OESTRUS BEHAVIOUR AND HORMONAL CHANGES IN NIGERIAN INDIGENOUS JENNIES DURING THE OESTROUS CYCLE AND POSTPARTUM PERIOD

BY

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**DEPARTMENT OF THERIOGENOLOGY AND PRODUCTION,
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ZARIA, NIGERIA**

SEPTEMBER, 2016

DECLARATION

I declare that the work in this dissertation, entitled '**Oestrus Behaviour and Hormonal Changes in Nigerian Indigenous Jennies during the Oestrous Cycle and Postpartum Period**', has been performed by me in the Department of Theriogenology and Production, under the supervision of Prof. P. I. Rekwot and Prof. E. K Bawa. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Rahilah HASSAN _____ _____
Name of Student Signature Date

CERTIFICATION

This dissertation titled '**OESTRUS BEHAVIOUR AND HORMONAL CHANGES IN NIGERIAN INDIGENOUS JENNIES DURING THE OESTROUS CYCLE AND POSTPARTUM PERIOD**', by Rahilah HASSAN meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This project is dedicated to God Almighty, my loving husband, adorable princess, to my outstanding mum and my dear sister.

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ABSTRACT

This study was carried out to evaluate oestrus behaviour patterns and hormonal changes during the oestrous cycle and postpartum period in indigenous Jennies. Fourteen (14) cycling Jennies aged 3.0 ± 1.0 years were used to study the oestrus behaviour and hormonal characteristics (Experiment I). Fifteen (15) jennies aged 4.5 ± 1.5 years and in the third trimester were used to study resumption of postpartum ovarian activities (Experiment II). The 14 jennies for oestrus behaviour patterns were randomly assigned to 4 groups as follows; Group 1 (n=2) were control for the study (not treated); Group 2 (n=4) were jennies treated with a single injection of 10mg of Lutalyse®; Group 3 (n=4) were jennies treated with double injection of 10mg of Lutalyse®, 11 days apart while Group 4 (n=4) were jennies treated with double injection of 375µg of Synchromate®, 11 days apart, while jennies for the postpartum studies were brought into the experiment as they foaled. The treated groups and postpartum jennies after foaling were observed for behavioural oestrus three times daily (8-10 am, 12-2 pm and 4-8 pm) for two oestrous cycles and parameters of oestrus assessed were; Time to onset of oestrus, duration of oestrus, oestrus response rate and intensity of synchronization. Blood was collected from the jennies for extraction of serum, for progesterone and oestradiol estimation using Enzyme-linked Immunosorbent Assays (ELISA). One-way analysis of variance was used to analyze the results. For oestrus behaviour patterns, the Time to Onset of Oestrus of 2.34 ± 0.32, 10.07 ± 4.91, 3.98 ± 0.95 and 1.78 ± 0.29 hours in groups 1, 2, 3 and 4, respectively were significantly different ($P<0.05$). The duration of oestrus was 48 ± 24.0, 24.0 ± 0.0, 78.0 ± 22.7 and 120 ± 16.97 hours in groups 1, 2, 3 and 4, respectively ($P<0.05$). Oestrus response rate

were 50, 25, 75 and 100% in groups 1, 2, 3 and 4, respectively. Group 4, had 100% intensity of synchronization within 48 hours while groups 1, 2 and 3 had 50, 50 and 75% intensity of synchronization ($P<0.01$). The characteristic oestrus behaviour of jennies were; tail raising from the perineum, opening and closing of mouth (mouth clapping), flehmen and winking of the vulva. The duration of oestrus was longer, onset of oestrus was shorter and intensity of synchronization was 100% in jennies treated with double injections of 375µg $PGF_{2\alpha}$ (Cloprostinol-Synchromate®). From this study it was established that the average oestrus period of Nigerian indigenous jennies was 9.75 ± 1.75 days and their average oestrous cycle length was 23.80 ± 3.07 days. Two different progesterone profiles were observed in the postpartum studies, these varied in time when foal heat was observed in the jennies. In the first profile (n=10), foal heat was observed at 28 days postpartum while in the second profile (n=5), foal heat was observed at 35 days postpartum. Thereafter normal cyclical ovarian activity resumed at 56 to 63 days postpartum. The oestrous cycle length postpartum was 28 days. It was concluded from this study that synchronization of jennies is possible using exogenous $PGF_{2\alpha}$. Double treatments with cloprostenol and dinoprost tromethamine produced similar effects on the oestrus characteristics in jennies. However, cloprostinol produced better response and was more efficient in oestrus synchronization. This study has shown that, plasma progesterone and oestradiol analyses is a valuable tool for the study of fundamental reproductive endocrinology and provide information regarding the postpartum reproductive events in Nigerian indigenous jennies. Foal heat occurred between 28 to 35 days postpartum followed by a normal cyclical ovarian activity between 56 to 63 days in Nigerian indigenous jennies. Consequently, Nigerian

jennies can be bred from day 56 to 63 days (8-9 weeks) postpartum so as to ensure optimum fertility.

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LIST OF ABBREVIATIONS

% → **Percent**

µg → **Microgram**

CL → **Corpus Luteum**

E_2 → **Oestradiol**

ELISA → **Enzyme Linked Immunosorbent Assay**

FH → **Foal Heat**

kg → **Kilogram**

mg → **Milligram**

ml → **Millilitre**

n → **Number of animals**

ng → **Nanogram**

P_4 → **Progesterone**

pg → **Picogram**

$PGF_{2\alpha}$ → **Prostaglandin $F_{2\alpha}$**

S.E.M → **Standard error of the mean**

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Donkeys are one of the ancient domesticated livestock species and are valued for their ability to survive under harsh conditions (Blench *et al*., 1990; Swai and Bwanga, 2008). However, they are often regarded as animals of low social status and neglected by research and developmental organizations (Starkey, 1995). There are 41.5 million donkeys worldwide (Desalegne *et al*., 2011), with a population of 0.8 million donkeys in Nigeria (Mabayoje and Ademiluyu, 2004). Cross-border movements by the pastoral Fulani from Niger, Chad, Burkina Faso, Mali and Cameroon, have increased the number of donkeys in Nigeria (Blench, 2004). In Nigeria, donkeys are concentrated mainly in the northern states because of the savannah type of vegetation and fewer disease vectors such as tsetse flies (RIM, 1992). About 16,000 donkeys are transported annually from the northern to south-eastern Nigeria, where they are slaughtered for food (ATNESA, 1997; Blench, 2004).

Donkey rearing in Nigeria started with the introduction of different donkey breeds through trans-Sahara caravan trade across the Nile via the Sudan and Chad (Fielding and Starkey, 2004). In Nigeria, donkeys help to transport people, carry water from deep wells and rivers, and serve herdsmen during seasonal migration throughout Nigeria. In urban areas, donkeys provide small-scale services, such as transportation of building materials and grains, particularly in the northern part of the country, hence the title "beasts of burden" (Blench, 2004).

The jenny (jennet) is very similar in many reproductive aspects to the mare and puberty is usually attained at 1–2 years (Blanchard *et al*., 1999). Although the oestrous cycle has been reported to range from 20 to 40 days (Fielding, 1988), oestrus usually lasts between 6 and 9 days, with ovulation occurring 5–6 days after the onset of oestrus (Vandeplassche *et al*., 1981). Oestrus behaviour in jennies has been characterized by; lowered head with neck extended forward, opening and closing of the mouth, ears back against the neck, hind legs splayed, one foreleg slightly back and the other slightly forward, tail raised from the perineum, and presentation of the perineum toward the jack (McCort, 1980; Clayton *et al*., 1981; Vandeplassche *et al*., 1981; Henry *et al*., 1991). Of these elements, the most conspicuous and unique to the jenny are mouth movements known as jawing, yawing, or clapping. This is the opening and closing of the mouth with the lips relaxed the head and neck lowered and extended forward, and the ears back against the neck (Clayton *et al*., 1981). With palpation or ultrasonographic evaluation, follicles >25–30 mm should be considered potentially ovulatory (Henry *et al*., 1987). Gestational length has been reported to be 372–374 days and foal heat usually occurs between 5 and 13 days postpartum (Clarke, 1983). The donkey cervix protrudes into the vagina, and this protrusion may preclude intrauterine ejaculation, make artificial insemination more difficult, and may be associated with a high incidence of post dystocia cervical adhesions (Vendramini *et al.,* 1998).

Ovarian activity, pregnancy and parturition appear to be less seasonal in domestic and feral donkeys than in wild asses (McCort, 1980; Ginther *et al*., 1987; Henry *et al*., 1987). The short-day anovulatory season in domestic Jennies is approximately 165 days, with a high incidence of anovulatory oestrous which is brief and frequent, the long-day ovulatory season is approximately 200 days, with an interovulatory interval approximately 24-25 days (Henry *et al*., 1987). The mean length of ovulatory oestrus is

about 6 days, with ovulation within the last l-2 days of oestrus (Ginther *et al*., 1987; Henry *et al.*, 1991).

When more than one jenny is in oestrus within a territory, they tend to form a sexually active group, reminiscent of sexually active female bovid (Henry *et al*., 1991). Behavioural features of dioestrus includes running away from the jack, turning away and moving off rather than stopping abruptly, swaying of the hips side-to-side and away from the jack, clamping of the head of the tail against the perineum while swishing the distal portion of the tail, kicking out with full extension of both hind legs, a grunting vocalization, and biting the jack (Henry *et al*., 1991).

The postpartum period is of great importance in management of reproduction in mares. The time from parturition to the resumption of cyclic ovarian activity is very short, the first fertile oestrus occurs as early as 6 to 20 days after foaling (Ginther, 1992). In case of lactational anoestrus, mares fail to ovulate and/or show overt oestrus during the postpartum period (Allen, 1985). Galisteo and Perez-Marinb (2010) reported that, foal-heat was detected after parturition in Spanish donkey, ovulation occurred at 13.2 ± 2.7 days postpartum, with foal-heat duration of 4.7 ± 1.7 days. The interval between the first and second ovulations and the duration of oestrus were prolonged to 23.8 ± 3.5 and 5.7 ± 2.2days, respectively. Different methods have been used to evaluate the postpartum period in Jennies such as ultrasonography (Dadarwal *et al*., 2004).

Synchronizing oestrus is usually achieved by using luteolytic agents such as prostaglandin $F_2\alpha$ or one of its analogues. Examples of these products are sold under the trade names of Lutylase ®, Estrumate ® and Prostamate® (Howey *et al*., 1983). Prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) acts in oestrous synchronization by terminating the luteal phase through regression of the corpus luteum. Dinoprost tromethamine (Lutylase®) contains naturally occurring Prostaglandin-$F_{2\alpha}$ (dinoprost) as the tromethamine salt

(Salverson *et al*., 2002). Cloprostinol (Estrumate®) contains the synthetic analogue of the naturally occurring $PGF_{2\alpha}$ (dinoprost) as the sodium salt (Kusina *et al*., 2000).

1.2 Statement of Research Problems

In comparison to other livestock, fewer studies have been reported regarding the oestrous cycle of the jenny. Despite the increased number of descriptive and experimental reports, the information on jenny reproduction remains inadequate. There is a paucity of information on oestrus behaviour and hormonal profiles during the oestrus cycle of Nigerian indigenous jennies. In particular, the data related to the monitoring of basic reproductive parameters such as the physiological occurrence of heat during the postpartum period is also insufficient. Similarly, the influence of exogenous hormones particularly prostaglandins for inducing oestrus behaviour and oestrus cycle characteristics in indigenous jennies, to the best of our knowledge have not been reported. A good farm management program cannot ignore the physiological interval within which the resumption of reproduction occurs with the appearance of the first heat after foaling, usually referred to as foaling heat.

1.3 Justification

Donkeys play a major role in the economy and livelihood of rural dwellers in Nigeria. The increasing consumption of donkey meat and their current dwindling numbers necessitates efforts geared towards enhancing their reproductive performance. Studies on their reproductive behaviours and characteristics are to the best of our knowledge not sufficient enough to help tackle the issue of the dwindling number of donkeys. Therefore, this study will provide baseline data required for the adoption of assisted reproductive technologies (ART) in enhancing the reproductive performance of indigenous donkeys.

1.4 Aim of the Study

The aim of this study was to evaluate the effects of synchronization with prostaglandin on oestrus and oestrus behaviour of Nigerian indigenous jennies and to evaluate oestrus and oestrus behaviours of postpartum jennies.

1.5 Objectives of the Study

To evaluate:

I. effect of single and double treatment protocols of synchronization on;
   a) onset of oestrus in jennies
   b) duration of oestrus
   c) oestrus behaviour
   d) hormonal profiles (Progesterone and oestrogen)

II. the onset of oestrus, oestrus behaviour and progesterone profiles of postpartum jennies.

1.6 Research Questions

I. Do synchronizing agents used have any effects on the onset of oestrus, duration of oestrus, oestrus response rate, oestrus behaviours and oestrus cycle length.

II. What is the parturition-oestrus interval in indigenous jennies?

1.7 Hypothesis

Null hypothesis:

I. Single and double treatment oestrus synchronization protocols with natural and synthetic prostaglandins have no effect on duration of oestrus, onset of oestrus, oestrus response rate and intensity of synchronization.

II. The onset of oestrus, oestrus behaviour and progesterone profiles have no effect on postpartum jennies

CHAPTER 2

LITERATURE REVIEW

2.1 Classification of Donkeys

Scientific classification

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Perissodactyla

Family: Equidae

Genus: *Equus*

Subgenus: *Asinus*

Species: *E. africanus*

Subspecies: *E. a. Asinus*

Trinomial name

Equus africanus asinus (Grubb, 2005)

The donkey or ass, *Equus africanus asinus*, is a domesticated member of the horse family, Equidae. The wild ancestor of the donkey is the African wild ass, *E. africanus* (Grubb, 2005).The donkey has been used as a working animal for at least 5000 years. There are more than 40 million donkeys in the world, mostly in underdeveloped

countries, where they are used principally as draught or pack animals (Desalegne *et al.*, 2011). Working donkeys are often associated with those living at or below subsistence levels, small numbers of donkeys are kept for breeding or as pets in developed countries (Anonymous, 2003a).

A male donkey or ass is called a jack, a female a jenny or jennet; (Oxford American Dictionaries, 2005) a young donkey is a foal. Jack donkeys are often used to mate with female horses to produce mules (Anonymous, 2010c).

Asses were first domesticated around 3000 BC, probably in Egypt or Mesopotamia, and have spread around the world. They continue to fill important roles in many places today (Rossel and Marshall, 2008). While domesticated species are increasing in numbers, the African wild ass and another relative, the Onager, are endangered as beasts of burden and companions, donkeys have worked together with humans for millennia (Rossel and Marshall, 2008).

2.2 Scientific and Common Names

Traditionally, the scientific name for the donkey is *Equus asinus asinus* based on the principle of priority used for scientific names of animals (Grubb, 2005). However, the International Commission on Zoological Nomenclature ruled in 2003 that if the domestic species and the wild species are considered subspecies of each other, the scientific name of the wild species has priority, even when that subspecies was described after the domestic subspecies. This means that the proper scientific name for the donkey is *Equus africanus asinus* when it is considered a subspecies, and *Equus asinus* when it is considered a species. From the 18th century, *donkey* gradually replaced *ass* (Anonymous, 2003b).

Donkeys vary considerably in size, depending on breed and management. The height at the withers ranges from 7.3 to 15.3 hands (31 to 63 inches, 79 to 160 cm), and the weight from 80 to 480 kg. Working donkeys in the poorest countries have a life expectancy of 12 to 15 years (Anonymous, 2012). In more prosperous countries, they may have a lifespan of 30 to 50 years (Anonymous, 1990).

Donkeys are adapted to marginal desert lands. Unlike wild and feral horses, wild donkeys in dry areas are solitary and do not form harems. Each adult donkey establishes a home range; breeding over a large area may be dominated by one jack (French, 1997). The loud call or bray of the donkey, which typically lasts for twenty seconds can be heard for over three kilometres and may help keep in contact with other donkeys over the wide spaces of the desert (French, 1997). Donkeys have large ears, which may pick up more distant sounds, and may help cool the donkey's blood (Canacoo and Avornyo, 1998). Donkeys can defend themselves by biting, striking with the front hooves or kicking with the hind legs (Whitehead *et al.*, 1991).

2.3 Present Status

About 41 million donkeys were reported worldwide, China has the highest with 11 million, followed by Pakistan, Ethiopia and Mexico (Waltraud *et al.*, 2008). Nigeria has about 0.8 million donkeys (Mabayoje and Ademiluyu, 2004). Some researchers believe the actual number is somewhat higher since many donkeys go uncounted (Starkey and starkey, 1997).

2.4 Domestication of Donkeys and Their Uses

Domestication of the donkey about 5,000 years ago transformed ancient societies and initiated the course of development of mobile pastoral communities in Africa and the expansion of ancient Egyptian and Sumerian land-based routes (Marshall, 2007; Rossel

and Marshall, 2008). Marshall (2007) stressed on the importance of African pastoralists use of donkeys for transport during long-distance movements, and for women's daily tasks, such as fetching of water or firewood, and in the sustainable management of rangelands. The relationship between humans and the ancestors of modern donkeys was established by pastoralist, who had to use the animals to assist them to survive the harsh Saharan terrain in Northern Africa more than 5,000 years ago (Kimura *et al.*, 2013). Today, donkeys play critical role in assisting African herders to cope with unpredictable climatic fluctuations, they provide greater mobility with which to face erratic rainfalls and are used for carrying firewood, loads, including water, house hold structures, goods and children (Marshall and Weissbrod, 2011). Donkeys have facilitated mobility; they have often been employed for travelling and trade, both of which are crucial in the establishment of new settlements and cultures (Bough, 2011). In India, donkeys have been used to carry arms and ammunition to the difficult terrains that mechanized vehicles cannot reach easily (Singh *et al.,* 2005). In the developed countries where their use as draught animals has disappeared, donkeys are used to sire mules, to guard sheep, for donkey rides, for children or tourists and as pets (Dohner, 2007).

The domestic donkeys owe most of their genes to two African ancestors, the Nubian wild ass (*Equus africanus africanus*) and the Somali wild ass (*Equus africanus somaliensis*), (Patton and Snyder, 2002; Arunguren-Mendez *et al.*, 2004; Beja-Perreira *et al.*, 2004; Marshall, 2007). According to studies carried out by Yilmaz *et al.* (2012), the donkey may be the first hoofed animal domesticated in Africa and also the first to be used for transportation. The donkey's domestication came before that of the horse or camel, and donkeys are the only domesticated species to have originated in Africa

(Beja-Pereira, 2004; Bough, 2011). Later, donkeys became the driving force for cultivating agricultural lands in Northern Africa (Bough, 2011).

The ultimate use of donkeys for human therapeutic purpose is in the field of onotherapy, whereby donkeys are used as a co-therapist in cognitive, behavioural, motor, brain damage or sensory speech impairment (a method frequently used for people, who have disabilities and discomforts such as Autism in children, schizophrenic patients, anxiety disorders and impulse control disorders. To achieve balance patients carry out exercises on a still or moving donkeys, to achieve coordination patients learn to guide a donkey and perform simple tasks using eyes, voice and body) (Karatosidi *et al*., 2013). Emotional/physical benefits of companion animals are now being established in psychiatric hospital and elderly units and evidences that the presence of animals can have a direct calming effect (Karatosidi *et al*., 2013). In ancient civilization, donkeys were not only used as pack animals, but their milk, skin and meat were useful. The milk was highly prized as it contains more sugar and protein than bovine milk, and is a good substitute for babies and the sick (Criscione *et al*., 2009). Donkeys have recently gained new application with the use of their milk to feed humans with allergic disorders. The Ragusana and Martina Franca breed from Sicily; Italy is used to produce milk for human consumption (Caldin *et al*., 2005).

2.5 Behavioural Activities of Donkeys

Donkeys have a notorious reputation for stubbornness, but this has been attributed to a much stronger sense of self-preservation than exhibited by horses learn (Anonymous, 2010a). Likely based on a stronger prey instinct and a weaker connection with man, it is considerably more difficult to force or frighten a donkey into doing something it perceives to be dangerous for whatever reason. Once a person has earned their

confidence they can be willing and companionable partners and very dependable in work (Clutton-Brook 1992). Although formal studies of their behaviour and cognition are rather limited, donkeys appear to be quite intelligent, cautious, friendly, playful, and eager to learn (Anonymous, 2010a).

2.6 Social Organization

Feral and free-ranging domestic donkeys *(Equus minus)* have a territorial social system (Klingel, 1977; Woodward, 1979; McCort, 1980; Henry *et al*., 1991) as opposed to the harem system typical of horses and some zebras (Klingel, 1975). The composition and degree of stability of territorial groups varies with particular populations studied. In some populations, each breeding male holds his own territory through which solitary females with their young pass (Woodward, 1979). Jennies in oestrus are bred by the breeding male holding the particular territory. Jennies travel, often over large areas, as solitary individuals with their young or in small, loosely affiliated groups of two or three Jennies and their young, subordinate non breeding males are more or less tolerated by the territorial breeding males as they pass through or reside in their territory. In contrast to this simple territorial system, populations have been identified in which Jennies tend to stay within particular territories and have a more stable affiliation with the breeding male and other Jennies in the territory, in a semi-harem type territorial breeding group (McCort, 1980). In some populations, there are groups in which subordinate males are allowed to breed some of the Jennies within the territory of a dominant jack, usually following mating by the dominant jack (McCort, 1980).

2.7 Adaptation of donkeys to arid conditions

The donkeys have evolved to live in some of the most unfavourable ecological zones and are adapted on feed that is high in cellulose and low in protein. Donkeys are well-adapted to low humidity and high temperature (Smith *et al.*, 2005). Domestic donkey

performs hard work under different agro climatic conditions and withstands shortage of feeds. Hardiness, including ability to survive under harsh environmental conditions and low-market value has made the donkey the cheapest and suitable means of transportation in developing countries. Donkeys are adapted to tough desert conditions and have an easier time surviving in drought-prone areas than do other livestock because of inherent tolerance for dehydration, low sweat rate and good thermoregulatory mechanism; cattle, for example, tend to perish in these conditions (Jacobs, 2001; Singh *et al*., 2005; Rossel and Marshall, 2008). Donkeys have the longest working life span than any other animal; they are relatively less disease prone, have excellent stamina, and their adaptable grazing behaviour allows them to consume harsher types of vegetation, such as bark and leaves from trees (Jones, 2001; Ashley *et al*., 2005; Merrifield, 2008; Bough, 2011). Donkeys are surprisingly well adapted to heavy burdens of internal parasites; and are known to be very resistant to most livestock diseases which affect horses (Youseph *et al*., 2005). They have higher threshold for thirst than other equine species in terms of water intake and latency to drink when fluid deficits develop (Grinder *et al*., 2006). Donkeys are not well adapted to damp areas, but can easily live in marginal desert lands and are adapted to areas that are arid, at least seasonally (Anonymous, 2011).

2.8 Breeds of Donkeys

Donkeys are one of the least-studied large domestic animals, even though they are economically important in many regions of the world, when compared to other livestock breeds, donkey breeds seldom have much importance, because donkeys are not held in high regards (Kimura *et al*., 2013). The breed history of this animals remains unknown or hidden (Kugler *et al*., 2008). Breeds are only developed in countries, where mules breeding had a need for large and strong sires. The common breed of donkeys include

Abyssinian donkeys, Anatolian donkey, large standard, standard breed, Mammoth Jack, Mary donkey, Miniature breed, Poitou, Amiata (Asino amiatino), Martina Franca, Ragusano, Catalonian donkey, Andalusian and Zamaro-leones breeds (Mason, 1996; Aranguren-Mendez *et al.,* 2004). Three breeds of donkeys in the Northern-eastern part of Nigeria: They are Goho, Jaba and Fari (Hassan *et al*., 2009).

2.9 Donkey Hybrids

A male donkey (jack) can be crossed with a female horse to produce a mule, a male horse can be crossed with a female donkey to produce a hinny (Anonymous, 2003). Horse-donkey hybrids are almost always sterile because horses have 64 chromosomes whereas donkeys have 62, producing offspring with 63 chromosomes. Mules are much more common than hinnies. This is believed to be caused by two factors, the first being proven in cat hybrids, that when the chromosome count of the male is the higher, fertility rates drop (as in the case of stallion and jennet). (Anonymous, 1990). Due to different mating behaviour, jacks are often more willing to cover mares than stallions are to breed Jennies, further, mares are usually larger than Jennies and thus have more room for the ensuing foal to grow in the womb, resulting in higher birth weights. It is commonly believed that mules are more easily handled and also physically stronger than hinnies, making them more desirable for breeders to produce and it are unquestioned that mules are more common in total number (Anonymous, 2003). The offspring of a zebra-donkey cross is called a zonkey, zebroid, zebrass, or zedonk (Anonymous, 2010b). Zebra mule is an older term, but still used in some regions today. The foregoing terms generally refer to hybrids produced by breeding a male zebra to a female donkey. Zebra hinny, zebret and zebrinny all refer to the cross of a female zebra with a male donkey. Zebrinnies are rarer than Zedonkies because female zebras in

captivity are most valuable when used to produce full-blooded zebras (Anonymous, 2009).

2.10 Feral Donkeys and Wild Asses

In some areas domestic donkeys have returned to the wild and established feral populations such as those of the Burro of North America and the Ainara donkey of Sardinia, Italy, both of which have protected status (Lucas-Zenk, 2011). In Australia, where there may be 5 million feral donkeys, they are regarded as an invasive pest and have a serious impact on the environment (Roots, 2007). They may compete with livestock and native animals for resources, spread weeds and diseases, foul or damage watering holes and cause erosion (Anonymous, 2011).

2.11 Management of Donkeys

Hooves

Donkeys hooves are more elastic than those of horses, and do not naturally wear down as fast, regular clipping may be required, neglect can lead to permanent damage (Anonymous 2, 1990). Working donkeys may need to be shod. Donkey shoes are similar to horse shoes, but usually smaller and without toe-clips (Starkey *et al.*, 1997).

Nutrition

The donkeys have evolved to thrive on highly fibrous, poor-quality foodstuffs, and are grazers as well as browsers. According to Burden (2011), they have different nutrient requirements with significantly lower energy and protein needs, when compared with horses. Little studies have been carried out to address the specific needs of the donkeys, traditionally referred to as the "small horse". The donkey is different from horse in its ability to thrive on highly fibrous feeds (Burden *et al*., 2013). In their native arid and semi-arid climates, donkeys spend more than half of each day foraging and feeding,

often on poor quality crops (Taylor, 1997). The donkey has a tough digestive system in which roughage is efficiently broken down by hind gut fermentation, microbial action in the caecum and large intestine (Taylor, 1997). While there is no marked structural difference between the gastro-intestinal tract of a donkey and that of a horse, digestion in the donkey is more efficient (Smith and David, 2008). It needs less food than a horse or pony of comparable height and weight, approximately 1.5 percent of body weight per day in dry matter (Wood *et al.*, 2005) compared to the 2–2.5 percent consumption rate possible for a horse (Hall *et al.*, 1992). Donkeys are also less prone to colic (Svendsen, 1997). The reasons for this difference are not fully understood; the donkey may have different intestinal flora to the horse, or a longer gut retention time,they are also less prone to colic because of different intestinal flora, when compared with horses or have a longer retention time (Smith and Pearson,2005). Donkeys obtain most of their energy from structural carbohydrates, donkey also need to be fed straw (preferably barley straw), supplemented with controlled grazing in the summer or hay in the winter (Wood*et al.*, 2005). Donkeys do best when allowed to consume small amounts of food over long periods, and can meet their nutritional needs on 6 to 7 hours of grazing per day on average dry land pasture that is not stressed by drought. When they are worked long hours or do not have access to pasture, they require hay or a similar dried forage, with no more than a 1:4 ratio of legumes to grass. Donkeys also require salt and mineral supplements, and access to clean, fresh water (Aganga *et al.*, 2000). In lush climate, donkeys are prone to obesity and are at risk of laminitis (Anonymous, 2009).

Throughout the world, working donkeys are associated with the very poor (Aluja *et al.*, 2004). Few receive adequate food, and in general donkeys throughout the third World are under-nourished and over-worked (Svendsen, 1997). In temperate regions, the forage available is often too abundant and rich and over-feeding may result to weight

gain and obesity, leading to metabolic disorders such as laminitis (Morrow *et al*., 2011), gastric ulcers (Burden *et al*., 2008), equine metabolic syndrome (DuToit *et al.,* 2010) and hyperlipidaemia (Burden *et al*., 2011).

The limited availability of feed, together with the constraint that the traditionally management system imposes on the natural foraging behaviour of donkeys, often leads to poor nutrition in donkeys (Carretero-Roque *et al*., 2005). It has been observed that donkeys have an efficient digestive system compared to horses, thus, feeding them as if they are small horses leads to excess energy intake, resulting in obesity (Wood *et al*., 2005). Donkeys in their natural habitat (arid and semi-arid environments) spend more than half of the day grazing and feeding mostly on poor-quality shrubs (Taylor, 1997). The digestive system of the donkey is more efficient than that of the horse, but there is no marked structural difference between the gastro-intestinal tracts of the two. A donkey needs less feed than a horse or pony of similar height and weight (David *et al.,* 2008).

2.12 Welfare of Donkeys

The well-being of animals is defined by a range of parameters including health, physiology and behaviour (Early *et al*., 2010). Working animals, including donkeys provide an essential transport resource in developing countries around the world. Many donkeys are owned by poor people and the animals are in a very harsh environment, so, their welfare is a cause for concern (Pritchard *et al*., 2005). A protocol was developed to assess the welfare of working horses, mules and donkeys using direct observation of health and behavioural parameters. The result obtained from the study was utilized for welfare interventions in working equids and to establish a welfare benchmark (Durham *et al*., 2003). Consideration of health and behaviour is critical when assessing welfare. In horses, direct observations of behaviour have been used to assess recovery from

internal surgery and orthoscopic surgery (Price *et al*., 2003). The practice of extensive breeding of donkeys has led to the belief that they do not need any welfare assessment. Recently in the developed countries, there has been gradual introduction of intensive and semi-extensive production system, especially in high productive dairy breeds. In view of the growing concern of the consumers about the conditions of the animals, this has led to improved welfare (Passantino, 2011).

According to Serpell (2004), individual and societal attitudes are central to the study of animal welfare and behaviour because of the connection between human attitudes towards animals and the level of humane treatment given. The owners and/or handlers are often the active decision-makers in determining the donkey's care; the care that is given is largely dependent on the owner's and society's perceptions of the animal's value and needs (Mcllwraith and Rollin, 2011). Thus, owner and societal views are paramount to animal wellbeing. Consequently, animal welfare scientists have incorporated other measures to create a more holistic view of an animal's relationship with people, and its' environment, to better understand how to assess animal welfare (Fraser, 2008). Thus, the practice of measuring quality of life, originally created by health practitioners for humans, has been adapted for measuring quality of life in non-human beings (McMillian, 2005).

Understanding the behaviour of the donkey may be useful in planning strategies and extension programmes to improve the physical and psychological welfare of the donkey (Murugan, 2006). Recent research efforts have been focused on serious welfare concerns of equids in developing countries (Burn *et al*., 2010). In developing countries, equids often carry out tasks under impoverished conditions for long hours each day. As a result, studies have showed that they have many physical and clinical problems, such as wounds, poor body conditions, high parasite loads and dental diseases (Tesfaye and

Curran, 2005; Burden *et al*., 2010). These problems reduce the work efficiency of the animals, and indirectly reduce the income of the often- very poor people, who rely on them. Despite growing information on working equine health, little is known about the animal welfare implications of the myriad physical conditions the animals accumulate (Broster *et al*., 2009). Scientists are continuously trying to achieve a clearer understanding of equine learning behaviour and its implication in training. Behavioural and learning processes in equids are likely to influence the usefulness of the animal as a domestic species (Murphy and Arkins, 2007).

2.13 Breeding

A jennet is normally pregnant for about 12 months, though the gestation period varies from 11 to 14 months and usually foal a single foal. Births of twins are rare, though less so than in horses mules (Anonymous, 1990). About 1.7 percent of donkey pregnancies result in twins; both foals survive in about 14 percent of those (Whitehead *et al.*, 1991). Although jennets come into heat within 9 or 10 days of giving birth, their fertility remains low and it is usual to wait one or two further oestrus cycles before rebreeding. Because of this and the longer gestation period, donkey breeders do not expect to obtain a foal every year, as horse breeders often do, but may plan for three foals in four years mules (Anonymous, 1990).

2.14 Sexual Behaviour of Jennies

Jennies are polyoestrus, showing a more pronounced seasonal pattern in some circumstances than in others. This difference has not yet been elucidated and may be related to climate differences between study sites (Henry *et al*., 1987). External manifestation of sexual receptivity or rejection by Jennies occurs upon visual and especially tactile contact with a jack. Occasionally, vocalization of the male only,

without visual contact, is enough to stimulate Jennies to show overt signs of oestrus (Ginther *et al*., 1987).

2.15 Oestrus Cycle of Jennies

The oestrus cycle is defined as the period from one heat to the next heat as well as the period of time the jenny prepares for the conceptus. The oestrous cycle can further be defined into two phases, oestrus phase and dioestrus phase (Vandeplassche *et al*., 1981). The oestrus phase is defined as the follicular phase or the period where the jenny is in heat and is receptive to the jack or stallion and an ovum (or egg) ovulates. The oestrus phase is similar to that of a mare and can average anywhere from 6 to 8 days. The average oestrus phase of mares is about 5 to 7 days (Henry *et al*., 1987). Dioestrus phase is the luteal phase, the period where the jenny prepares for pregnancy due to a structure on the ovary that forms after an ovum or egg has been released or ovulates, known as the corpus luteum which produces a hormone (Progesterone) that signals pregnancy. Progesterone helps prevent her from coming into heat or displaying signs of heat (Ginther *et al*., 1987), also the lower progesterone production of the jenny may lead to early embryonic loss, in addition to other reasons not directly related to reproductive biology (Kugler *et al*., 2008). The dioestrus period in the donkey is on average 18 to 19 days which according to research is approximately 2 to 3 days longer than dioestrus in mares which is approximately 14 to 15 days. Therefore, the oestrus cycle of the horse, which includes both the oestrus and dioestrus period is about 19 to 22 days and the oestrus period of the jenny is longer by about 25 to 26 days (McKinnon and Voss, 2003). Other differences associated with the oestrous cycle in donkeys include the behavioural differences, cycling throughout the year, and the increased number of ovulation or twins in mammoth jennies when compared to horses. When jennies display signs of oestrus or being in heat typically the jenny will open and shut her mouth,

known as mouth clapping, as well as increase braying and she will allow other jennies to mount her or she will mount other jennies. This type of behaviour is also seen in cows and heifers when they come into heat. Although like the mare the jenny will also display signs such as frequent urination, winking of the vulva and raising her tail (McKinnon and Voss, 2003). Some studies have indicated that jennies are less likely to display signs of oestrus when being teased by stallions, also stallions have often been documented as not being very interested in jennies but most mares will still show signs of heat when being teased or bred to jacks. In addition when breeding jennies to stallions the conception rate is much lower than when breeding mares to jacks. Mares bred to jacks typically have normal fertility rates according to researchers but when making the reciprocal cross, the conception rate varies from 10 to 14.7% (Allen, 1982).

Another major difference when comparing the length of oestrus cycles of jennies and mares is the seasonality issue. Most mares are considered to be polyoestrus, meaning they come into heat when the photoperiod increases or daylight becomes longer and as daylight decreases they become less reproductively active (Ginther *et al.,* 1987). Light triggers the retina in the eye of the mare to signal to the hypothalamus in the brain to release hormones to other parts of her body that will prepare her to come in oestrus and when the light is decreased this signals to the hypothalamus to stop signalling the rest of the body and she does not come in heat. A majority of the mares do not show signs of oestrus in the winter months beginning around November through early February (depending on the location) due to a decrease in daylight, and this period is called anoestrus. However, jennies, especially mammoth jennies seem to be less sensitive to the length of daylight and continue to cycle throughout the winter months (Allen, 1982). A study conducted at Texas A&M University reported that the length of oestrus cycle throughout 4 seasons (winter, spring, summer, and fall) in 33 jennies were similar

among all seasons, oestrus cycle lengths was 23.3 ± 2.6 days and the length of oestrus was 5.9 ± 2.9 days (Blanchard *et al*., 1999). Other researchers have reported similar findings in mammoth jennies but not in standards (Ginther *et al.*, 1987).

Some researchers have reported that standard jennies do experience an anoestrus period during the winter months, meaning they do not show signs of heat in the winter (Henry *et al*., 1987). It has been documented that jennies are more sensitive to the nutritional availability than the photoperiod in regards to when they come on heat (Lemma, 2006). A study conducted in the Southern hemisphere such as West Africa, indicated that jennies do come on heat all year except during the rainy season when daylight is decreased and forage availability is maximized. The days of oestrus are longer when compared to the dry season when the daylight is increased and forages become limited due to lack of water. This research therefore indicates that in donkeys the availability of food or nutrients has a stronger implication over the oestrus cycle than the photoperiod like in mares (Lemma, 2006).

Not only have differences been reported in jennies in terms of when they come in oestrus and for how long but there can also be a higher chance for twins especially in mammoths. Several researchers indicated that standard jennies had multiple ovulations (meaning more than 1 egg or ovum is ovulated at a time) but only 5.3 to 31.8% of the time. However, other researchers indicated that mammoth jennies had approximately 61% multiple ovulations which are much higher than standards (Blanchard *et al*., 1999). Multiple ovulations or twins are seen in horses and most often it's associated with larger breeds of horses, so the same maybe true for the mammoth donkeys since they are a larger breed of donkey. It's important to keep in mind that mares and jennies who produce multiple ovulations can likely pass this onto their foals. Studies have indicated that multiple ovulations are not correlated to the season of the year and they either

happen on the same day or can be separated by as much as 11 days apart, the jenny will not go out of oestrus or heat until the second ovum has ovulated (Ginther *et al*., 1987; Blanchard *et al*., 1999).

Embryo transfer in donkeys has proven to be less successful when compared to success rates in mares. Typically in surgically placed embryos in mares the results average around 73% and nonsurgical from 40 to 72%. In jennies, nonsurgical transferred embryos were only 20% successful; meaning 5 to 7 days after the transfer the embryo was still alive (Panzani *et al*., 2006). Interestingly, efforts to save endangered breeds such as the Baudette Poitou breed, have led researchers to try alternative methods for transferring embryos such as placing donkey embryos into mares. This method has proven to be more successful and yielded higher pregnancy rates about 70% (Allen *et al*., 1985). One of the first successful embryo transfers from a Poitou donkey was conducted in 2002 by Dr. Angus McKinnon in Australia at Monash University using a Standard bred mare as the recipient. Complications have also been experienced when trying to preserve other endangered European donkey breeds such as the Zamorano-Leones, a rare Spanish breed of donkeys.

2.15.1 Oestrus

The particular elements of oestrus in the jenny include: lowered head with neck extended forward, opening and closing of the mouth, ears back against the neck, hind legs splayed, one foreleg slightly back and the other slightly forward, tail raised from the perineum, and presentation of the perineum toward the jack (McCort, 1980; Clayton *et al.*, 1981; Vandeplassche *et al*., 1981; Henry *et al.*, 1991). Of these elements, the most conspicuous and unique to the jenny are mouth movements known as jawing, yawing, or clapping. This is the opening and closing of the mouth with the lips relaxed the head and neck lowered and extended forward, and the ears back against the neck.

Certain elements of donkey oestrus portray ambivalence, these include the tendency for quick movement forward a few strides away from the jack followed by an abrupt stop and solid oestrus stance, abbreviated double hind-leg lifts reminiscent of kick threats, and mild tail swishing and hip swaying. All of these can occur in a single precopulatory interaction that ends in full oestrus stance and copulation (McCort, 1980; Clayton *et al*., 1981). The transition from the muted non receptive aspect to the clearly receptive counterpart typically appears to be the key behaviour eliciting mounting by the jack (McDonnell, unpublished observations).

When more than one jenny is in oestrus within a territory, they tend to form a sexually active group, reminiscent of sexually active female bovid (Henry *et al*., 1991).

2.15.2 Dioestrus

This includes running away from the jack, turning away and moving off rather than stopping abruptly, swaying of the hips side-to-side and away from the jack, clamping of the head of the tail against the perineum while swishing the distal portion of the tail, kicking out with full extension of both hind legs, a grunting vocalization, and biting the jack (Clayton *et al*., 1981).

2.16 Breeding Programs and General Reproduction

Jennies can be pasture or hand bred, whenever a hand breeding program is employed (either with natural breeding or artificial insemination), an effective teasing program will enhance its success (Blanchard *et al.*, 1999). Texas workers demonstrated that oestrus synchronization protocols useful in horses seem effective in the donkey (Vandeplassche *et al*., 1981). If natural breeding systems are used, the jenny should be mated the second day of oestrus, and then at 48-h intervals until the end of “standing heat” (Ferlding, 1988).

2.17 Postpartum Period

In recent years, the reproductive physiology of the donkey has attracted increasing attention of veterinary practitioners and researchers considering the farming relevance of this specie and the risk of extinction that involves several donkey breeds (Carluccio *et al*., 2006). This state of emergency is compounded by the lack of adequate information regarding their reproductive physiology which strongly limits a proper and aimed breeding management program (Carluccio *et al*., 2013). In particular, the precise detection of how and when the reproductive functions resume during the puerperium period is relevant for sound reproductive capacity. The growing demand of this primary jenny and/or mare product is related to its importance for human feeding, in particular, in allergy sufferers and newborn infants (Iacono *et al*., 1992; Mancuso *et al*., 2004). On the basis of currently available data, a fertile oestrus could appear in 5 to 13 days after foaling even if it has been described in a widely variable range in mares and Jennies (from 60% to 90%) followed by a second oestrus in 21 days (Pugh 2002; Galisteo *et al*., 2010; Sharma *et al*., 2010). In fact, to maximize the number of foals produced per dam lifetime and, as a consequence, the economic return of broodmares of one foal per mare per year should be produced. This reproductive target could be achieved only if pregnancy, lasting 335 to 340 days, would start in 25 to 30 days after foaling. Another aspect that must be clarified is to define the foal heat fertility in mares. Although foal heat spontaneously occurs (Panasophonkul *et al.,* 2007) because of a high pregnancy rate expression in feral pony populations (McDonnell 2000) however, a definitive evaluation of this crucial reproductive parameter in farming household mares is still debated (McKinnon *et al.,* 1988: Sharma *et al*., 2012). In particular, the results describing the incidence of pregnancy losses in foal heat-bred mares and the factors affecting foal heat pregnancy rate, remain inconclusive for the reduced case studies

(Makek *et al*., 1998; Sarwar *et al*., 1998), because of the heterogeneity of the mare breeds considered (Ishii *et al*., 2001; Blanchard *et al*., 2004), the management systems employed (Lowis *et al*., 1991; Morris *et al*., 2003) and the low accuracy of the transrectal palpation technique still used (Gilbert *et al*., 1992; Arott *et al*., 1994).

2.18 Importance of Donkey Milk

Donkey's milk is a very ancient product, used since the dawn of time. Its amazing properties have been known ever since (Karatosidi *et al*., 2013). It is said that Cleopatra, queen of Egypt, took care of great beauty and velvety skin by taking donkey milk-bath. Donkey's milk is used in the cosmetic industry to make soap and other cosmetic products because it contains active anti-ageing anti-oxidant and regenerative components (Karatosidi *et al*., 2013). Donkey milk has recently generated scientific interest because of its attractive nutrient and functional components. It is considered a valid substitute for infants with severe IgE-mediated cow's milk-protein allergy (Carrocio *et al*., 2002; Monti *et al*., 2007). Donkey milk is a potent vasodilator, making it potentially useful in the prevention of atherosclerosis (Tafaro *et al*., 2007) and has been shown to exert suppressive effect against human lung tumour *in vitro* (Mao, 2009). Presently, the milk is mostly used in Asia, Africa and Eastern Europe where donkeys are still bred traditionally.

The milk is predominantly rich in whey proteins, such as α – lactalbumin and β-lactoglobulin. The total casein content is about 8.7 g/L (Salimei *et al*., 2004). Donkey's milk is particularly rich in lysozyme, which is a well-known natural anti-microbial agent, and may contribute to the low bacterial concentration in donkey's milk (Malacarne *et al*., 2002; Salimei *et al*., 2004; Chiavari *et al*., 2005). Antimicrobial activity of isolated donkey's milk component is effective against pathogens such as *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli*

(Nazzaro *et al*., 2010). Biomolecules released during the proteolytic hydrolysis of the milk plays an important role to the antimicrobial activity in donkey milk, and also critical in the host defence system (Nazzaro *et al*., 2010). Donkey's milk may be considered a valid alternative for infant nutrition to powdered milk, soya bean milk or other formulas because its lipids and proteins are very close to human milk (Vincenzetti *et al*., 2008). The milk also contains high levels of calcium and phosphorous, and sixty times the amount of vitamin C found in cow's milk (Polidori *et al*., 2010). The milk also does not need pasteurization because it does not contain any natural bacteria and research has found that donkey milk contains immunoglobulins that boost the immune system, therefore helping individuals with reduced immune systems (Polidori *et al*., 2010).

2.19 Oestrogen

Oestrogens are important for a number of physiological processes, including reproductive events such as growth, differentiation and function of tissues in the reproductive tracts of females (Katzenellenbogen, 1996; Osborne *et al.*, 2000). Estradiol targets female reproductive tissues such as the mammary glands, uterus, vaginal and ovaries (Katzenellenbogen, 1996; Osborne *et al.,* 2000). Oestrogens is a part of the steroid hormone family and are mediated through oestrogen receptor (ER), which has a hormone binding site of 250 amino acids (Parker, 1993; Katzenellenbogen, 1996; Osborne *et al.*, 2000).

They function as a ligand-activated transcriptional complex that shares a common structural and functional organization of ligand binding, protein dimerization and transcriptional activation (Parker *et al.*, 1993; Katzenellenbogen, 1996; Osborne *et al*., 2000). Oestrogen influences gene expression by diffusing through the cell and binding to the nucleus which causes a dimerization of the receptors and facilitates the promoter

region of the gene to begin gene transcription ovaries (Katzenellenbogen, 1996; Osborne *et al*., 2000). There are many sources of ooestrogen releases; however, from a reproductive standpoint in the female tract, the ovarian follicle is the major sources of estradiol synthesis (Spicer and Echternkamp, 1986; Richards and Hedin, 1988). Oestrogen has been shown to increase GnRH receptor mRNAs for GnRH synthesis (Yasin *et al.,* 1995). It has been proven that oestrogen will also induce follicle atresia (Hutz *et al.,* 1988).

2.20 Progesterone

Progesterone is a steroid hormone responsible for the maintenance of the female reproductive tract (Rueda *et al.,* 2000; Williams and sigler, 1998). Progesterone actions are mediated through specific intracellular progesterone receptors (Williams and Sigler, 1998). In mammals, progesterone functions to support the uterus and ovary in releasing mature oocytes facilitate implantation and maintenance of pregnancy, to assist in mammary development, and milk secretion and mediation in sexual behaviour in the brain (Graham and Clarke, 1997; Rueda *et al*., 2000). Progesterone receptors are influenced by the release of oestrogen in most target tissues brain (Graham and Clarke, 1997; Williams and Sigler, 1998).

Progesterone is synthesized and secreted from the ovary as the primordial follicles are the main site for cyclic hormonal changes in the oestrus cycle (Graham and Clarke, 1997; Rueda *et al*., 2000). Once the follicle ruptures, the corpus luteum is formed and becomes the major endocrine organ for the release of progesterone cycle (Graham and Clarke, 1997; Rueda *et al*., 2000). A number of hormones such as Luteinizing hormone, $PGF_2\alpha$, Follicle stimulating hormone and prolactin all influence progesterone's release from the corpus luteum (Graham and Clarke, 1997; Rueda *et al*., 2000). Progesterone

can lengthen the oestrus cycle by extending the luteal phase and this prolongs both morphological and functional follicular dominance (Sirios and Fortune, 1988).

2.21 Oestrus Synchronization

Oestrus synchronization is the manipulation of the reproductive process so that females can be bred with normal fertility during a short, predefined interval (Mitchell *et al*., 1976). This control facilitates breeding in two important ways: it reduces and in some cases eliminates the labour of detecting oestrus (heat), and it allows the producer to schedule the breeding. If the majority of a herd can be induced to exhibit oestrus at about the same time, the producer can arrange for a few days of intensive insemination. Although the total amount of labour involved with insemination may not be reduced, it is concentrated into a shorter period (Howey *et al.*, 1983). A successful oestrus synchronization program requires an understanding of the oestrus cycle and research has dramatically increased the number of synchronization options. A producer has many choices to pick from to tailor a synchronization protocol to his operation, his production goals and his available labor (Weems *et al*., 2006). One of the oldest ways to synchronize oestrus is by using a luteolytic agent such as prostaglandin $F_2\alpha$ or one of its analogues, which causes the regression of the corpus luteum (Weems *et al*., 2006).

Prostaglandin $F_2\alpha$ is only effective if administered after day 7 of the oestrus cycle i.e. luteal phase (Mitchell *et al*., 1976). Examples of these products are sold under the trade names of Lutylase®, Estrumate® and Prostamate® (Howey *et al.*, 1983). Synchrony of oestrus and fertility with these products is good with cyclic females, such as virgin heifers, but will not induce oestrus cycles in non-cycling cows following calving (postpartum anoestrus). The hormone treatments used in these protocols are the least expensive; however, they often require skilled labour for oestrus detection. Another way of creating oestrus synchrony is by using gonadotropin-releasing hormone (GnRH) or

an analogue, which causes ovulation of a large follicle. This product is sold under the trade name Cystorelin®, Fertagyl® or Factrel®. Treatment with GnRH is combined with prostaglandin F2α, as these hormones have different functions. Synchrony of oestrus and fertility with a combination of GnRH and prostaglandin F2α are good for cyclic females (Howey *et al.*, 1983).

2.22 Prostaglandin-$F_{2\alpha}$ and its Analogue in Reproduction

Prostaglandin F_2 and its analogues have been used to induce oestrus and terminate pregnancy and in the management of reproductive disorders in domestic animals (Squires, 2008). The side effects vary by the type of prostaglandin, the dose level, the dosage regimen and the species. In mares PGF_2 causes transient side effects (locomotor disturbances, respiratory distress, tachycardia and sweating) at doses lower than 100 mg but the effects are profound at doses of 400mg and above (Pharmacia and Upjohn Company, 2004). No side effect was recorded in a regimen of 0.5 mg PGF_2 given 24 hours apart compared to a 10 mg single dose (Irvine *et al.*, 2002). Sweating, increased respiration and defecation were reported after administration of fluprostenol at 250 -500 µg (Ousey *et al.*, 1984) and except for swelling at the site of injection no side effect was recorded after administration of fenprostalene at a dose of 250 µg (Bosu *et al.*, 1983). Cloprostenol at a dose of 0.075 mg was luteolytic with no side effects (Carluccio *et al.*, 2006). 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).

2.22.1 Dinoprost tromethamine

This product contains naturally occurring Prostaglandin-$F_{2\alpha}$ (dinoprost) as the tromethamine salt. Each milliliter (ml) contains dinoprost tromethamine equivalent to 5

milligram (mg) dinoprost with 16.5 mg of benzyl alcohol added to as preservative. 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).

2.22.2 Cloprostinol

This product contains the synthetic analogue of the naturally occurring $PGF_{2\alpha}$ (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 for 250µg either twice at a 5 to 14 day interval (Jainudeen and Hafez, 2000; Kusina *et al*., 2000).

2.23 Assessment of Body Condition

Body condition is a useful indicator of a donkey's nutritional status and its well-being. It can be used to compare donkeys of different sizes and on different management systems where individuals cannot be reliably identified (Pearson and Ouassat, 2000). Although condition scoring is a subjective technique, with practice, people doing it can obtain a high level of repeatability on different occasions and between different scorers (Pearson and Ouassat, 2000).

2.23.1 Body Condition Scoring System

A nine-point system was selected as it gives distinguishable steps that can be described and used to account for the wide range of body conditions that are shown by donkeys around the world from temperate to tropical areas (Nicholson and Butterworth, 1985). It avoids the use of half-points, which seem to be common when applying a five point system to livestock condition scoring. An arrangement in which three main categories

are first defined, which are then each sub-divided into three to give nine possible options gave repeatable results as well as being easy to teach and explain to others (Nicholson and Butterworth, 1985).

Description of the body condition scores for donkeys on a scale from 1 to 9.

1. Very thin (Emaciated); Animal markedly emaciated; bone structure easily seen over body; little muscle present; animal weak, lethargic.

2. Thin Animal emaciated; individual spinous processes, ribs, hooks (tuber coxae), pins (tuber ischii), shoulder blades and spine all prominent, sharply defined; some muscle development; neck thin; prominent withers; shoulders sharply angular.

3. Less thin; Vertebral column prominent and individual spinous processes can be felt, little fat, but superspinous muscle over spinous processes apparent; ribs, pins, (tuber ischii) and hooks (tuber coxae) prominent; loin area and rump concave; little muscle or fat covering over withers and shoulders.

4. Less than moderate; Vertebral column visible; pins (tuber ischii) can be felt but not visible, hooks (tuber coxae) rounded but visible; rump flat rather than concave; ribs can be felt but not obvious; withers, shoulders and neck have some muscle and fat cover; shoulder blades less clearly defined.

5. Moderate; Superspinous muscles developed and readily apparent, vertebral column can be felt; hooks (tuber coxae) rounded; rump rounded, convex; pins (tuber ischii) not visible; some fat can be felt in shoulder area region and at base of neck; can feel ribs, but not visible.

6. More than moderate; Cannot feel spinous processes easily; back becoming flat well covered; rump convex and well-muscled; some fat can be felt on neck, base of neck and shoulder area; neck filled into shoulder; hooks (tuber coxae) just visible.

7. Less fat; Back flat, cannot feel spinous processes, hooks (tuber coxae) just visible; fat on neck and shoulder area beginning to expand over ribs; flanks filling, neck thickening.

8. Fat; Animal appears well covered with body rounded with fat and bones not discernible; flanks filled, broad back.

9. Very fat; (obese) Bones buried in fat; back broad or flat, in some cases crease along the backbone; large accumulations of fat on neck, over shoulder area and ribs; flank filled with fat.

Notes: 1-3 frame obvious. 4-6 frame and covering balanced. 7-9 frame not as obvious as covering (Pearson and Ouassat, 1996).

The spinous processes are the bony points rising from the spine or backbone. The superspinous muscle is the muscle along each side of the backbone. Individual donkeys can deposit their body fat in different areas of the body, so the individual neck, shoulders, ribs, rump, and flank condition should be assessed and combined to give an overall condition score (Pearson and Ouassat, 1996).

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Area

This study was carried out at the Donkey Farm of the Equine and Camel Research Programme of the National Animal Production Research Institute (NAPRI), Shika, Ahmadu Bello University, Zaria. NAPRI is located in the Northern Guinea Savannah Zone of Nigeria between 11˚N and 12˚N and 7˚E and 33˚E, at an elevation of 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 between 20-37 % (Kowell, 1986).

3.2 Experimental Animals and Management

The study was conducted using jennies of the Equine and Camel Research Programme of National Animal Production Research Institute (NAPRI). Fourteen (14) cycling Jennies aged 3.0 ± 1.0 year were used for oestrus behaviour and hormonal profile characteristics during the oestrous cycle (Experiment I). Fifteen (15) jennies aged 4.5 $\pm$ 1.5 years and in the third trimester, were used to study resumption of postpartum activities (Experiment II). The jennies were kept outdoors in a group and fed *Digitaria smutsii* (wooly finger grass), concentrate rations at 1.2kg/jennies/day and hay as basal diet, water was provided *ad libitum*.

3.3 Methodology

3.3.1 Experiment I: Oestrus behaviour and hormonal profile characteristics during the oestrous cycle

Fourteen (14) cycling Jennies aged 3.0 ± 1.0 years with mean body weight of 90.6 ± 6.5kg and mean body condition score of 3.5 ± 0.2 were used for this study. Cyclicity was determined based on two rectal palpations (30 days apart), ultrasound scans done twice and two blood samples were also obtained twice (30 days apart) to confirm cyclicity.

3.3.2 Experiment II: Postpartum ovarian activity in jennies

Fifteen (15) jennies aged 4.5 ± 1.5 years and in the third trimester, with mean body weight of 119.6 ± 26.5kg and mean body condition score of 3.5 ± 0.6 were used for this study. The jennies joined the study as soon as they foaled. Five (5) ml of blood was obtained from the jennies via jugular veni-puncture using a 5 ml syringe (18 Gauge needle) on day of foaling and thereafter twice weekly for progesterone profile, until the observation of overt oestrus behaviours. The blood samples were transported to laboratory on ice pack where they were centrifuged at 2000×G to obtain serum. The serum samples were stored at -20 ^{0}C until analysis for progesterone (P_4) concentrations using Enzyme Linked Immunosorbent Assay (ELISA) kits from Monobind Limited, Lake Forest, CA 92630, USA.



Plate 1 Rectal palpation in a jenny.

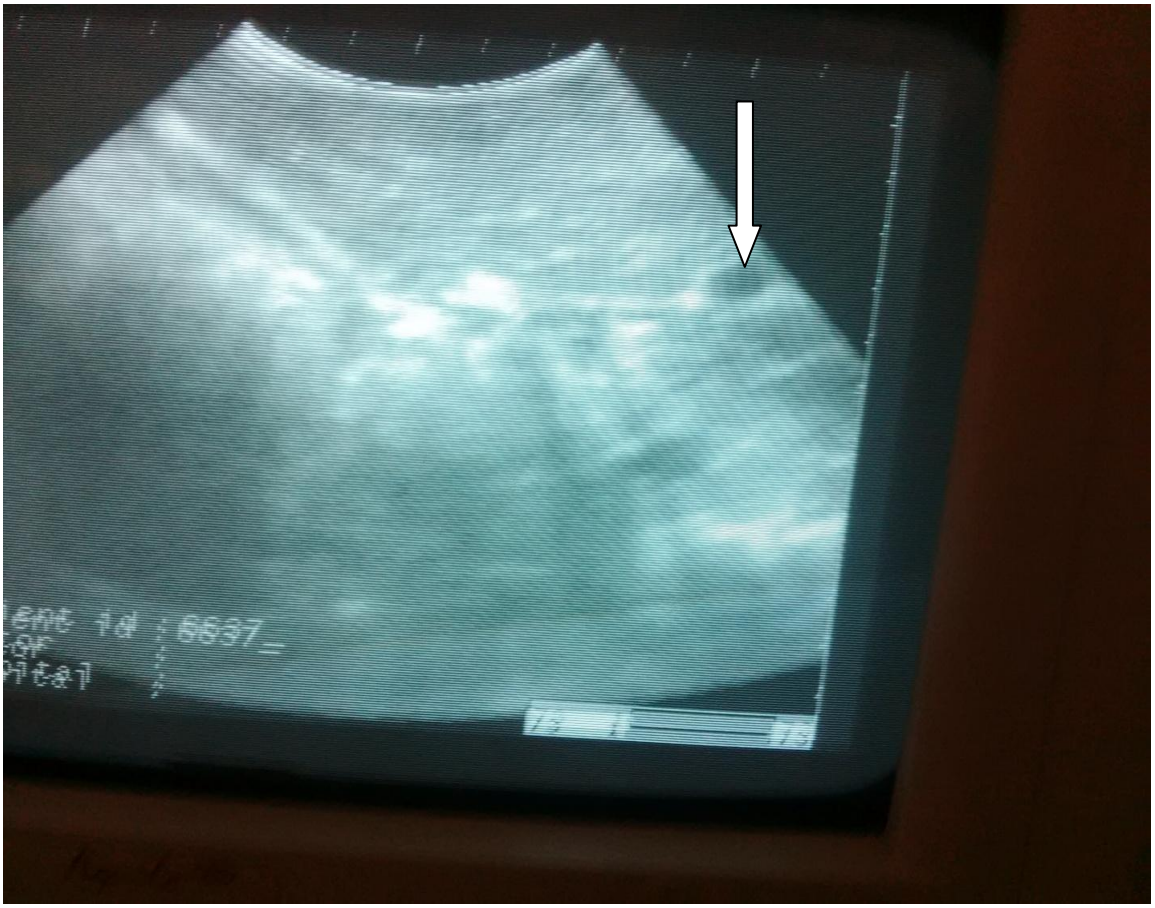
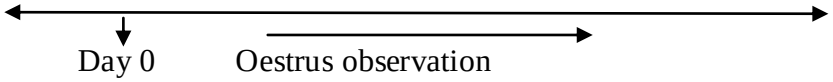


Plate 2. Ultrasonograph of a cycling jenny, showing a follicle (arrowed).

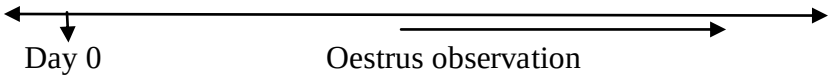
3.4 Experimental Design

The animals were randomly assigned to 4 groups;

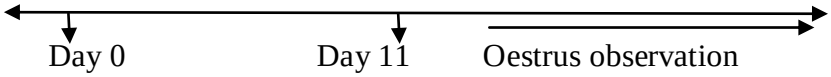
Group 1: Two jennies were used to detect natural oestrus and served as control



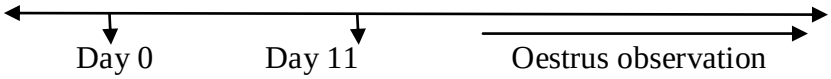
Group 2: Four jennies were synchronized with a single intramuscular injection of 10mg of $PGF_{2\alpha}$ (Dinoprost tromethamine- Lutalyse®).



Group 3: Four jennies were synchronized with double intramuscular injection of 10mg of $PGF_{2\alpha}$ (Dinoprost tromethamine- Lutalyse®) 11 days apart.



Group 4: Four jennies were synchronized with double intramuscular injection of 375µg of $PGF_{2\alpha}$ (Cloprostinol-Synchromate®) 11 days apart.



3.5 Oestrus Detection

Following the administration of the first and second injections of $PGF_{2\alpha}$ on days 0 and 11, respectively, treated jennies were observed for behavioural oestrus beginning on day 1 (after 1st treatment and control group) and day 12 (after 2nd treatment), respectively. Jennies were observed for oestrus behaviours for two hours, three times daily (8-10 am, 12-2 pm and 4-6 pm) for the period of the experiment.

Parameters evaluated were, lowered head with neck extended forward, opening and closing of the mouth, ears back against the neck, standing to be mounted, tail raised

from the perineum, vulva winking, mucous discharge and presentation of the perineum toward the jack. In addition Jennies were exposed to a jack to aid oestrus detection. The following oestrus behaviour and characteristics to be determined:

1. Duration of oestrus- Time interval between the first and last standing oestrus, expressed as Mean ± S.E.M

2. Onset of oestrus- Time interval from last $PGF_{2\alpha}$ administration to the time when the jennies first expressed standing oestrus.

3. Oestrus response rate- Number of jennies that were visually observed to be mounted by a jack following 1^{st} and 2^{nd} $PGF_{2\alpha}$ injection compared to the total number of jennies injected.

4. Intensity of synchronization- Number of jennies in oestrus as a result of synchronization within 48 hours.

5. Physical manifestation of oestrus- Number of animals that showed the various oestrusbehaviours.

3.6 Blood Sampling

Five (5) ml of blood each were obtained from the jennies via jugular veni-puncture using a 5 ml syringe (18 Gauge needle) on the day of synchronization, then twice weekly for 8 weeks, to determine the progesterone and estradiol concentrations. The blood samples were decanted into vacutainers and transported to the laboratory. Blood samples were centrifuged at 2000×G and serum harvested. Serum samples obtained were appropriately stored at -20˚C until analysis for progesterone and estradiol concentrations using an Enzyme-linked immunosorbent assays (ELISA) technique.

3.7 Hormone Assay

Concentrations of progesterone and oestradiol were measured in the serum using enzyme-linked immunosorbent assays (ELISA). The ELISA kits were obtained from

Monobind® Inc, USA. The kits were used according to the manufacturer's specifications**.** The oestradiol AccuBind™ Microplate (EIA) test system had a sensitivity of 8.2 pg/ml serum calibrator and using the 95% certainty statistic to calculate the minimum dose. The between assay precision coefficient of variation for the low, normal and high pooled controlled serum samples were 9.9%, 8.5% and 7.5%, respectively.

The Progesterone AccuBind® ELISA test system had a sensitivity of 0.105 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 95% certainty statistic to calculate the minimum dose. The between assay precision coefficient of variation for the low, normal and high pooled controlled serum samples were 8.9%, 7.5% and 6.4%, respectively. The within assay precision coefficient of variation for the low, normal and high pooled controlled serum samples were 15.3%, 3.8% and 6.1%, respectively.

3.7.1 Oestradiol Assay Procedure

1. The micro plates well for each serum reference was formatted and the control specimen duplicated with unused microwell strips returned.
2. 0.025 ml (25µl) of the appropriate serum reference control or specimen was pipetted into the assigned well.
3. 0.050 ml (50µl) of oestradiol biotin reagent was added to all wells
4. The microplate was swirled gently for 20-30 seconds to mix, then covered and incubate for 30 minutes at room temperature
5. 0.05ml (50µl) of oestradiol enzyme reagent was added to all the well.
6. The microplate was swirled gently for 20-30 second to mix, covered and incubate for 90 minutes at room temperature.

7. The content of the micro plate was then discarded and blot dried with adsorbent paper.
8. 350µl of wash buffer was added and washed thoroughly, repeated two additional times.
9. 0.1 ml (100µl) of substrate solution was added to all the wells and incubated at room temperature for twenty (20) minutes.
10. 0.050 ml (50µl) of stop solution was added and gently mixed for 20 seconds.
11. The absorbance in each well was read at 450 nm (using a reference wavelength of 620-630 nm) within thirty minutes of adding the step solution.

3.7.2 Progesterone Assay Procedure

1. The micro plates well for each serum reference was formatted and the control specimen duplicated with unused microwell strips returned.
2. 0.025 ml (25µl) of the appropriate serum reference control or specimen was pipetted into the assigned well.
3. 0.050 ml (50µl) of progesterone-enzyme reagent was added to all wells
4. The microplate was swirled gently for 20 seconds to mix.
5. 0.05 ml (50µl) of progesterone biotin reagent was added to all the well.
6. The microplate was swirled gently for 20-30 second to mix, covered and incubate for 60 minutes at room temperature.
7. The content of the micro plate was then discarded and blot dried with adsorbent paper.
8. 350 µl of wash buffer was added and washed thoroughly, repeated two additional times.
9. 0.1 ml (100µl) of substrate solution was added to all the wells and incubated at room temperature for 20 minutes.

10. 0.050 ml (50µl) of stop solution was added and gently mixed for 20 seconds.
11. The absorbance in each well was read at 450 nm (using a reference wavelength of 620-630 nm) within thirty minutes of adding the step solution.

3.8 Data Analysis

One way analysis of variance model was used to analyse the data.

$y_{ij} = \mu + t_i \pm e_{ij}$

Where;

y_{ij} is the individual observation

μ is the overall mean

t_i is the effect of the ith (1,2,3 and 4) group

e_{ij} is the random error

Oestrus response rate, intensity of synchronization and physical manifestation of oestrus were expressed as percentages. Chi square was used to compare between the physical manifestations of oestrus and the different groups. Data on progesterone and oestradiol profile values of postpartum and oestrus cycle, duration of oestrus and time to onset of oestrus, were expressed as Mean ± S.E.M. Comparisons between the groups were carried out using one way ANOVA and T- test. The differences were considered significant when $P < 0.05$, highly significant when $P<0.01$ and not significant when $P > 0.05$. SAS system for windows 9.0 was used for the analysis.

CHAPTER 4

RESULTS

4.1 Onset of Oestrus

Mean time to onset of oestrus (Mean ± S.E.M) was 2.34 ± 0.32, 10.07 ± 4.91, 3.98 ± 0.95 and 1.78 ± 0.29 hours for groups 1, 2, 3 and 4, respectively (as shown in figure 4.1). Jennies in group 4 had the shortest time to onset of oestrus. However, the Time to onset of oestrus were significantly different between the four groups ($P<0.05$).

4.2 Duration of Oestrus

Mean duration of oestrus (Mean ± S.E.M) was 48.0 ± 24.0, 24.0 ± 0.0, 78.0 ± 22.7 and 120 ± 16.97 hours for groups 1, 2, 3 and 4, respectively (as shown in figure 4.2). Jennies in group 4 had longer duration of oestrus (120 ± 16.97 hours). However, the durations of oestrus were significantly different between the four groups ($P<0.05$).

4.3 Oestrus Response Rate

Oestrus response rates was 50, 25, 75 and 100% for groups 1, 2, 3 and 4, respectively (as shown in figure 4.4). Jennies in group 4 that were treated with double injections of 375µg $PGF_{2\alpha}$ (Cloprostinol-Synchromate®) had 100% oestrus response rate. However, the oestrus response rate were significantly different between the four groups ($P<0.01$).

4.4 Intensity of Synchronization

Group 4 (double injections Synchromate), had 100% intensity of synchronization within 48 hours while groups 1, 2 3 had 50, 50 and 75% intensity of synchronization (as shown in figure 4.4). However, the differences in the intensity of synchronization was highly significant between the four groups ($P<0.01$).

4.5 Postpartum Activities

Progesterone (P_4) profiles for jennies that exhibited foal heat at 28 and 35 days postpartum are presented in figure 4.5. Mean serum P_4 levels for the 2 groups were not significantly different ($P > 0.05$) mean serum P_4 concentration was 2.44 ± 0.05 ng/ml (jennies with FH 28 days) and 5.72 ± 0.1 ng/ml (jennies with FH 35 days) on day 0 postpartum, and then dropped to 0.44 ± 0.23 ng/ml at 28 days and 1.20 ± 0.08 ng/ml 35 days after parturition. P_4 concentrations increased to 21.36 ± 3.51 ng/ml on day 42 and 19.5 ± 6.92 ng/ml on day 49 for both groups then declined to1.9 ± 0.2 ng/ml on day 56 and 3.6 ± 1.2 ng/ml on day 63, respectively.

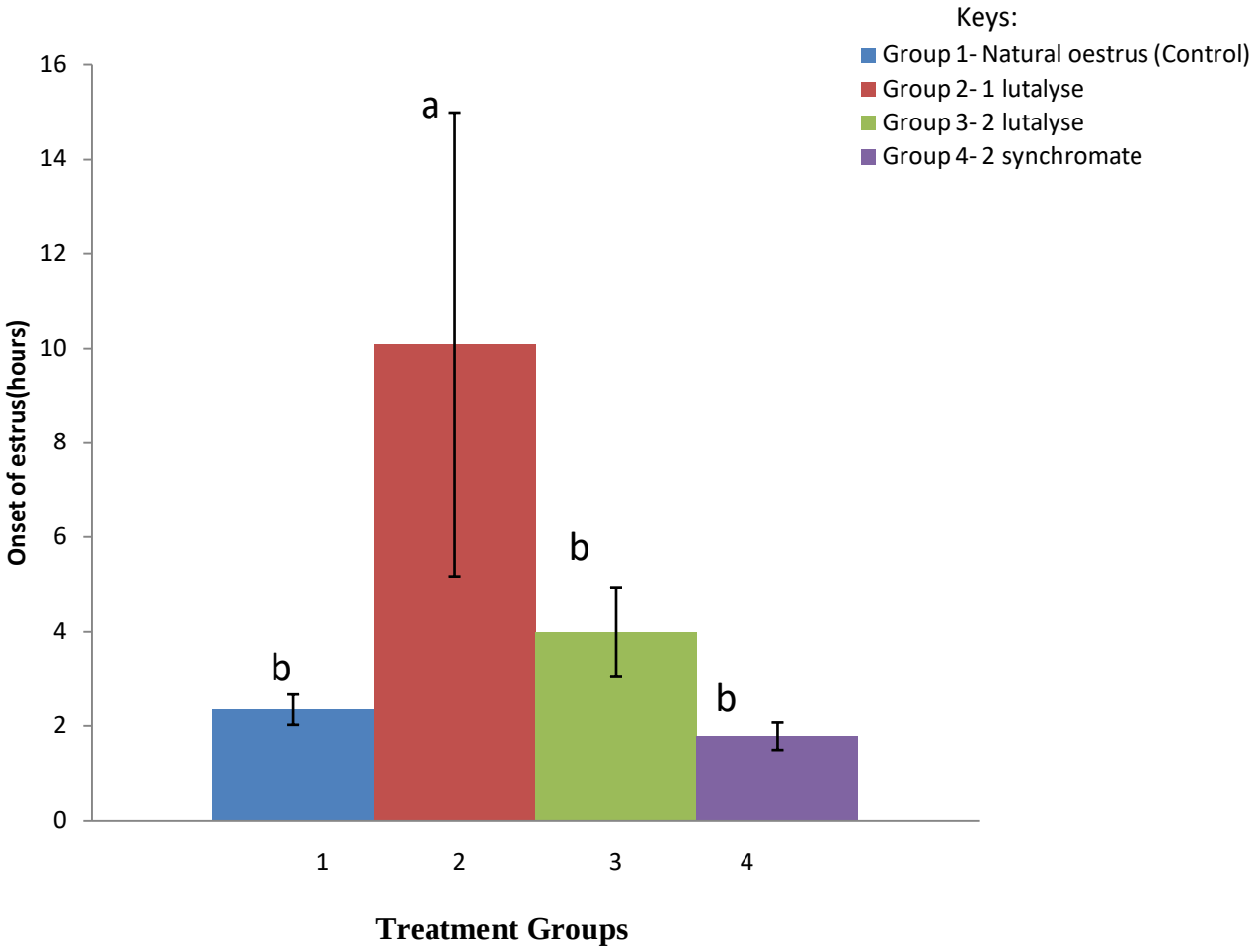


Figure 4.1 Onset of oestrus of jennies in the four treatment groups.

[a,b] Different letters indicate significant differences (P<0.05).

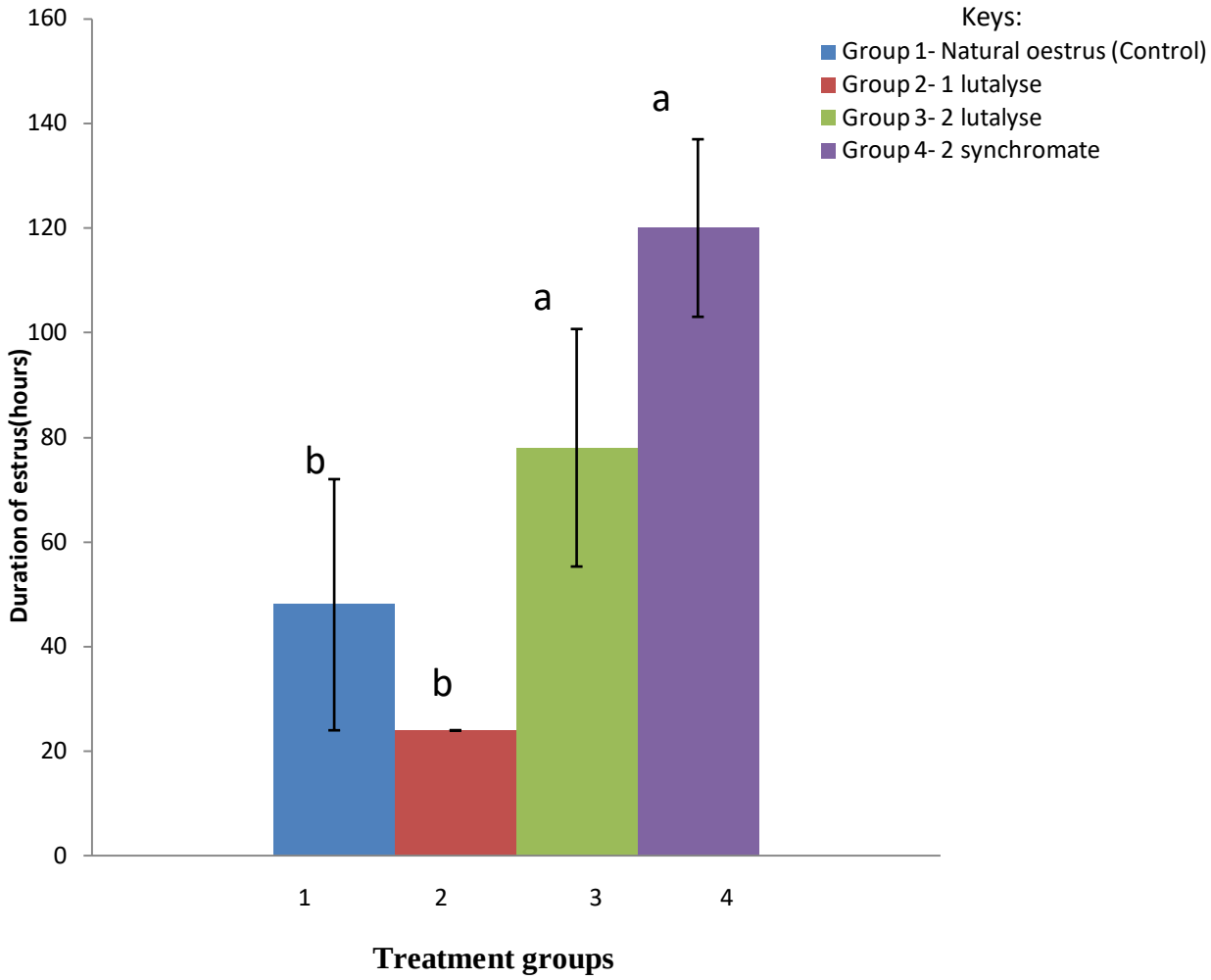


Figure 4.2 Duration of oestrus of jennies in the four treatment groups.

[a,b] Different letters indicate significant differences (P<0.05).

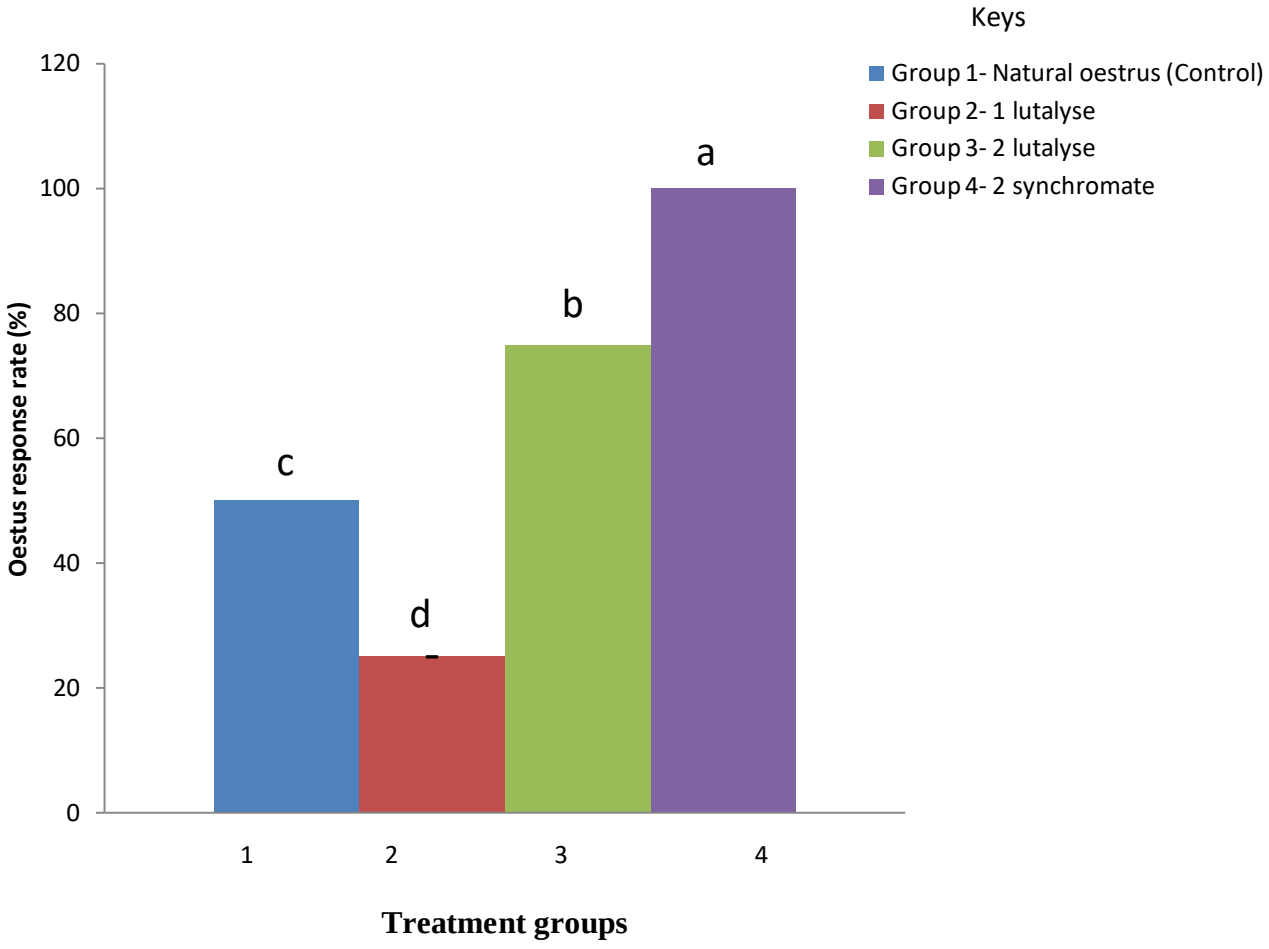


Figure 4.3 Oestrus response rates of jennies in the four treatment groups.

[a,b,c,d] Different letters indicate significant differences (P<0.01).

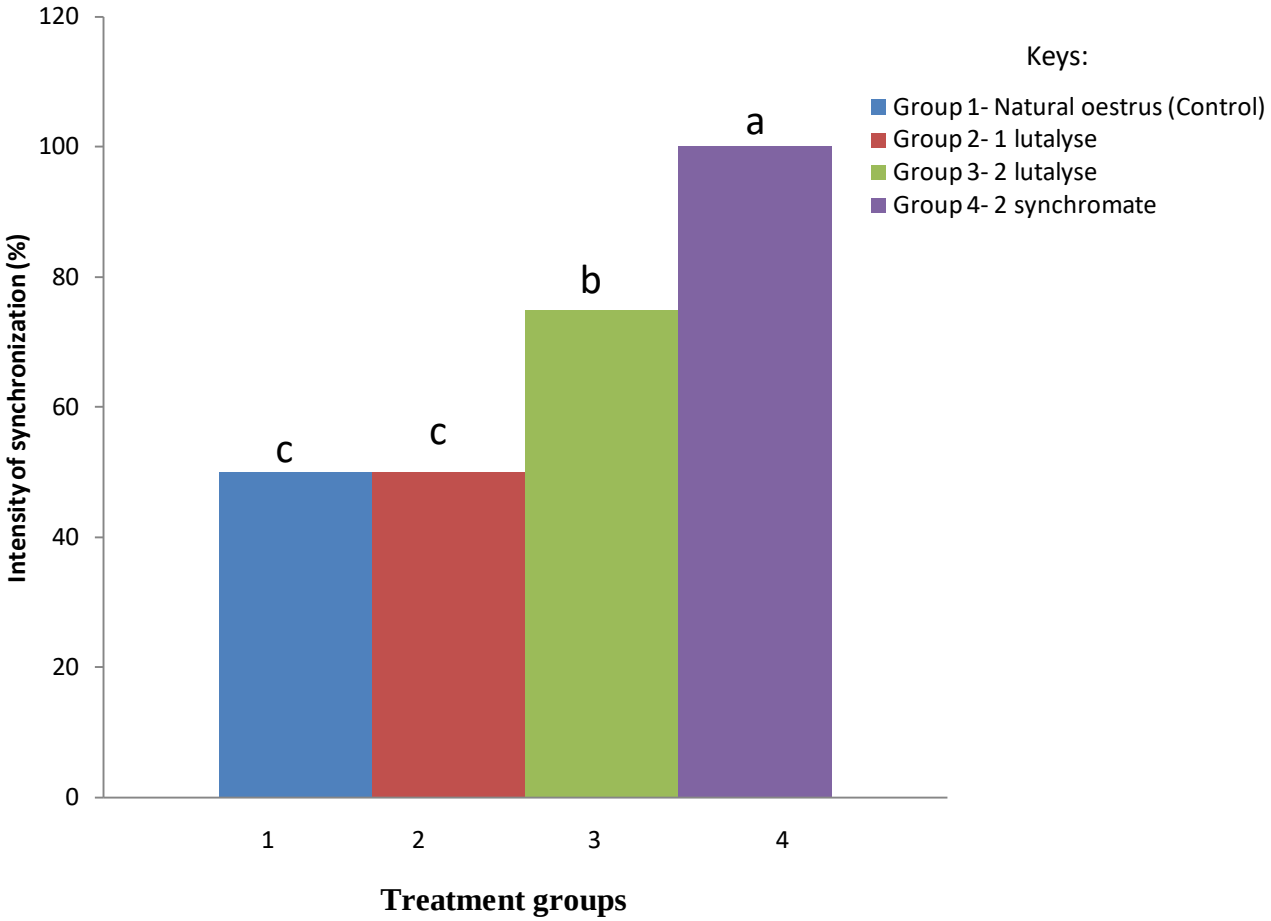


Figure 4.4 Intensity of synchronization of jennies in the four treatment groups.

[a,b,c] Different letters indicates significant differences (P<0.01).

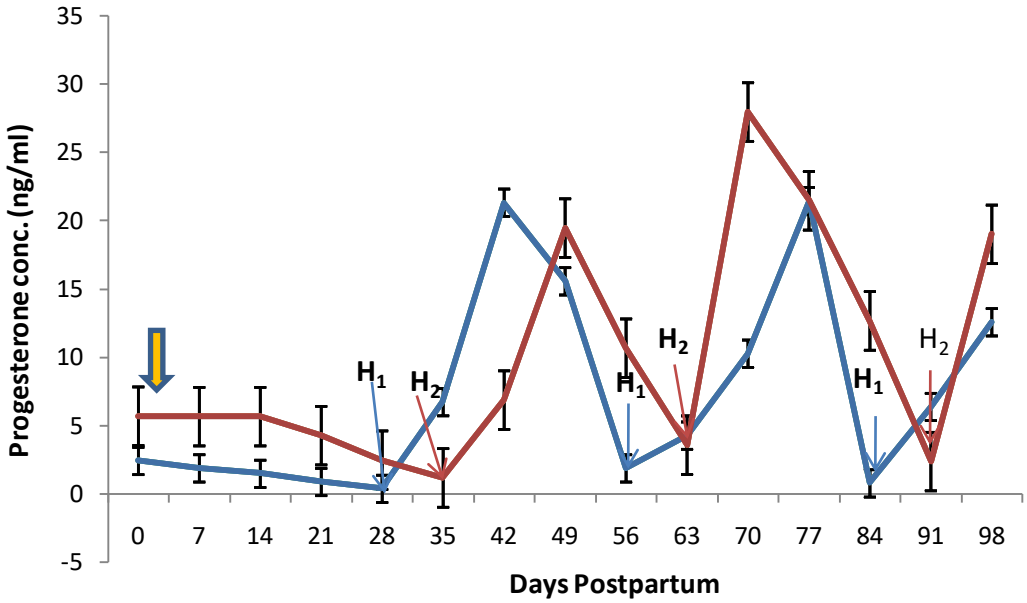


Figure 4.5 Serum Progesterone profiles for jennies exhibiting foal heat at 28 (10) and 35 days postpartum.

Keys:

F- Foal heat

H- Oestrus/Heat

H_1- Heat for the first profile (FH-28 days)

H_2- Heat for the second profile (FH-35 days)

4.6 Percentage of frequency for oestrus behaviour

Percentages of frequency for oestrus behaviour are shown in Table 4.1while plates 3-8 show various characteristics of oestrus behaviour. Jennies in group 4 (double treatment synchromate) had higher frequencies of oestrus behaviour than those in groups 1, 2 and 3. The characteristic oestrus behaviour of jennies observed were; tail raising from the perineum, opening and closing of mouth (mouth clapping) and flehmen and winking of the vulva. Percentage of frequency for oestrus behavior was significantly different between the four groups ($P<0.01$).

Table 4.1 Percentage frequency of oestrus characteristics in jennies treated with $PGF_{2\alpha}$

Treatment groups

Parameters	Grp1 (NT)(%)	Grp2 (1LT)(%)	Grp3 (2LT)(%)	Grp4 (2SH)(%)	LS
Head lowering	60^{c}	40^{c}	60^{b}	70^{a}	***
Mouth clapping	70	50^{b}	80^{a}	90^{a}	***
Ears backward	60^{b}	50^{b}	70^{a}	80^{a}	***
Standing to be mounted	60^{b}	50^{b}	70^{a}	80^{a}	***
Tails raising	70^{a}	50^{b}	90^{a}	100^{a}	***
Vulva winking	60^{a}	40^{b}	80^{a}	90^{a}	***
Mucous discharge	20^{b}	10^{c}	40^{a}	40^{a}	***
Presentation of perineum	10^{b}	0^{c}	10^{bc}	20^{a}	***
Flehmen	70^{a}	60^{b}	80^{a}	90^{a}	***

^{a,b,c} Values on the same row with different superscripts are highly significantly different ($P<0.01$).

LS= Level of Significance**.**



Plate 3 Flehmen in an oestrus jenny.



Plate 4 Posturing of perineum towards the jack in an oestrus jenny.



Plate 5 Mouth clapping (opening and closing mouth) in an oestrus jenny.



Plate 6 Ears backward in an oestrus jenny.



Plate 7 Tail raising from the perineum in an oestrus jenny.



Plate 8 Standing to be mounted in an oestrus jenny.

4.7 Progesterone and Oestradiol Profiles for Group 1 (Control group)

The serum P_4 and E_2 profiles for group 1 (jennies that served as control) to detect natural oestrus as shown in Figure 4.7. Mean P_4 concentration rose from 1.35±1.08 ng/ml on day 0 to 2.01±1.80 ng/ml on day 3 then declined to 0.18 ± 0.07 ng/ml on day 10 increasing to 2.44 ± 2.22 ng/ml on day 18, while E_2 concentration decreased from 11.81 ± 4.94 pg/ml on day 0 to 8.55 ± 1.63 pg/ml on day 14 then increasing to 11.10 ± 3.05 pg/ml on day 25.

4.8 Progesterone and Oestradiol Profiles for Group 2 (Single treatment lutalyse)

The serum P_4 and E_2 profiles for group 2 (Single treatment lutalyse) as shown in Figure 4.8. The progesterone and oestradiol concentrations were inversely related. Mean serum P_4 concentration at the start of the study day 0, was 0.88 ± 0.61 ng/ml and mean serum E_2 concentration was 63.2 ± 49.94 pg/ml. The P_4 concentration rose to 6.45 ± ng/ml on day 3 after $PGF_{2\alpha}$ treatment and E_2 declined to 62.71 ± 45.79 pg/ml.

4.9 Progesterone and Oestradiol profiles for Group 3 (Double treatments lutalyse)

The serum P_4 and E_2 profiles for group 3 (Double treatments lutalyse) as shown in Figure 4.9. The progesterone and oestradiol concentrations were inversely related. Mean P_4 concentration increased from 6.53 ± 5.59 ng/ml at first $PGF_{2\alpha}$ injection to 9.10 ± 4.82 ng/m 11 days later when the second $PGF_{2\alpha}$ was injected while the E_2 concentration decreased from 18.98 ± 8.36 pg/ml to 18.46 ± 10.39 pg/ml. P_4 concentration started to decline after the second $PGF_{2\alpha}$ injection from day 11 at 9.10 ± 4.82 ng/m to 0.50 ± 0.10 on day 18 and E_2 concentration rose from 18.46 ± 10.39 pg/ml to 20.65 ± 11.6 pg/ml on day 21.

4.10 Progesterone and Oestradiol profiles for Group 4 (Double treatments synchromate)

The serum P_4 and E_2 profiles for group 4 (Double treatments synchromate) as shown in Figure 4.10. The progesterone and oestradiol concentrations were inversely related. Mean P4 concentration was 5.36 ± 2.64 ng/ml on the day of first PGF2α injection then declined to 0.83 ± 0.59 ng/m 11 days later, while E2 concentration decreased from 9.02 ± 2.58 pg/ml to 7.21 ± 2.82 pg/ml. The second PGF2α was injected on day 11, P4 concentration rose from 0.83 ± 0.59 ng/m to 5.60 ± 2.20 ng/ml on day 18 then declined on day 32 having P4 concentration of 0.35 ± 0.12 ng/ml, E2 concentration increased from 7.92 ± 1.08 pg/ml after the second treatment to 12.73 ± 3.08 pg/ml on day 32.

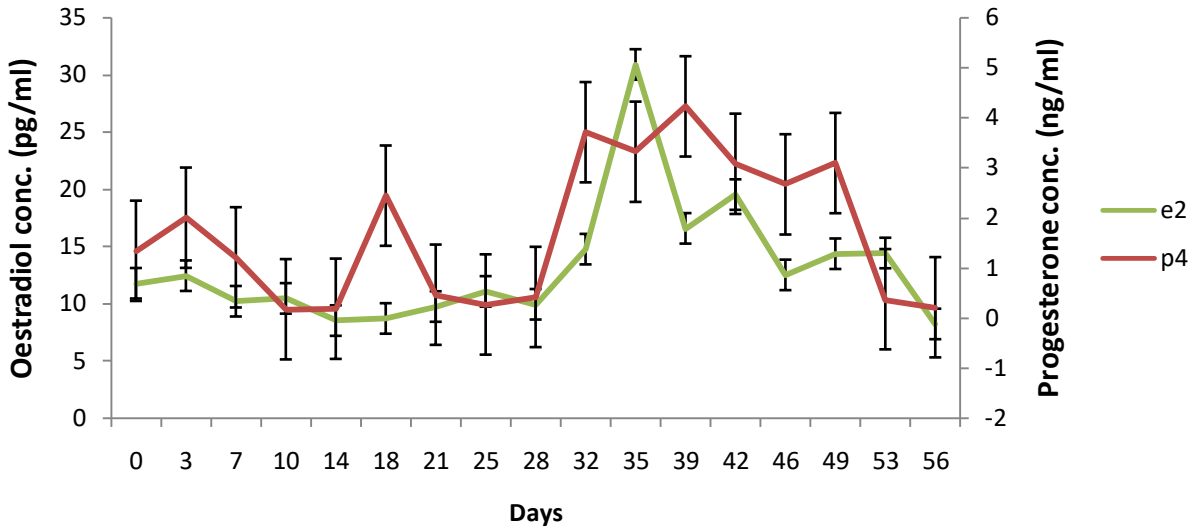


Figure 4.6 Progesterone and oestradiol profiles of jennies in the control group (i.e. not treated).

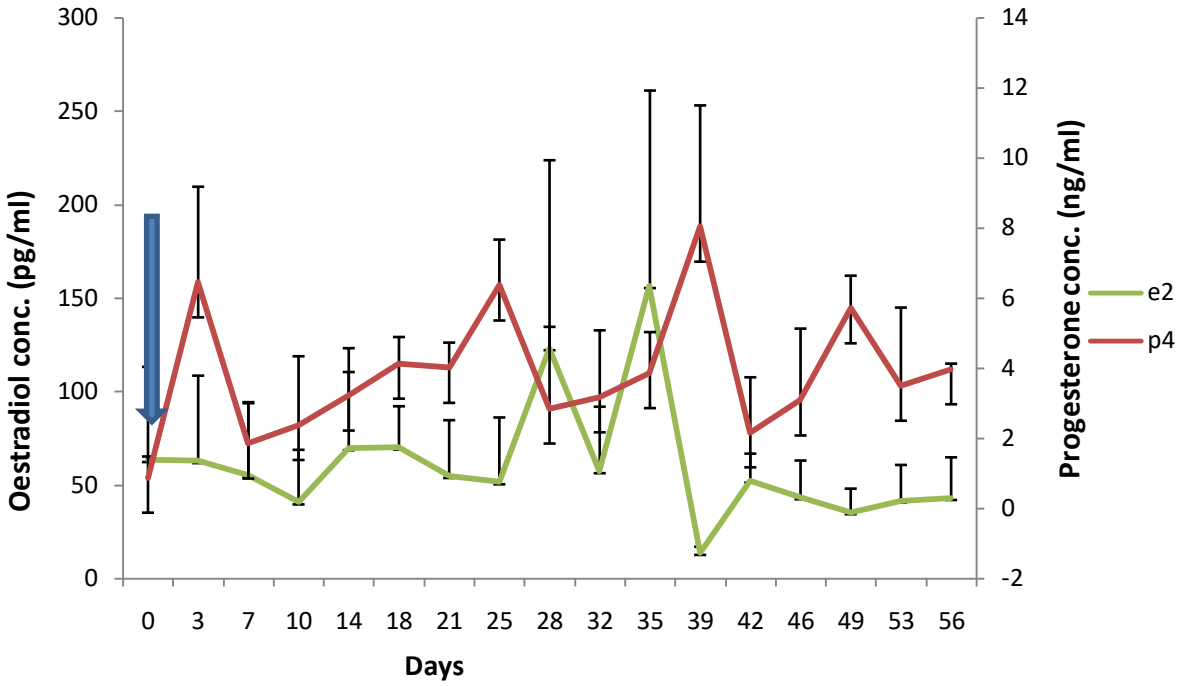


Figure 4.7 Progesterone and oestradiol profiles of jennies treated with a single injection of 10 mg of $PGF_{2\alpha}$

Key:

Day 0= Day of first injection of 10mg of Lutylase

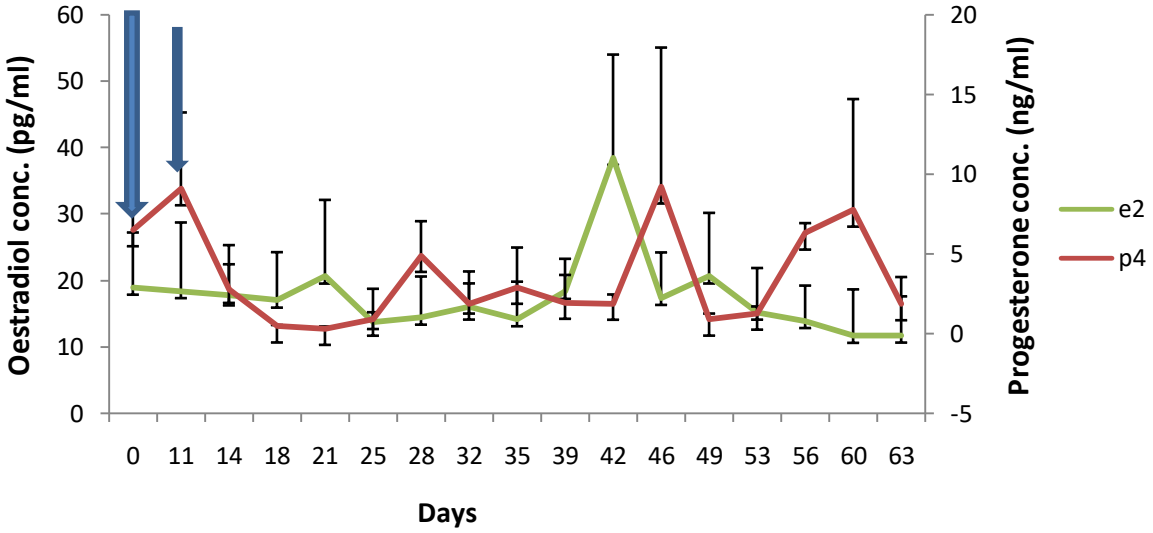


Figure 4.8 Progesterone and oestradiol profiles of Jennies treated with double injections of 10 mg of $PGF_{2\alpha}$

Keys:

Day 0= Day of first injection of 10mg of Lutalyse®.

Day 11= Day of second injection of 10mg of Lutalyse®.

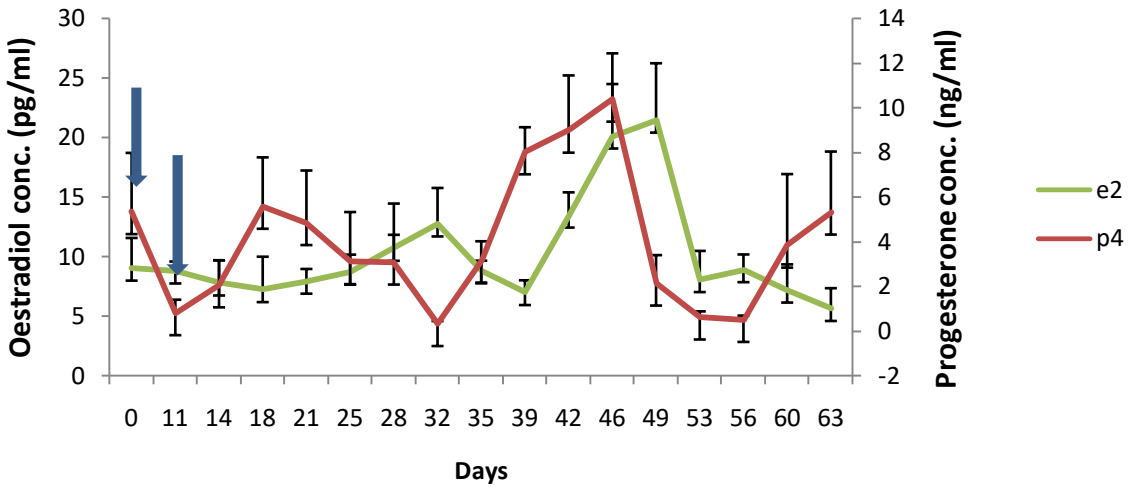


Figure 4.9 Progesterone and oestradiol profiles of jennies treated with double injections of 375µg of $PGF_{2\alpha}$

Keys:

Day 0= Day of first injection of 375µg of Synchromate®

Day 11= Day of first injection of 375µg Synchromate®

CHAPTER 5

DISCUSSION

In this study, luteolysis and onset of oestrus was earlier in group 4 (double treatment synchromate) and later in group 2 (single treatment lutalyse). It was established that the time to onset of oestrus in the four treatment groups in this study were shorter than the work of Blanchard *et al*., (1999) and Getachew, (2014), who observed that heat was induced 105.6 hours and 86.4 hours post $PGF_{2\alpha}$ (5mg Dinoprost tromethamine) administration. This difference could be attributed to the differences of dosage of $PGF_{2\alpha}$, the different protocols used and also the sensitivity of the *corpus luteum* to the different treatments. The observed shorter time to onset of oestrus in group 4 (Synthetic $PGF_{2\alpha}$) as compared to groups 2 and 3 (Natural $PGF_{2\alpha}$) in this study contradicts the work of Recai *et al.,* (2013) who reported that sheep treated with natural $PGF_{2\alpha}$ had shorter time to onset of oestrus than those treated with synthetic $PGF_{2\alpha}$, this difference could be as a result of different dosages used in the studies and also the species differences.

The duration of oestrus was 48, 24, 78.0 and 120 hours in Groups 1, 2, 3 and 4, respectively, and this differs from the report of Meira *et al.,* (1995) and Henry *et al*., (1991) who reported a longer duration of oestrus. This finding also differs from the earlier report of Blanchard *et al.*, (1999) who established a longer duration of oestrus of 134.4 hours after $PGF_2\alpha$ treatment using 150mg $PGF_2\alpha$ and 10mg estradiol -17β.

The 100% oestrus response rate and intensity of synchronization in this study corroborates the findings of Ozturkler *et al.,* (2003) and Godfrey *et al.,* (1997), who also observed 100% oestrus response rate in ewes treated with the synthetic $PGF_{2\alpha}$ than the natural $PGF_{2\alpha}$.

It was observed that tail raising, opening and closing of mouth (mouth clapping), flehmen and winking of the vulva were the most consistently observed signs of oestrus in jennies from all the groups, corroborating the works of Vandeplassche *et al*., (1981), Henry *et al*., (1991) and Taberner *et al*., (2008).

The jennies used for this study were in their different reproductive phases, the progesterone (P_4) and oestradiol (E_2) levels of all the groups were inversely related, as P_4 peaks, E_2 decline and this agrees with the normal physiology of these hormones. A decline in P_4 levels occurred on day 7 after the first treatment with $PGF_{2\alpha}$ (Lutalayse®), similar to the work of Gordon, (1999) who reported that the *corpus luteum* of the ewe was receptive to $PGF_{2\alpha}$ or its analogues between days 5 and 14 of the oestrous cycle. The mean P_4 concentration was 6.53 ng/ml and 5.36 ng/ml on day 0 in groups 3 and 4, and this corroborates with the work of Henry *et al*., (1991) who reported that plasma P_4 concentration greater than 3 ng/ml indicated that the detected ovulations resulted in active *corpus luteum*. In this study, a sharp decline in P_4 levels and rise in E_2 after the first and second injections of $PGF_{2\alpha}$ (Lutalayse® and synchromate®) and the rise in E_2 levels, indicate successful luteolysis of existing *corpora lutea* and negative feedback of E_2. This corroborates the work of Ataman and Akoz, (2006) who reported that prostaglandins and its analogues have been known to induce luteolysis of the corpus luteum in domestic animals.

It was established from this study that the oestrus period of groups 1, 2, 3 and 4, was 8, 8, 8 and15 days, respectively. The oestrous cycle length of groups 1, 2, 3 and 4 was 25, 35, 21 and 17 to 21, these findings are similar to the work of Henry *et al*., (1987), Blanchard *et al*., (1999), Taberner *et al*., (2008) and McKinnon and Voss, (2003), who established that the oestrus period can occur between 6 to 8 days, and the oestrus cycle from 25 to 26 days.

The second study on postpartum resumption of cyclicity has established progesterone assay, as an important tool in the determination of on-set of postpartum ovarian activity (PPOA) in jennies. Progesterone (P_4) concentration declined from 2.44 ng/ml and 5.72 ng/ml on the day of foaling to 0.4 ng/ml 28 days postpartum (10 jennies) and 1.2 ng/ml on day 35 (5 jennies) postpartum, respectively, indicating that after parturition *corpus luteum* luteolysis results in removal of the negative feedback of P_4 on the hypothalamus causing folliculogenesis to resume, invariably causing P_4 decline on days 28 and 35 and foal heat on days 28 and 35 in the 2 groups of jennies, followed by a second oestrus between 56-63 days. This is in contrast to the reports by Pugh (2002) and Umberto *et al*., (2013) who reported that foal heat in the jennies could appear in 5 to 13 days after foaling followed by a second oestrus in 21-35 days. This also differs from the works by Kalender, *et al.,* (2012) and Galisteo and Perez-Marinb (2010). From this study, it was evident that the length of the oestrous cycle postpartum in Nigerian jennies is 28 days, which differed from the results reported by of Blanchard *et al.,* (1999), Nishikawa and Yamazaki, (1999) and Galisteo and Perez-Marinb (2010) who reported a shorter oestrous cycle length of 22.8, 23.3 and 23.8 days, respectively, in jennies. The difference between the results in this study and the earlier studies could be as a result of breed differences, environment (tropics and temperate) and nutrition. Galisteo and Perez-Marinb (2010) reported that breed difference, different farm conditions under which animals are housed and the variable modalities used to collect reproductive parameters could cause a huge difference in the resumption of postpartum activities and postpartum oestrous cycle length in donkeys.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

1. It was concluded from this study that foal heat occurred between 28 and 35 days and normal cyclical ovarian activity in indigenous jennies can occur between 56 and 63 days postpartum.
2. Nigerian jennies can be bred from day 56 to 63 days (8-9 weeks) postpartum so as to ensure optimum fertility.
3. Synchronization of jennies is possible using exogenous $PGF_{2\alpha}$. Double treatments of cloprostenol and dinoprost tromethamine produced similar effect on the oestrus characteristics in jennies.
4. Cloprostinol had a better response, therefore, it is more efficient in oestrus synchronization.
5. This study has shown that, measurement of plasma progesterone and oestradiol concentrations provides valuable information on the postpartum reproductive events in Nigerian jennies.

6.2 Recommendations

1. Nigerian indigenous jennies should be bred from 8-9 weeks postpartum, to ensure optimum fertility
2. Double injection of cloprostenol (Synthetic $PGF_{2\alpha}$) should be used by farmers and researchers in synchronizing donkeys.

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APPENDICES

Appendix 1. Mean postpartum activities of the two groups

2.44	1.9	1.5	0.9	0.4	6.75	21.36	15.6	1.9	4.3	10.3	21.48	0.8	6.4	12.6
5.72	5.69	5.69	4.3	2.5	1.2	6.9	19.5	10.7	3.6	28	21.5	12.7	2.4	19.04

Appendix 2. Prostaglandins

A. Cloprostenol sodium (250 µg Synchromate)

B. Dinoprost tromethamine (5 Mg Dinoprost tromethamine)



A **B**

Appendix 3. Oestrus characteristics

Parameters	**1**	**2**	**3**	**4**	**LS**
Onset of oestrus (hours**)**	2.34±0.32^{b}	10.07±4.91^{a}	3.98±0.95^{b}	1.78±0.29^{b}	NS
Duration of oestrus (hours**)**	**48±24.0^{b}**	**24.0±0.0^{b}**	**78.0±22.7^{a}**	**120±16.97^{a}**	**
Oestrus response rate (%)	**50^{c}**	**25^{d}**	**75^{b}**	**100^{a}**	***
Intensity of synchronization (%)	**50^{c}**	**50^{c}**	**75^{b}**	**100^{a}**	***

Appendix 4. Progesterone and oestrogen profile values

Group 1 (Lutalase 1 Dose)
Progesterone

1.48	3.72	3	4.34	3.89	3.37	4.73	5.05	5.18	5.08	5.03	4.58	3.74	5.14	4.78	5.73	4.13
0.2	0.57	0.26	0.34	0.44	0.35	0.38	0.44	0.4	0.56	0.65	0.94	1.35	1.64	1.02	1.59	1.3
0.3	0.35	0.19	0.41	0.24	0.22	0.44	0.4	0.32	0.44	0.43	0.33	0.44	0.36	0.25	0.41	0.21
0.27	9.17	0.71	0.42	2.56	4.89	3.29	7.67	0.53	1.26	2.7	11.51	0.6	1.03	6.65	1.28	3.81
		Oestrogen														
113.2	108.5	94.31	68.82	123.2	47.8	84.67	86.11	223.8	91.9	261.1	10.21	66.77	63.08	48.03	60.73	64.82
7.1	22.78	11.29	15.17	25.39	16.45	12.53	9.38	17.35	14.6	32.84	22.55	37.68	18.85	19.27	18.16	27.86
4.77	7.45	5.35	6.74	15.82	14.39	14.04	12.14	10.44	12.76	31.85	22.23	20.92	8.93	6.5	6.36	7.01
13.32	16.92	16.31	12.38	16.04	92.13	24.81	16.61	22.4	22.73	51.63	16.95	37.78	23.66	22.66	22.4	21.13

Group 2 (Lutalase 2 Doses)
Progesterone

0.94	4.28	0.04	0.4	0.19	0.45	2.74	0.66	0.39	0.23	1.34	18	1.31	0.84	6.98	14.76	3.6
12.12	13.92	5.61	0.59	0.5	1.39	7.1	3.21	5.46	3.73	2.51	0.43	0.54	1.75	5.66	0.76	0.18
1.12	1.26	1.05	1.84	1.25	1.03	1.76	1.15	1.3	1.02	0.83	1.07	1.32	1.72	1.4	1.82	1.59
0.25	0.2	0.12	0.24	0.27	0.1	0.14	0.26	0.21	0.34	0.2	0.33	0.43	0.44	0.4	0.57	0.31
			Oestrogen													
10.62	8.07	12.92	9.68	9.05	8.75	8.22	10.8	8.52	13.32	22.9	10.54	11.03	8.45	8.57	4.69	5.81
27.34	28.86	22.56	24.38	32.25	18.87	20.73	21.48	19.94	23.38	54.13	24.34	30.3	22	19.35	18.78	17.74
25.71	43.33	24.32	32.53	26.53	25.31	28.89	20.44	28.08	30.61	45.8	35.28	40.73	22.08	27.15	23.82	23.73

3.49	4.46	4.04	7.1	7.37	7.02	6.73	6.11	7.53	7.44	13.13	10.09	12.79	3.71	6.31	5.71	6.68

Group 3 (Sychromate 2 Doses)
Progesterone

9.77	0.21	4.01	9.4	7.78	0.78	0.42	0.48	1.58	9.5	13.69	13.19	4.51	0.45	0.5	0.51	4.83
0.63	0.27	0.15	5.6	0.25	7.59	8.36	0.11	3.54	5.86	5.34	6.35	0.22	0.31	0.23	10.25	10.28
5.7	2.02	2.1	1.79	6.58	1	0.53	0.45	4.46	8.77	8.01	11.55	1.75	1.15	0.86	0.83	0.9
0.39	0.48	0.3	0.44	0.46	0.35	0.34	0.31	0.67	0.41	0.5	0.38	0.43	0.45	0.4	0.5	0.44
			Oestrogen													
5.26	7.84	4.26	4.23	8.64	6.15	11.82	18.68	7.81	5.18	17.32	28.58	25.35	5	8.05	3.04	2.4
7.83	10.47	11.01	12.84	9.32	11.41	11.92	11.1	10.6	6.81	10.9	13.69	11.86	6.25	7.13	7.91	6.06
13.97	8.05	8.05	4.57	5.81	8.48	8.31	8.41	7.96	8.91	12.21	18.08	27.15	12.92	11.49	10.58	8.43
21.56	20.23	14.78	15.09	20.17	19.49	20.23	13.8	13.65	10.24	10.86	28.76	25.11	13.09	13.63	14.55	11.7

Group 4 (Natural oestrus)

Progesterone

0.27	0.21	0.21	0.25	0.18	0.23	0.26	0.2	0.14	0.44	0.27	0.25	0.3	0.43	0.42	0.33	0.23
2.42	3.8	2.22	0.11	0.2	4.66	0.68	0.35	0.71	6.98	6.37	8.2	5.86	4.91	5.77	0.43	0.21
0.13	8.47	0.3	0.19	0.42	0.45	0.37	0.61									
			Oestrogen													
6.87	11.08	9.89	8.87	6.92	6.67	10.84	8.05	5.5	6.11	20.88	10.54	16.7	6.96	4.82	3.89	8.69
16.74	13.85	10.58	12.1	10.18	10.8	8.71	14.14	14.44	23.45	40.9	22.65	22.38	18.11	23.94	25	7.82
9.26	12.01	7.59	7.6	9.66	11.21	7.31	10.17									

Appendix 5. Progesterone and oestrogen profile mean± s.e.m

Group 1 (Lutalase 1 **Dose**)

Progesterone

	VAR00001	VAR00002	VAR00003	VAR00004	VAR00005	VAR00006	VAR00007	VAR00008	VAR00009	VAR00010	VAR00011	VAR00012	VAR00013	VAR00014	VAR00015	VAR00016	VAR00017
Mean	.875	6.45	1.85	2.38	3.22	4.13	4.01	6.36	2.85	3.17	3.86	8.04	2.17	3.08	5.71	3.50	3.97
Std. D	.85560	3.85373	1.61927	2.77186	.94045	1.07480	1.01823	1.85262	3.28805	2.70115	1.64756	4.90025	2.22032	2.90621	1.32229	3.14663	.22627
S E M	.605	2.73	1.15	1.96	0.67	0.76	0.72	1.31	2.33	1.91	1.17	3.46	1.57	2.05	.93	2.23	.16

Oestrogen

	VAR00018	VAR00019	VAR00020	VAR00021	VAR00022	VAR00023	VAR00024	VAR00025	VAR00026	VAR00027	VAR00028	VAR00029	VAR00030	VAR00031	VAR00032	VAR00033	VAR00034
Mean	63.26	62.71	55.31	40.6	69.62	69.965	54.74	51.36	123.10	57.3	156.36	13.580	52.27	43.37	35.35	41.56	42.97
Std. D	70.62583	64.75684	55.15433	39.90911	75.77356	31.34604	42.32741	49.14392	142.41131	48.91058	148.11766	4.76590	20.49903	27.87415	17.93930	27.10340	30.89350
S E M	49.94	45.79	39.0	28.22	53.58	22.16	29.93	34.75	100.7	34.58	104.73	3.37	14.50	19.71	12.68	19.16	21.84

Group 2 (Lutalase 2 Doses)
Progesterone

	VAR00001	VAR00002	VAR00003	VAR00004	VAR00005	VAR00006	VAR00007	VAR00008	VAR00009	VAR00010	VAR00011	VAR00012	VAR00013	VAR00014	VAR00015	VAR00016	VAR00017
Mean	6.53	9.10	2.82	.495	.345	.920	4.92	1.93	2.92	1.98	1.92	9.21	.925	1.30	6.32	7.76	1.89
S. D	7.90545	6.81651	3.93858	.13435	.21920	.66468	3.08299	1.80312	3.58503	2.47487	.82731	12.42387	.54447	.64347	.93338	9.89949	2.41831
S E M	5.59	4.82	2.78	.095	.155	.470	2.18	1.27	2.53	1.75	.585	8.78	.385	.455	.660	7.000	1.710

Oestrogen

	VAR00018	VAR00019	VAR00020	VAR00021	VAR00022	VAR00023	VAR00024	VAR00025	VAR00026	VAR00027	VAR00028	VAR00029	VAR00030	VAR00031	VAR00032	VAR00033	VAR00034
Mean	18.98	18.46	17.74	17.03	20.65	13.81	14.47	16.14	14.23	18.35	38.52	17.44	20.665	15.225	13.96	11.73	11.77
Std. D	11.82283	14.70075	6.81651	10.39447	16.40488	7.15592	8.84591	7.55190	8.07516	7.11349	22.08294	9.75807	13.62595	9.58130	7.62261	9.96313	8.43578
S E M	8.36	10.39	4.82	7.35	11.6	5.06	6.25	5.34	5.71	5.03	15.6	6.90	9.63	6.77	5.39	7.05	5.96

Group 3 (Sychromate 2 Doses)
Progesterone

	VAR00001	VAR00002	VAR00003	VAR00004	VAR00005	VAR00006	VAR00007	VAR00008	VAR00009	VAR00010	VAR00011	VAR00012	VAR00013	VAR00014	VAR00015	VAR00016	VAR00017
Mean	5.36	.833	2.08	5.60	4.87	3.12	3.10	.346	3.19	8.04	9.01	10.4	2.16	.64	.53	3.86	5.34
Std. D	4.57911	1.02812	1.93003	3.80500	4.04578	3.86981	4.55274	.20551	1.47096	1.92573	4.26446	3.57107	2.17419	.45004	.31607	5.53333	4.71048
S E M	2.64	.59	1.11	2.20	2.34	2.23	2.63	.12	.85	1.11	2.46	2.06	1.26	.26	.18	3.19	2.72

Oestrogen

	VAR00018	VAR00019	VAR00020	VAR00021	VAR00022	VAR00023	VAR00024	VAR00025	VAR00026	VAR0 0027	VAR00028	VAR00029	VAR00030	VAR00031	VAR00032	VAR00033	VAR00034
Mean	9.02	8.78	7.77	7.2133	7.9233	8.6800	10.6833	12.7300	8.7900	6.9667	13.4767	20.1167	21.4533	8.0567	8.8900	7.1767	5.6300
Std. D	4.47528	1.46159	3.38349	4.87580	1.86151	2.63570	2.05598	5.32550	1.56930	1.86993	3.39226	7.65108	8.35668	4.25789	2.29817	3.82312	3.03791
S E M	2.58380	.84385	1.95346	2.81504	1.07475	1.52172	1.18702	3.07468	.90604	1.07960	1.95852	4.41735	4.82473	2.45829	1.32685	2.20728	1.75394

Group 4 (Natural oestrus)

Progesterone

	VAR00001	VAR00002	VAR00003	VAR00004	VAR00005	VAR00006	VAR00007	VAR00008	VAR00009	VAR00010	VAR00011	VAR00012	VAR00013	VAR00014	VAR00015	VAR00016	VAR00017
Mean	1.3450	2.0050	1.2150	.1800	.1900	2.4450	.4700	.2750	.4250	3.7100	3.3200	4.2250	3.0800	2.6700	3.0950	.3800	.2200
Std. D	1.52028	2.53851	1.42128	.09899	.01414	3.13248	.29698	.10607	.40305	4.62448	4.31335	5.62150	3.93151	3.16784	3.78302	.07071	.01414
S.E.M	1.07500	1.79500	1.00500	.07000	.01000	2.21500	.21000	.07500	.28500	3.27000	3.05000	3.97500	2.78000	2.24000	2.67500	.05000	.01000

Oestrogen

	VAR00018	VAR00019	VAR00020	VAR00021	VAR00022	VAR00023	VAR00024	VAR00025	VAR00026	VAR00027	VAR00028	VAR00029	VAR00030	VAR00031	VAR00032	VAR00033	VAR00034
Mean	11.8050	12.4650	10.2350	10.4850	8.5500	8.7350	9.7750	11.0950	9.9700	14.7800	30.8900	16.5950	19.5400	12.5350	14.3800	14.4450	8.2550
Std. D	6.97914	1.95869	.48790	2.28395	2.30517	2.92035	1.50614	4.30628	6.32153	12.26123	14.15628	8.56306	4.01637	7.88424	13.51988	14.92702	.61518
S.E.M	4.93500	1.38500	.34500	1.61500	1.63000	2.06500	1.06500	3.04500	4.47000	8.67000	10.01000	6.05500	2.84000	5.57500	9.56000	10.55500	.43500

Appendix 6. The African wild ass

