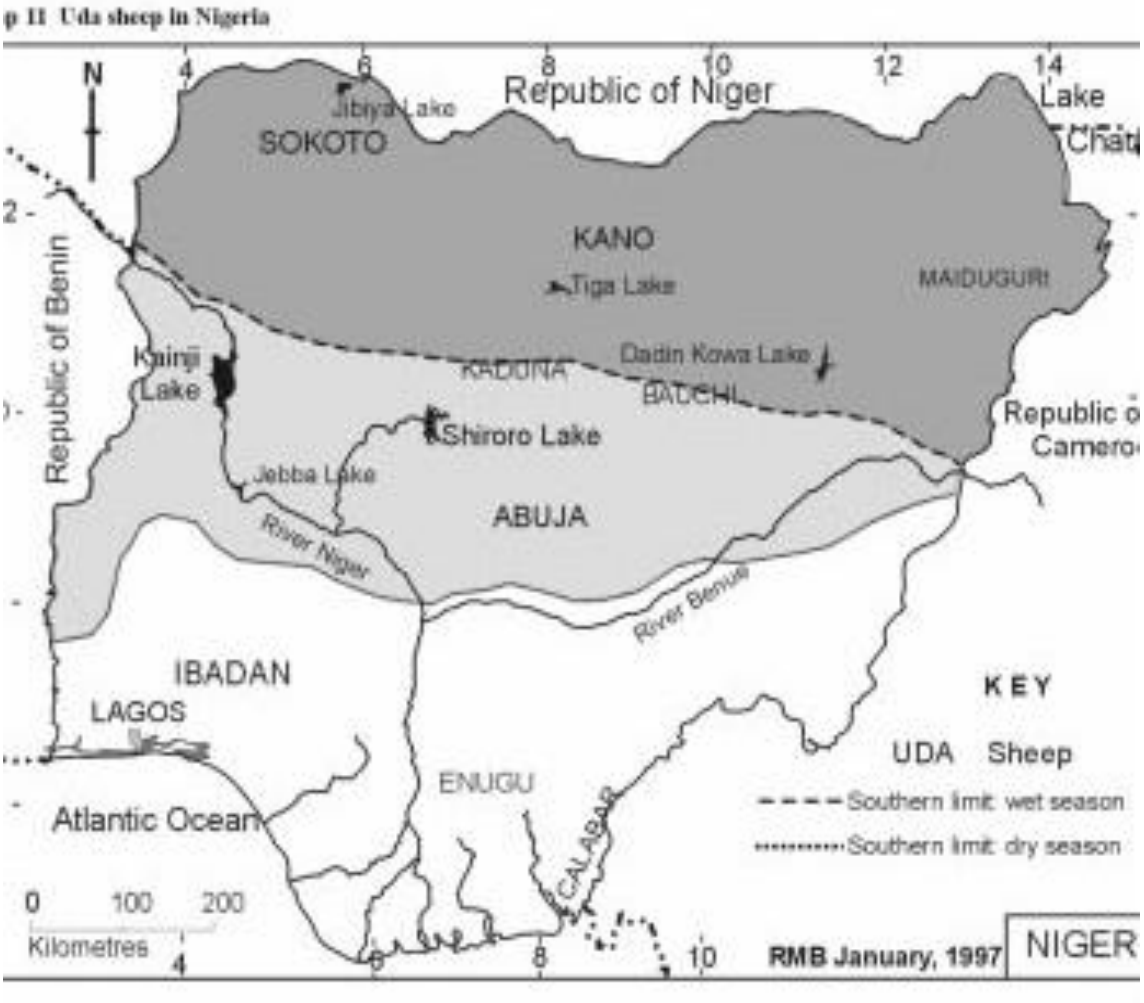
APPENDICES

Appendix 1: Distribution of Yankasa sheep in Nigeria

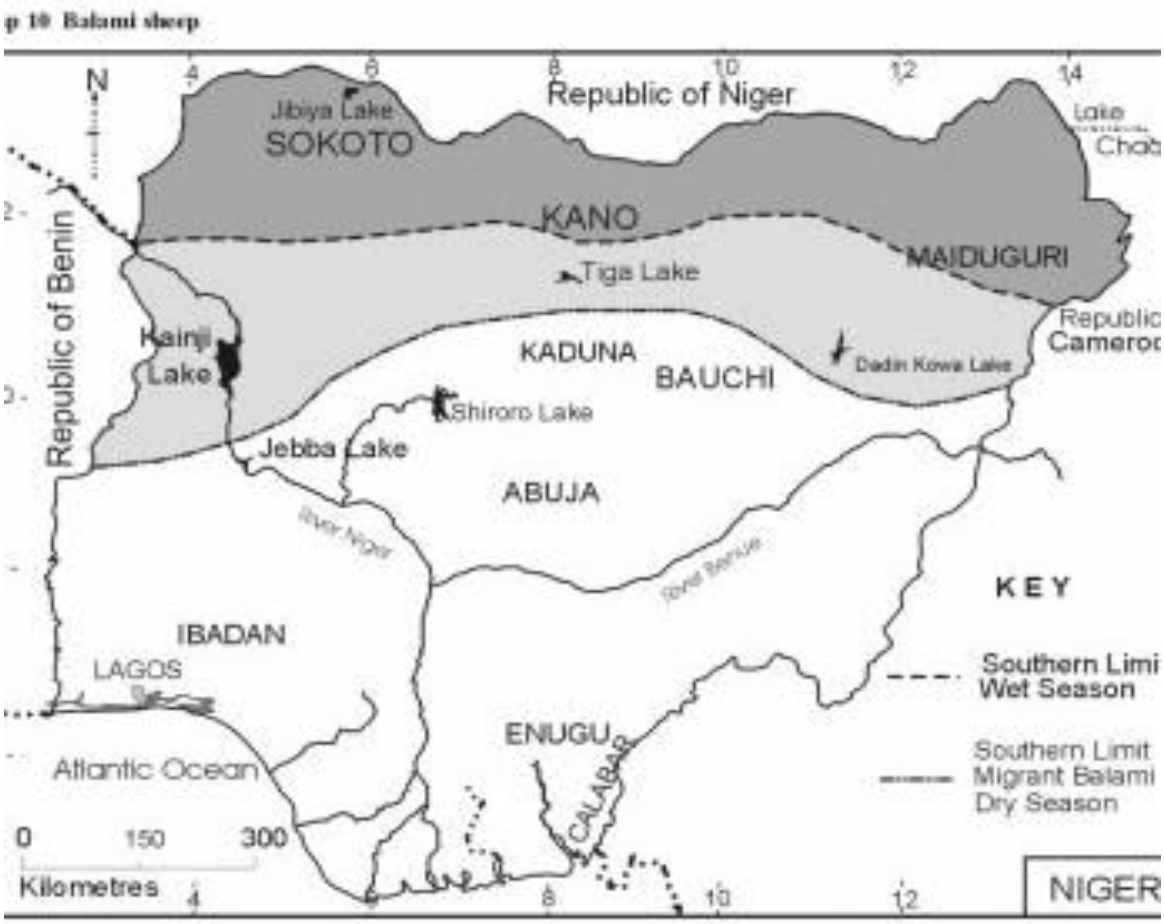
Source: Blench, 1999

Appendix II: Distribution of Uda sheep in Nigeria



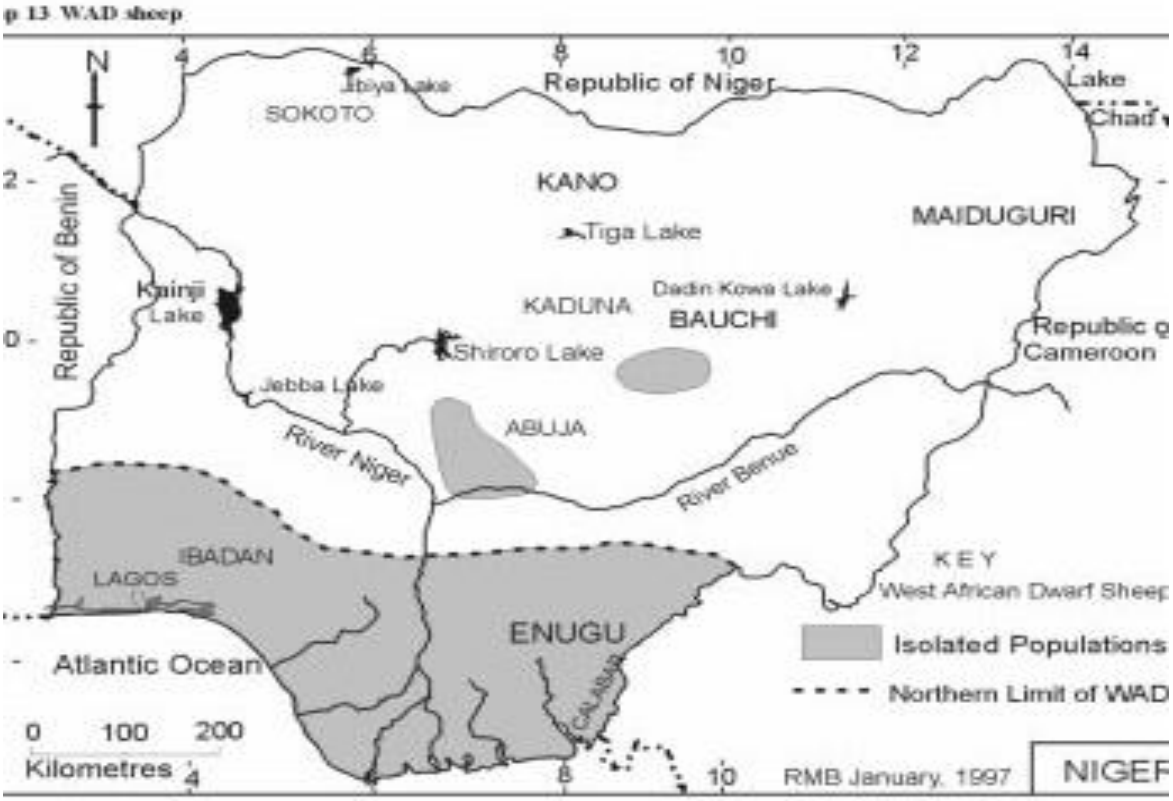
Source: Blench, 1999

Appendix III: Distribution of Balami sheep in Nigeria



Source: Blench, 1999

Appendix IV: Distribution of West African Dwarf sheep in Nigeria

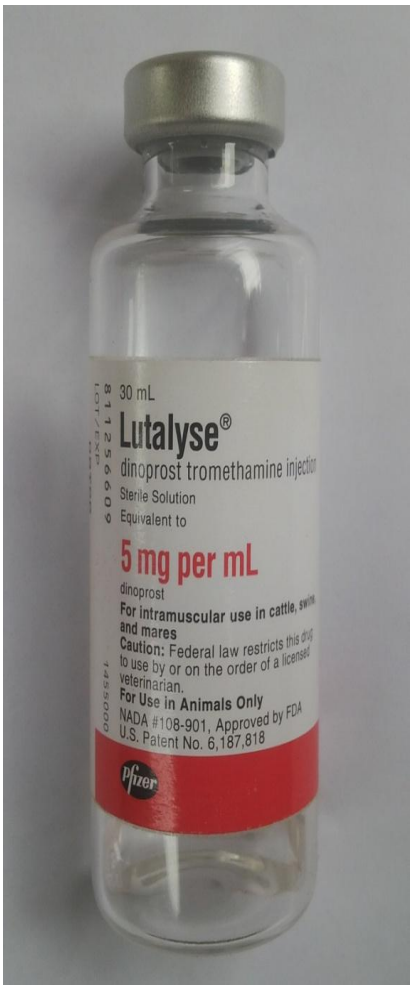


Source: Blench, 1999

Appendix V: prostaglandins

A-Cloprostenol sodium (estroPLAN®)

B- Dinoprost tromethamine (Lutalyse®)

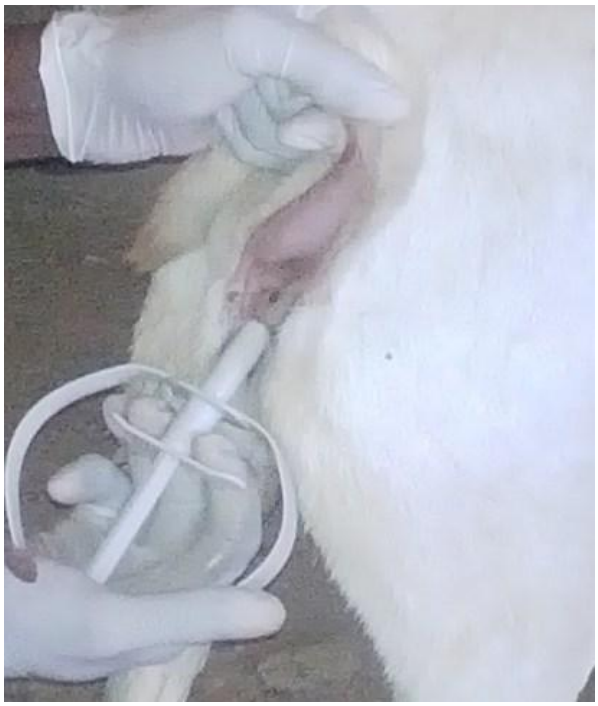


A

B

Appendix VI: Insertion of progestagens.

A-Insertion of EAZI-Breed™ CIDR® device into the vagina of a Yankasa ewe.



A

Appendix VII: Yankasa Ewe exhibiting oestrus behaviour by assuming urination posture.

Appendix VIII: Yankasa Ewe standing to be mounted by a ram during oestrus detection and breeding.