Hormonal profiles and the reproductive tract morphology of the Brown Bush-baby (Galago garnettii) during induced and non-induced oestrous cycles.

by

CAROLINE NJERI KURIA, B.Sc. (NAIROBI)

DEPARTMENT OF ANIMAL PHYSIOLOGY

UNIVERSITY OF NAIROBI.

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

CAROLINE NJERI KURIA, B.Sc.

Department of Animal Physiology

University of Nairobi, Kenya.

This thesis has been submitted for examination with our approval as her supervisors.

Supervisor 1:

G OTIANG'A-OWITI, BVM, M.Sc., Ph.D.

Department of Veterinary Anatomy

University of Nairobi.

Supervisor 2:

R.M ELEY, M.Sc. Ph.D.

Institute of Primate Research

National Museums of Kenya, Nairobi.

Supervisor 3:

Prof. J.M.Z Kamau, BVM, M.Sc, Ph.D.

Department of Animal Physiology

University of Nairobi.

DEDICATION

I dedicate this work to the following people:

My parents, George Thiong'o and Nelly Wanjiku,

My son, Thiong'o,

and to George Kamau.

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ABSTRACT

The aim of the study was first to characterize endocrine profiles of the brown bush-baby (Galago garnettii) during the oestrous cycle and during pregnancy. The second part looked at the effect of inducing gonadotropins on their oestrous cycles, hormonal profiles and morphology of the reproductive tract.

Oestrus cycles of Galago garnettii were analyzed from daily observations of their external genitalia. Their plasma oestradiol and progesterone hormones were quantified by radioimmunoassay at various stages of the cycle.

Vaginal oestrus was successfully induced by using FSH:LH-hCG combination. During treatment, blood samples were collected on alternate days and later analyzed. A pre-treatment of progesterone (5mg daily for 5 days) was first given, followed by 2mg FSH:LH administered for 5 days. A single dose of 250 I.U. of hCG administered at the end of the treatment caused membrane regression in the colony-bred animals while the captured animals required a second injection of this dose given on the next days.

Reproductive cycles of the non-induced animals did

not show regularity from observations of their external genitalia. However, blood samples showed a pattern in their steroid levels. Peak levels of progesterone (mean±sd) of 45±10nmol/l were detected in the luteal phase that lasted for 19±5 days. A follicular phase with low progesterone (3±1nmol/l) lasted 23±9 days. Oestradiol peaks of 969±265 pmol/l were seen at the end of each phase. Two pregnant animals exhibited maximum levels of 1257 nmol/l and 3113 pmol/l of progesterone and oestradiol respectively.

A correlation was deduced between the state of the external genitalia, behaviour and steroidal profiles. It was also evident that the inducing hormones exerted their effects on the reproductive tracts of all the animals.

CHAPTER ONE

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The order Primate can generally be divided into the simians (higher primates) which include man, monkeys and apes, and the prosimians (lower primates) comprising of the tarsiers, lemurs, bush-babies and tree shrew. In the field of primate physiology, studies of simian primates dominate mainly because these are diurnal animals and live in a sensory world similar to that of man where vision dominates. Most of the species are terrestrial and thus accessible for observation. Evolutionary studies show that the simians are the closest kin to man; showing great similarity to man with respect to morphology and behavioral patterns. As a result of these similarities simians have been used as models in various human research studies.

On the other hand, prosimians are almost exclusively arboreal, usually nocturnal and thus their sensory world is very different from that of man. In these primates, olfaction and auditory senses predominate. They occupy a very different ecological niche, are less accessible and more difficult to study especially in the field. The

evolutionary trend shows that they are further placed from man and that they differ greatly in their morphology and behavior. As a result of this, prosimians have received less attention than their simian counterparts. Studies on primates would however be incomplete if prosimians were to be ignored totally. Interest in prosimians has been aroused by the hope of tracing the evolutionary sequence of primates and also in the search for more models for humans in biomedical research. As ethical and technical constraints limit many types of experimentation in humans, non-human primates are becoming increasingly useful as surrogates or models for human biomedical research.

Many factors influence the choice of a particular animal for experimental purposes. These include the size of the animal, adaptability to life in captivity, the ease of handling, rate of breeding, phylogenetic proximity to man and their availability. Even if these conditions are favourable, in order to identify an appropriate model, basic information about their reproductive patterns, morphology, physiology, endocrinology and embryonic development is essential.

One area of specific interest is reproduction. The non-human primate reproductive system provides an excellent model for studying basic physiological

processes applicable to humans [Sakai & Hodgen (1988)]. Although non-human primates have been used as models for studying various aspects of human reproduction, no single one of them can be described as ideal [Hearn (1980)]. As such there is need to continue to try and characterize more primates that may serve as models for human reproduction.

Information has been obtained on the reproductive cycles of various non-human primate species but most of it is derived from anthropoids [Robinson & Goy (1986)]. Of these, the great apes such as the orangutan and chimpanzee have been shown to have cycles that closely resemble those of the human [Hearn (1980)]. However, the cost of their acquisition and maintainance is becoming prohibitively expensive, especially in long-term projects. In addition, most of them are also among the endangered species. Some researchers have therefore turned to using alternative models such as laboratory rodents, domestic animals, and <u>in vitro</u> systems, to investigate biological questions which may have direct relevance to man. The search for a suitable model need not necessarily be limited to higher primates if a prosimian form can be found that parallels humans in the same way already shown in the higher primates. In general the ideal animal would be a small primate that is easy to maintain,

has a short gestation period and produces more than one offspring per year.

Progesterone profiles of Galago crassicaudatus were shown to be similar to those of the chimpanzee during pregnancy [Izard & Fail (1988)]. This raises the potential of the genus Galago as an alternative model in certain aspects of human endocrine functions.

Conservation and management of captive species of non-human primates is dependent upon adequate breeding success. Techniques that help to supplement natural breeding contribute towards this goal and these include artificial insemination (AI), embryo transfer (ET) and in vitro fertilization (IVF). AI has been used widely, especially in domestic animals and some primates [Bennet (1967 a & b), Hardin (1973), Wright (1980), Bavister et al (1984)]. However, for these techniques to work adequately, precise timing of ovulation is necessary. Natural ovulation in some cases may be difficult to detect, or detection can be delayed thus resulting in poor conception rates. To improve the rate of conception, ovulation induced by exogenous agents can supplement artificial insemination. This ensures precise timing of ovulation and that the event does indeed take place. Oestrus or ovulation induction using a combination of gonadotrophins and/or steroid

hormones is currently being addressed in captive non-human primates. This could lead to a new direction in trying to improve their breeding performance under captive conditions. If their response to ovulation induction shows some resemblance to the human reaction, this could open a new field of study in these animals.

1.2 Literature review

1.2.1 Prosimian taxonomy

Most primate systematists have adopted a scheme of classification that divides primates into lower (prosimians) and higher (simian) primates [Simpson (1945), Hill (1953), Napier & Napier (1967), Kingdon (1971)]. A general outline of primate classification is as shown in fig 1.

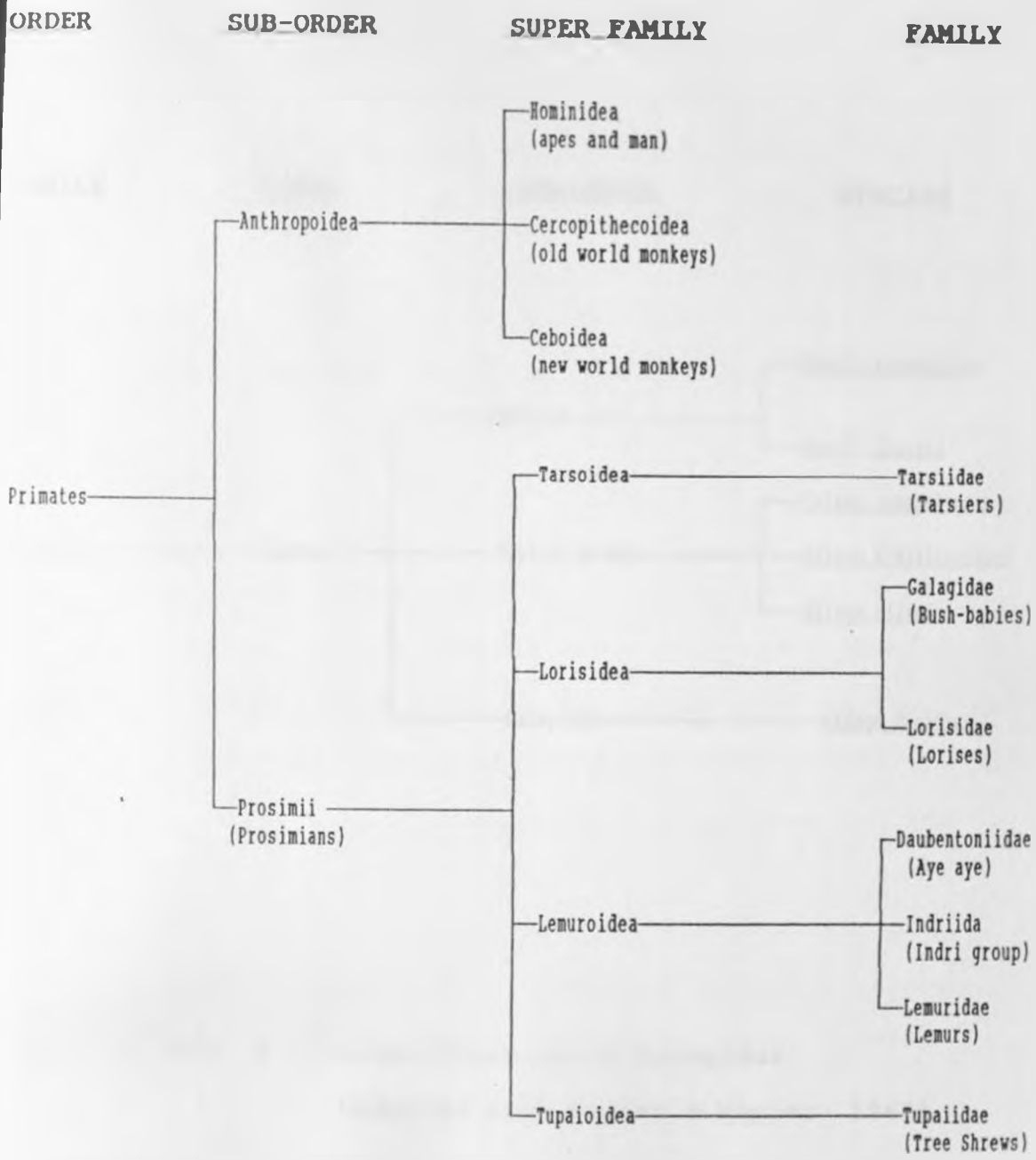

Fig 1 A classification of the order Primates (adapted from Schreir et al 1967).

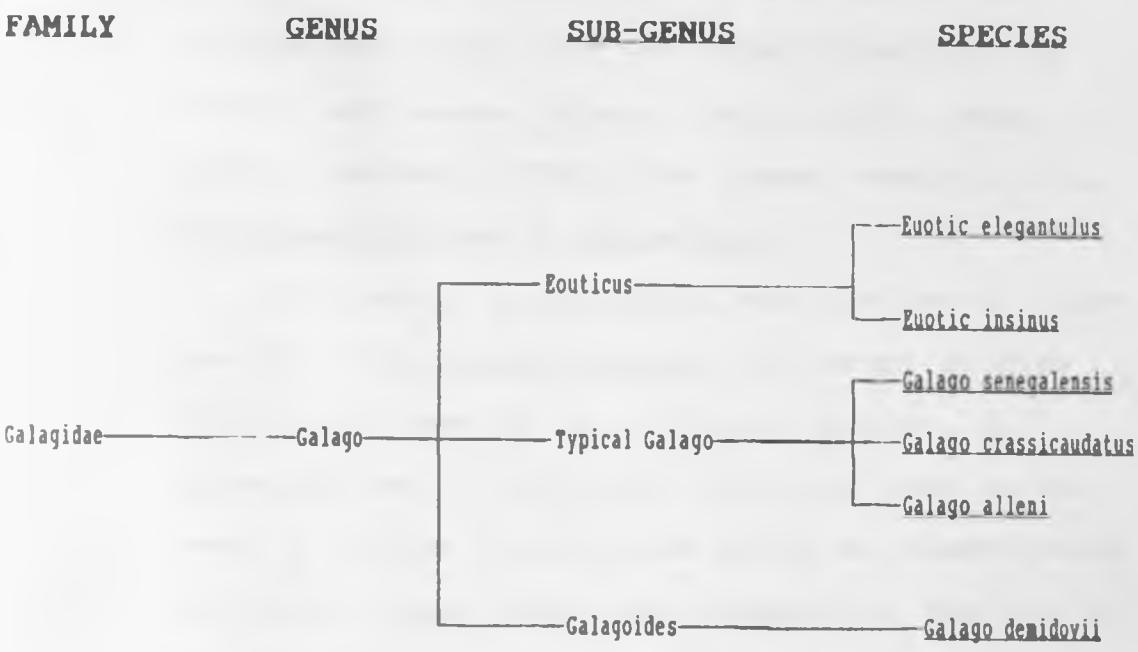

Fig 2 Classification of Galagidae
(adapted from Napier & Napier 1967).

Classification of bush-babies has been a subject of great controversy with respect to the number of genera and species. Napier & Napier (1967) recognized three sub-genera within the genus Galago (fig 2). Recent studies, based largely on size, regroup the Galago into two major divisions; the Greater and Lesser Galagos [Oslon (1979), Nash (1983), Bearder (1975)]. The former consists of G. crassicaudatus and G. garnettii.

Previously, G. garnettii was considered a sub-species of G. crassicaudatus, but recent studies distinguish them as two different species. G. garnettii had a light brown colour of coat on the back, a lighter ventral side which was almost white and black finger tips, when compared to the grey to black coat of G. crassicaudatus [Hill (1953), Oslon (1979)]. G. crassicaudatus weighed between 1200g-2000g while G. garnettii was 900g-1200g [Izard & Fail (1988), Haines (1982)]. Molecular biology studies also showed fixation of different alleles at loci of some enzymes in the two species, indicating a lack of gene exchange between them [Masters & Dunn (1988)]. In addition to the different morphological features, the animals were shown to occupy different niches [Oslon (1979)]. G. garnettii was thus qualified as a separate species from G. crassicaudatus.

The Lesser Galago encompasses all the remaining species of this genus which include G. senegalensis, G. demidoyii, G. alleni, Euoticus elegantulas and their various sub-species.

1.2.2 Distribution and habitat

G. crassicaudatus was reported to occupy the coastal area of East Africa while G. garnettii occupied the coast of South Africa, showing no overlap of the two [Hill (1953)]. Classification based on this distribution was adapted in subsequent studies [Eaton et al (1973), Nash (1983)]. However, more detailed observations differentiating the two by their morphology, behaviour and habitat showed that G. garnettii also occupies the central and southern regions of Kenya, the east coast of both Kenya and Tanzania, and the southern Tanzania regions [Oslon (1979)]. G. crassicaudatus was shown to occupy mainly central and southern Africa and it's habitat does not cover the Kenyan territory. It is therefore apparent that G. crassicaudatus is restricted to the drier savanna with open woodlands, while G. garnettii takes to the forested environs of the coast, mountains and river valleys [Oslon (1979)].

The latter occupies small niches and only infrequently descends to the ground either to feed

or to move between trees. On the other hand, G. crassicaudatus makes frequent use of the ground for both activities.

1.2.3 Ecology and behaviour

Bush-babies are nocturnal arboreal animals. They acquired this commonly used name from the similarity in some of the vocalization of the Greater Galago to that of a crying human baby. The name is now used to cover all members of this genus [Oslon (1979)].

Their social organisation consisted of either solitary animals, mating pairs with or without young, or a female with young [Vincent (1969), Bearder (1975)]. The exception seemed to be the Lesser Bush-baby which was found to live in groups of up to six members of the same family [Bearder (1969)].

These animals are omnivorous in the wild, feeding on insects and small birds in addition to their basic fruit and gum diet. It has been observed that when the diet is deficient in protein, G. senegalensis will not breed or will turn to feeding on their young. This phenomenon has been suggested for all insectivorous prosimians [Doyle (1974)].

Members of the Galagines are mainly leapers, aided by their elongated hind-limbs. The Greater

Bush-baby however defies this major characterization and is intermediate between the galaginae leaping mode and crawling locomotion of Lorises [Charles-Dominique (1974)].

Communication is by olfactory and auditory signals [Anderson (1969)]. Urine washing of the hands and feet is used for territorial demarcation and as a means of communication, and the two phenomenon are fairly common in these animals [Andrew & Clopman (1976)]. Other calls of social significance such as clucking vocalisation during courtship or threatening sounds when aggression is observed, have also been studied [Tandy (1976)].

Behavioral studies on bush-babies in the wild have extensively been carried out [Charles-Dominique (1974; 1977), Bearder (1975)]. These include their diet, mating behaviour, social interactions, predator-prey behaviour, agonistic and territorial behaviour, and their breeding patterns. Studies have also been done in both semi-natural and laboratory conditions [Buettner-Janusch (1964), Doyle et al (1971), Hendrickx & Newman (1978), Eaglen & Simpson (1980)].

Day and night reversals have enabled the study of their behaviour in both day and night situations. Reversal was achieved by providing white fluorescent light for day and 60 watt red bulb for nights. Best

illumination procedures have been shown to be 11-12 hours of daylight followed by 12-13 hours of darkness (night) [Manley (1966), Doyle et al (1967)].

Husbandry techniques for maintenance of colonies in captivity have been developed [Haines (1982)]. These studies have shown that in addition to other types of behaviour,
(a) breeding seasonality shown by bush-babies in the wild is absent in captivity,
(b) agonistic behaviour is enhanced by overcrowding or by caging of conspecifics together and
(c) that the animals adapt easily to captive conditions and thus breeding colonies can be established.

1.2.4 Reproductive behaviour

Most prosimians are reported to experience more than one oestrus period in a year (polyoestrus) under both laboratory and wild conditions. Post partum oestrus has been observed in some members of this sub-order [Hill (1953), Ioannou (1966)].

In the wild, seasonal patterns of high birth occurence at a particular time of the year are considered to be the norm. This was shown to be most common in areas with marked seasonal changes in climate conditions where peaks coincided with warm

weather [Bearder (1969), Vincent (1969), Nash (1983)]. This was hypothesised to be either due to "true" seasonality, a phenomenon where the animals exhibit mechanisms of sexual restriction, or due to oestrus synchrony. Some prosimians were shown to exhibit sexual mechanisms of restriction in both sexes. Females went through long anoestrus periods while males exhibited testicular retraction and atrophy outside the mating season [Vincent (1969)]. Seasonality attributed to oestrus synchrony has been suggested to result from either visual or olfactory signals, age of the females, or even from alteration of day lengths [Jolly (1974)].

In G. demidovii studied in Gabon (West Africa), females were found to ovulate only three times per year and the males were sexually active only during this time. A period of sexual inactivity was observed outside this season [Vincent (1969)].

Seasonal breeding was also seen in most sub-species of G. senegalensis, where primary conceptions peak occurred between December and March. Post-partum oestrus, after a gestation period of 120-130 days, was a normal feature in this species and was seen to occur between April and August [Zuckerman (1932), Lowther (1940), Hill (1953), Butler (1957; 1967 a), Cooper (1966), Doyle et al (1967), Bearder (1969)].

In a study carried out in S. Africa on G.s moholi, a birth peak was observed in October and early November when the rainfall is highest and food plus nesting materials are in abundance [Bearder (1969)]. Post-partum oestrus in G.s moholi resulted in a high proportion of infants between January and February. This second birth peak was about one gestation length from the last peak [Cooper (1966), Manley (1966), Bearder (1969) Doyle et al (1971)]. Bimodal breeding was also shown in G. demidovii where oestrus peaks observed in May-July and September-October periods preceded the birth seasons observed in September-October and January-February periods [Vincent (1969)]. G. alleni and Euoticus elengatulus also showed two periods of sexual activity [Sanderson (1940), Vincent (1969)].

In captivity, births occured outside the seasonal pattern found in the wild [Zuckerman (1953), Doyle et al (1971), Darney & Frankling (1982)]. In Kenya, where day length is fairly constant, births have been recorded in every month of the year in a captive colony of G. garnettii in the Institute of Primate Research (IPR). It is however noted that a higher proportion occurs in the May/June and November/January periods [Njuguna et al (1984)].

Only one season of sexual activity has been reported for G. crassicaudatus, with most conceptions occurring between June-November; this results in one birth peak [Smithers (1966), Vincent (1969), Bearder (1975)]. In studies carried out in S. Africa and Zambia for G.c. umbrosus, a birth peak was detected between September and October in one study, August and September in another, and yet a third study placed it in November [Ansell (1960), Jolly (1974), Bearder (1975)]. The extended birth peaks between August-November shown in these studies could either cover two consecutive birth periods if post-partum oestrus is allowed for in this species, or could be due to the different locations of studies.

Unlike the Lesser Galago, observations of G. crassicaudatus females, in the wild or in captivity, do not postulate post-partum oestrus even though some reports dispute this conclusion [Cooper (1966), Doyle et al (1971), Eaton et al (1973), Bearder (1975), Buettner-Janusch (1964)].

Although studies on captive colonies of G.c. pangienensis did not show pronounced seasonality, most conceptions occurred in January and February, while a secondary peak was seen in June and July; this peak could be attributed to post-partum oestrus [Cooper (1966)]. Studies so far show that breeding

occurs seasonally when natural day and night cycles are employed, while under a 12 hrs day/12 hrs night cycle the animals bred throughout the year [Cooper (1966), Eaton et al (1973)]. Thus seasonality of G. crassicaudatus in captivity seems to be dependent on laboratory conditions employed.

1.2.5 Reproductive anatomy and physiology

The female genitalia of bush-babies comprises internally of a pair of gonads or ovaries, paired fallopian or uterine tubes, a single uterus and a vagina. Externally, the genitalia are represented by a vulvar opening, clitoris and labial folds. The internal morphology varies among and between species with respect to the size, shape, and the reproductive status of the animal. A vivid description of the male and female genitalia of the Lesser Bush-baby, which is quite characteristic of the genus, has been given [Haines et al (1976). The anatomy of some other members of the Lorisidae have also been described and reviewed [Nayak (1933), Sanderson (1940), Hill (1953) and Dail (1971)].

1.2.5.1 Sexual cycles

In mammals, two sexual cycles are recognized (a) oestrus and (b) menstrual cycles. A follicular and luteal phase are evident in both types of

cycles. In the follicular phase, the main activity is the development and maturation of follicles, while in the luteal phase, the corpus luteum predominates.

In the oestrous cycle, the first day of the cycle is the day that oestrus behaviour is first exhibited. During this time, a behavioral change at the transition from the follicular to the luteal phase is observed; the animal is said to be on oestrus or "on heat". The luteal ends with the demise of the corpus luteum, and the beginning of the follicular phase. There is no outward behavioral sign to show the transition from luteal to follicular phase in these cycles.

Menstrual cycles are only experienced by higher primates. Although not all primates show overt menstruation, many old world monkeys, apes and man, experience this process. In this type of sexual cycles, a prominent feature is the shedding of the uterine endometrium at the end of the luteal phase. This is exhibited externally as menstrual blood and marks the first day of the cycle. A follicular phase follows and this ends in the ovulation process.

Oestrus behaviour is not always as obviously shown in "menstrual cycles" as in the "oestrus" animals. However, in some species, sexual interest from both sexes is greatly magnified around the

ovulation period (proceptivity and receptivity) [Robinson & Goy (1986)].

The events of mammalian sexual cycles can also be monitored by vaginal cytology, in addition to the other physiologic and behavioral changes observed [Darney & Franklin (1982), Hendrickx & Newman (1978), Jarosz et al (1977), Njuguna et al (1984)]. It is also noted that not all animals experience spontaneous ovulations, and variations of these cycles do exist. In some animals such as rabbits, cats, camels and other induced ovulators, the ovulation process is a reflex action, induced by mating or physical probing of the genital region.

Almost all prosimians are reported to be non-menstruating primates. However, there is one report where bleeding was documented in tree-shrews [Conaway & Sorenson (1966)]. Captive animals exhibited bleeding after a 20-22 day long period but menstruation did not appear during their regular cycle of about 10-12 days, thus the bleeding observed could have been due to some physical trauma or stress.

In absence of menstruation, the reproductive status of prosimian primates can be detected by visual examination of their external genitalia or by vaginal cytology. The vaginal orifice is completely sealed by an external membrane at all times except

during oestrus and at parturition [Manley (1966), Valerio et al (1972 a), Eaton et al (1973)]. Specific cyclic changes in other parts of the external genitalia which include colour and swelling of labial folds as well as the vulvar region [Hendrickx & Newman (1978)]. The vaginal epithelial cell layers also show cyclicity in cornification, keratinization, or nucleation of cells. Vaginal cytology has been used in evaluation of the stage of oestrous cycle (Rosenblum et al (1967), Jarosz et al (1977), Njuguna et al (1984)].

Using vaginal cytology and changes in the external genitalia, some researchers have characterized the prosimian sexual cycles and divided them into several phases [Manley (1966), Butler (1967 a & b), Valerio et al (1972 a), Hendrickx & Newman (1978)]. These phases are similar to those found in rodents and other laboratory animals and include pro-oestrus; this is the period prior to oestrus. During this phase, the labial folds are swollen but the vaginal orifice is still sealed [Hendrickx and Newman (1978)]. In the following phase, oestrus, the vaginal orifice is opened and early, mid and late oestrus stages are recognized. Early oestrus is when the membrane has just regressed. In mid-oestrus, the opening is maximum and a slight vaginal discharge is seen and

extends throughout this stage [Lowther (1940), Sauer & Sauer (1963), Anderson (1969)]. In addition, a pink to pale red colouration of the labial folds is observed during this phase. In late oestrus, the orifice starts to seal, the vagina is dry and the labial folds begin to lose their colouration. Dioestrus follows the late oestrus phase and during this time, the orifice becomes completely sealed as the membrane is fully formed; all reproductive activity is quiescent in dioestrus.

Oestrus was further divided into vaginal and behavioral oestrus. Vaginal oestrus covers the period when the orifice is opened while behavioral oestrus is the period when the female allows the male to mount and intromission is attained [Doyle et al (1967), Eaton et al (1973)].

Oestrous cycle lengths for prosimians have been reported by several authors. Among the prosimians, the Lemur catta has an average of 39 days and the Periodicticus potto has 38-39 days to 50 days [Petter-Rousseaux (1962), Ioannou (1966), Manley (1966), Evans & Goy (1968), Van Horn (1975)]. Reproductive cycle length for G.c. crassicaudatus in captivity, based on the state of their vaginal orifice, was 44 days in one study and 50.3 days in another study [Valerio et al (1972 b), Eaton et al (1973)].

Estimates of the oestrous cycle lengths for the Lesser Bush-baby are relatively more variable. Studies based on vaginal cytology estimated a mean of 39 days [Petter-Rousseaux (1962)]. Observations of the external genitalia alone gave an estimate of 31.7 days, while sexual behaviour monitored in addition to observing changes in the external genitalia gave an oestrous cycle length of 43.5 days [Lowther (1940), Manley (1966)]. Divergence of the oestrous cycle lengths in the different studies was therefore noted when different aspects of the cycle were monitored.

1.2.5.2 Endocrinology

The stereotype patterns of the plasma concentrations of oestrogen, progesterone and the pituitary gonadotropins; Luteinizing hormone (LH) and Follicular Stimulating hormone (FSH), obtained for humans are as shown in fig 3.

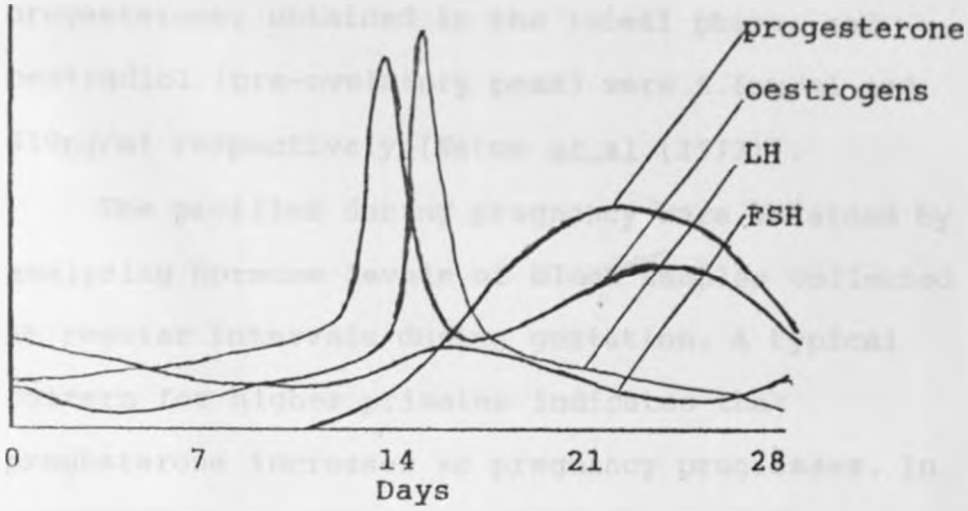

Fig 3 A schematic diagram of the reproductive hormones (concentrations are not to scale) during the human ovarian cycle

Most primates exhibit similar hormonal patterns during their sexual cycles [Robinson & Goy (1986)]. Whereas neuroendocrine characterization of prosimian cycles has been attempted [Anand Kumar (1972)], studies on endocrine profiles have only been done for G.c. crassicaudatus and some three species of Lemur [Eaton et al (1973), Bogart et al (1977), Izard & Fail (1988)].

In G.c crassicaudatus, blood samples from captive animals were analyzed for circulating progesterone and oestradiol during the oestrous cycle. These levels were correlated to the state of the external genitalia, and behavioral oestrus.

Analysis of these hormones revealed an oestrous length of about 40 days. Peak levels of progesterone, obtained in the luteal phase, and oestradiol (pre-ovulatory peak) were 8.6ng/ml and 519pg/ml respectively [Eaton et al (1973)].

The profiles during pregnancy were obtained by analyzing hormone levels of blood samples collected at regular intervals during gestation. A typical pattern for higher primates indicates that progesterone increases as pregnancy progresses. In chimpanzees, a 20- to 22-fold increase in this hormone over the luteal peak levels was seen by week twelve of gestation [Reyes et al (1975)]. In man, maximum levels were seen during the third trimester [Johansson (1969)]. Analysis of progesterone in pregnant G. crassicaudatus gave a pattern that closely resembled that of the chimpanzee and the human [Izard & Fail (1988)]. These studies showed similarity between endocrine characteristics of Galagos to those of other primates. However, owing to the difficulty in obtaining urine from these animals, and their small size that limits the quantity and duration of blood collection for endocrine analysis, information in this aspect is fragmentary. Consequently to date virtually all data on cycle lengths are only based on observations of the external genitalia and vaginal cytology.

1.2.5.3 Ovulation

Although it is not clear as to what actually causes the release of the oocyte, the reproductive hormones, mainly estrogens, gonadotrophins and their releaser hormones, play a considerable part. Prior to ovulation, peak oestrogen levels are followed by gonadotrophin peaks (fig 3). These gonadotrophin surges normally result in ovulation while high levels of progesterone are known to suppress this event. A balanced interaction of the various reproductive hormones and an ovarian response is required for a normal functioning reproductive cycle with ovulation, corpus luteum development and intact endocrine functions [Oelsner et al (1978)]. Failure of the coordinated interaction of these hormones often leads to ovulation failure.

The cycle can be manipulated with exogenous agents in an attempt to simulate a normal cycle. Synthetic hormones and other exogenous agents with activities similar to these natural hormones have been used for this purpose. They have been known to cause follicular growth and ovulation or to inhibit these processes [Simpson & Van Wagenen (1963), Bennet (1967 a & b), Roussel et al (1969), Dukelow (1970; 1971; 1979), Jarosz et al (1977), Oelsner et al (1978), Shepherd et al (1979), Wang & Gemzell (1980), Wright (1980), Vemura et al (1982). Agents

used to induce or inhibit ovulation include exogenous gonadotropins, gonadotropin releasing hormones, ovarian steroids, prostaglandins, analogues to these hormones as well as their inhibitors. The mechanism of action of these agents and results obtained in different species vary considerably. As an example, prostaglandins that are found to be effective in most domestic animals have little or no effect on most primates.

A lot of work has been done using gonadotropins for induction of single or multiple ovulations in a number of species including primates. In a study (in humans) on the relationship of weight to successful induction of ovulation with an anti oestrogen, clomiphene citrate (CC), there was a linear relationship between body weight and dose required to induce ovulation [Shepherd *et al* (1979)]. The squirrel monkey (*Saimiri sciureus*), which has a similar weight to *Galago garnettii*, has received a great deal of attention in studies with gonadotropins. Human chorionic gonadotropin (hCG) and pregnant mare serum gonadotropin (PMSG), have been successful in inducing ovulation in these animals. The doses used for ovulation induction in the squirrel monkey have also been successfully used to produce similar results in *G. crassicaudatus* [Dukelow *et al* (1971)]. In the squirrel monkey,

superovulation was successfully induced by a single dose of hCG, following treatment with PMSG [Bennett (1967 a & b)]. A procedure limiting the number of ovulations per animal to single or double ovulations in this species was later carried out [Dukelow (1970)].

Seasonality was found to affect the response to ovulation induction procedure [Jarosz et al (1977)]. Peak response in squirrel monkeys was achieved during the breeding season, while less success was observed outside this season. A study to improve the ovulatory response of captive animals during these months was carried out [Kuehl & Dukelow (1975)].

Ovulation induction in bush-babies has also been done on G.s. senegalensis [Darney & Franklin (1982)]. Ovarian response to exogenous gonadotropins was monitored by examination of the external genitalia. Vaginal oestrus was interpreted as signalling physiologic response [Dukelow (1971), Darney and Franklin (1982)]. No data on G. garnettii exist.

Oestrogens have been used to follow the response to the gonadotropins during ovulation induction [Wang & Gemzell (1980)]. Their levels during an induction procedure helps avoiding hyperstimulation or multiple births. Excessive ovarian stimulation by gonadotropins could result in

development of ovarian cysts [Coulam *et al* (1983), Schenker & Weinstein (1978)]. There is also the possibility of producing antigenicity to hCG when the hormone is used repeatedly for ovulation induction. Repeated subjection of the animal to this treatment may therefore result in refractoriness to the hormone and is thus discouraged.

1.3 Aims and objectives

The study looks at the basic physiology of the Brown bush-baby with a hope of giving a better understanding of it's reproductive system.

The objectives of this study are:

1. Characterization of the endocrine profiles of the brown bush-baby during the oestrous cycle and during pregnancy. Emphasis will be laid on oestradiol and progesterone hormones. In addition the profiles obtained will be correlated with the state of the external genitalia.
2. To study the effects of exogenous inducing agents on the oestrous cycles, hormonal profiles, and the morphology of the reproductive tracts.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Experimental animals

Two groups of bush-babies were used in this study; Captive (CB), refering to those that had been residing in the Institute of Primate Research (I.P.R) Karen, Kenya . These were colony born, and their ages ranged between five and nine years. The wild-caught (WC) bush babies had just been trapped prior to the beginning of the project. These were spotted during the day on tree branches in riverine areas along the slopes of Kilimambogo Hills, and only descended to feed at night, during which time they were trapped. Based on colour, size, distribution and habitat, this study classified the animals as G. garnettii (fig 4); a separate species from G. crassicaudatus [Bearder (1975), Oslon (1979)].

Although the study was primarily on females, males were also observed. These were used in sex determination, detection of behavioral oestrus, and breeding.

Fig 4 Two bush-babies (Galago garnettii species) trapped from the slopes of kilimambogo hills

The colony consisted of twelve females and eleven male bush-babies. Out of these, seven females and three males had been maintained in captivity in I.P.R for at least two years. Six of the females and the three males were colony born. Of the trapped animals, there were nine females and ten males but four females and four males died prior to the start of the project. Two females were pregnant at the time of capture but one died following an abortion. The other one survived but the pregnancy had terminated since palpation at a later date revealed no foetus. A postmortem performed on the dead animals indicated death was due to stress.

After a period of conditioning (80-100 days) the animals underwent a physical examination. The males were examined by weight and size, testicular descent, colour and size of scrotum/testicles and dental eruption. Females were also examined on size, weight and reproductive state (vaginal orifice state or pregnancy, if any) and the ages also estimated. No abnormalities were observed. Judging from adult weight of colony born animals and data based on weights by Haines (1982), all animals except one male, had attained adult size.

2.2 Housing facilities and animal care

The animals were maintained in semi-natural conditions in a dark room with a single window that was continuously shuttered. Lighting was not regulated but when required, it was provided by a sixty watt bulb and the cages were covered by sacks to reduce the amount reaching the animals. The cages measured 25"x25"x30", and contained a small sleeping site (7"x7"x12"). This was made of solid materials which did not allow any light to penetrate. Sacks were provided at the bottom of the cages and also in the sleeping sites. Initially, the animals were caged individually but in the second part of the project some males were introduced into female cages when vaginal opening was noted in the latter.

Each unit had a food dish and water bottle with automatic valves. The animals were fed a diet of high protein monkey pellets (Unga Feeds, Nairobi) supplemented by some fruits which included bananas and oranges. Water was provided ad libitum.

2.3 Handling and blood collection

The animals were easy to handle and heavy leather gloves provided adequate protection from possible bites. The animals were tattooed on the inside of a hind limb for identification.

Each female was examined on a daily basis for a patent vaginal orifice. The following code was developed to determine the state of vaginal opening (VO).

0 - Orifice completely closed,
1 - pin-hole opening towards opening,
2 - medium sized opening towards opening,
3 - complete opening with hyperaemia,
4 - medium sized opening towards closing,
5 - pin-hole opening towards closing,
6 - complete closure immediately after oestrus,
8 - complete closure during pregnancy,
9 - opening after parturition.

Observations were recorded daily in a cycle chart (appendix). Vaginal swabs of oestrus females, those that were coupled with males for breeding, were taken at 09.00hrs during the oestrus period. These were observed for the presence of any spermatozoa.

Prior to bleeding animals were anaesthetized using ketamine (xylazine hydrochloride; Ketaset® , Fort Dodge Laborataries IOWA) and then weighed. The CB animals received 10mg of ketamine, while the WC animals received 20mg. The dosages used rendered the animals managable for the bleeding and weighing purposes. Blood collection was done at specific times as outlined in table 1. A sample was taken each time the external genitalia showed a change of state.

The animals were given a rest of approximately one month during which time they were not bled. Regular bleeding then resumed every Monday and Friday as shown in table 2.

Blood samples of approximately one ml were obtained in non-heparinized tubes from the femoral vein (fig 5). These were left to clot overnight in a fridge at 4° C. Serum was harvested by centrifugation at 4° C (at 2000g) for 10 minutes. It was then frozen at -20° C until time for assay.

Table 1: Dates of sample collection, as determined by the VO state

Animal no	62	90	106	109	113	128	145
Dates: 1990							
October 8	1	8	8	0	0	0	3
12	2	-	-	0	0	-	3
15	-	8	8	-	-	0	-
16	3	-	-	-	-	-	3
18	-	-	-	0	0	-	-
19	4	-	-	-	-	-	3
22	-	8	8	-	-	1	-
25	5	-	-	-	-	-	-
26	-	-	-	0	0	2	4
29	-	8	8	-	-	-	-
30	0	-	-	-	-	0	0
November 5	0	8	9	0	0	0	0
12	0	1	3	0	0	0	0
19	0	4	5	0	0	0	0
26	0	5	5	0	0	0	0
December 3	0	5	5	0	0	0	0
8	2	3	5	0	0	2	0
10	3	3	5	0	1	3	0
21	6	4	5	1	4	4	0

Table 2: Dates of sample collection as determined by regular sampling on Mondays and Fridays.

Animal no	62	90	106	109	113	128	145
Dates 1991							
February 21	2	3	3	5	5	5	0
25	3	3	4	0	0	5	2
March 3	3	4	4	0	0	5	2
4	4	4	5	0	0	5	5
8	5	5	5	0	2	5	5
11	0	5	5	0	3	0	0
15	0	5	5	0	3	0	0
18	0	5	5	0	3	0	0
22	0	5	5	0	4	0	0
25	0	5	5	0	5	0	0
30	0	1	5	0	5	0	0
April 2	0	2	5	0	0	0	0
5	0	2	5	0	0	0	0
8	0	2	5	0	0	0	0
12	0	4	5	0	0	0	0

Fig 5 Photograph showing the collection of blood from the femoral vein of an anaesthetized animal.

2.4 Experimental set up

The first part of the project was designed to characterize the progesterone and oestradiol profiles during the oestrous cycle. The seven females maintained in the Institute were used for this part. Past records from I.P.R. on their ages and oestrous cycles, together with results obtained in the course of the present study, enabled the determination of some of their reproductive cycle characteristics. Regularity of the cycles was presumed when over 50% of the cycles of an individual animal were within the mean ± s.d. of all the animals.

In the second part, in which the effects of inducing (exogenous) gonadotropins on the reproductive tract were studied, a pilot experiment was carried out prior to the main project. This involved two animals, one was cycling CB (Gal 113), and the other a non-cycling WC (Gal 163). The first animal had shown regular cycles for a considerable length of time. The treatment was commenced when the labial folds were swollen, as normally seen when the vaginal orifice is about to open even though the orifice was still closed, hence VO- 0. This was on day 34 since the last oestrus. The second animal had never shown vaginal oestrus since its capture and abdominal palpations did not indicate pregnancy.

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Hormone assays on samples collected one month previously revealed relatively low levels of both progesterone and oestradiol, and this confirmed that the animal was not pregnant. In these two animals, a standard ovulation regime for ovulation induction was used [Dukelow (1970; 1979), Jarosz et al (1977)]. The procedure involved five days of two mg human postmenupausal gonadotropin (Humegon®, N.V Organon Oss, Holland) per day, followed by a single dose of 250 IU hCG (Pregnyl®, N.V Organon Oss, Holland). Intramuscular (IM) route was used in all the injections given.

The results of the pilot experiment indicated vaginal oestrus on the cyclic animal within 24 hours after the hCG injection. The second animal did not show signs of oestrus on the day following the hCG injection. A second dose of hCG (250 IU given 24 hrs after the last one) resulted in the animal showing membrane regression within 24 hours following this injection. It was also noted that this animal did not exhibit oestrus as described previously (stage 3 of VO) which was observed in its counterpart, but exhibited a stage that was more characteristic of metoestrus. The results obtained here were used to determine the induction procedure carried out in the main project in part two of the study.

The induction study, had two groups of animals where Group one was the control group. This consisted of five animals that were not induced. The other, Group two, had four animals that were induced (see table 3).

Table 3. Animals used for the study on oestrus induction

	Animal no	source	Culled on day from last VO
Group one	90	CB	79
	106	CB	39
	113	CB	69 (induced)
	169	WC	unknown
	177	WC	unknown
Group two	109	CB	287
	145	CB	269
	163	WC	58 (induced)
	173	WC	Unknown

2.5 Oestrus induction procedure

Progesterone; 4-pregnene-3,20-dione (Sigma Chemical Company, St. Louis Mo USA) was dissolved in pure corn oil (Elianto®). Each animal in Group two received a daily injection of five mg of progesterone in 0.25 mls corn oil for five days. The hormone was first dissolved in a few drops of alcohol (99% ethanol) then mixed thoroughly with the oil. The solution was left uncovered overnight to allow the alcohol to evaporate. Before injection, the mixture was warmed up to 37°C and any remaining

alcohol allowed to evaporate. These were administered intramuscularly (IM) between 1100hrs and 1200hrs daily.

On the sixth day, daily HMG treatment commenced. Injections of this hormone were given via the same route and time indicated earlier, to each animal for the next five days.

This treatment was followed by a single dose of hCG given on the eleventh day of treatment in the cyclic females, after at least 24 hours following the last Humegon® injection. Those animals that did not respond by the following day and were given a second dose of the gonadotrophin.

2.6 Collection of the reproductive tract

The animals were weighed and blood samples collected from them prior to sacrifice. Each animal was anaesthetised with ketamine and then euthenised with 0.5-1ml Euthatol®. A cut was made through the ventral abdominal wall and the internal organs exposed. The reproductive tract was identified and carefully dissected out. The fat surrounding the tract was then trimmed off as well as the hair around the external genitalia. The ovaries were exposed from the ovarian capsule and the state of follicular development was noted. Linear measurements of the whole tract were taken using

calipers. Aspects measured were the intercornal-vulval length, the vaginal and cervical diameters. Diameters were calculated from measurements obtained of the outer circumference of the vagina and cervix. The whole tract was then fixed by immersion in 5% formalin.

2.7 Fixation, processing and examination

After fixation for a period of one day, the reproductive tract was cut into several divisions. The ovaries, uterus and vagina were identified and subsequently processed for routine histology. Briefly, the tissues were dehydrated through ascending grades of alcohol (50%,70%,90%,100%), cleared using methyl benzoate, infiltrated in liquid paraffin before embedding them in paraffin wax. They were sectioned at 7μm, mounted and stained with Haematoxylin and Eosin stains. They were subsequently studied the light microscope.

Photographs of the gross specimen, macro- and micro-sections were taken at the appropriate stages using ordinary camera and an ortholux II (fitted with an orthomat unit) camera respectively.

2.8 Hormone assays

The amount of oestradiol and progesterone in each sample was determined or quantified in

duplicate by radioimmunoassay (RIA), using the method of Sufi et al (1990). Briefly the procedure was as follows:

The RIA reagents provided by the World Health Organisation (WHO) were reconstituted. These reagents included the progesterone (P_4) or oestradiol (E_2) antiserum, the respective standards and tracers, charcoal reagent, gelatin and dextran reagents. Anti-progesterone and anti-oestradiol serum was provided in lyophilized form and stored at 4° C until required for assay. The standards were provided in ethanolic solution at a concentration of 250 nmol/l and 150 nmol/l for P_4 and E_2 hormones respectively. These were also stored at 4° C until required for assay. A serial dilution was carried out with steroid buffer on the reconstituted standard solutions containing 2500 fmol/ml and 1500 fmol/ml of P_4 and E_2 respectively. This was used to obtain a standard curve.

Tracer was provided in sealed ampoules containing 3.7 MBq(100 µci) and 9.25 MBq(250 µci) for P_4 and E_2 respectively. A stock solution was prepared by transferring the contents of the ampoules into a 10ml (P_4) or 25ml (E_2) volumetric flask and making up the volume with toluene:ethanol (9:1 vol ratio) solution. When needed, 150 µl of the stock solution was transferred into a vial and the

solvent evaporated. 10 mls of steroid buffer was then added to the residue, mixed vigorously and given about 30 minutes for the tracer to be completely redissolved.

The serum stored at -20° C was thawed and aliquoted for assay. Extraction was carried out using 3 ml of diethyl ether, and efficiency monitored by obtaining recovery percentages for each sample. 100 µl of recovery tracer was added to culture tubes containing 20 µl serum for P_4 determination or 150 µl for E_2. Extraction was carried out twice for E_2. Duplicate aliquotes of 500 µl of extracted sample was used for hormone assay. The prepared assay tubes, various reagents and samples were aliquoted as shown in table 4. Free and bound hormone was separated by dextran coated charcoal reagent.

The assay sensitivity for the hormones was calculated at 3 S.D and found to be 0.12 nmol/l for P_4 and 32 pmol/l for E_2. Intra and inter assay coefficient of variation for P_4 were 7.3 % and 8.1 % (0.5 nmol at 3 s.d, n=15) respectively, while for E_2 5.2 % and 6.1 % (200 pmol at 3 s.d, n=12) respectively. Recovery percentages ranged between 80-90 % for E_2 and up to 60-70 % for P_4.

Table 4: Summary of contents of assay tubes.

	Buffer	STD or Sample	Tracer	Antiserum		Charcoal reagent
Total	800 µl	-	100 µl	-	INCUBATE at 4°C	-
NSB	600 µl	-	100 µl	-		200 µl
Bo	500 µl	-	100 µl	100 µl		200 µl
STD or Sample	-	500 µl	100 µl	100 µl		200 µl

2.9 Statistical analysis

Mean hormone levels were used to give the general pattern of the combined cycles. In the morphological study, Students' t-test was used to determine the statistical significance on the mean differences.

CHAPTER THREE

RESULTS

3.1 Problems encountered

There were several problems encountered in meeting the objectives laid out.

1. Failure to obtain a larger sample size, for the cycles that were not manipulated, which would give less biased results. This resulted from death of some of the trapped animals that were to be used, and failure of membrane regression in those that did not die. The CB animals that had previously shown vaginal oestrus also ceased to exhibit membrane regression once they begun to be physically handled.
2. Blood collection was reduced to two samples per week since a higher frequency (three sample/week), resulted in anaemia (Gal 106).
3. Breeding failure. This resulted in the failure to obtain pregnant animals, for characterizing steroid hormones from the onset of pregnancy. However, two animals were pregnant when the project commenced. They were palpated and confirmed to be in the late stages of gestation. These were used to quantify hormone levels in late pregnancy.

3.2 General sexual behaviour

Oestrus cycles lengths of females were calculated from daily records of their external genitalia. Cycle length was determined from the first day of membrane regression (stage 1) through to the next when it regressed. Past records of colony born (CB) animals in the Institute together with the records obtained in the course of the study (a total of two year period) were used to estimate mean cycle length of bush babies in this colony. The mean of all cycles (n=44) was 53.5 ± 18.4 days, while the mean of means was 51.1 ± 6.7 days. Data on cycle lengths from two animals (Gal 128 & 145) that had shown long anoestrus periods (≥ 200 days, see table 5), were excluded from these calculations.

Table 5. Mean cycle and oestrus lengths (in days) of the CB bush-babies

Animal	x Cycle length	(N)	x oestrus Length	(N)
62	44.2 ± 9.6	11	18.77± 1.88	13
90	44.4 ±14.4	5	23.5 ± 7.45	8
106	59.5 ±24.5	6	35.43±12.38	7
109	54.5 ± 9.3	8	22.78± 5.09	9
113	53.1 ±18.8	12	25.5 ± 8.05	12
128	124 ±59.2	3	32.25± 4.35	4
145	110.6 ±26.4	3	19.67± 4.5	3

Mean of all animals
Cycle length 53.5 ±18.4
Oestrus length 24.6 ±5.42

CB animals had cycles ranging from 44 to 72 days. The cycles for the individual animals were said to be regular if the length of ≥ 50% of the cycles were within the overall mean ± s.d (53.5 ± 18.4). This regularity was lost once the animals were subjected to experimental handling, after which some animals had their orifice closed for over 200 days. Animals acquired from the field did not show any signs of cyclicity and thus remained with their orifice closed except for those induced with exogenous gonadotropins.

During oestrus, mature male adults helped in determination of behavioural oestrus. At this time they showed interest in the oestrus female. The male was observed to continually follow the female, sniffing at the female's genital region, making grunting vocalisation and in several occasions attempting to mount the female. However, no mating was observed during the entire period of study.

Induction of vaginal oestrus was successful in all treated animals and two of these females received some attention from mature males that had been introduced into their cages. Membrane regression was observed at least 24 hours after the hCG injection, but stage 3 of oestrus where hyperaemia and female receptivity that normally occurs, was not attained.

3.3 Hormonal profiles

3.3.1 Profiles of non-induced animals

The hormonal profiles for the non-induced, non-pregnant cycles are as shown in fig 6(i-v). The hormonal profiles plotted were standardised from the day of vaginal opening (VO). They varied in each individual animal, but a general pattern obtained by plotting the mean levels of all the animals on a given day of the cycle, gave results indicative of cyclic behaviour for all of them.

The P_4 levels ranged from 10 to 60 nmol/l. A distinct luteal phase with levels peaking at 45.7±10.8 nmol/l and lasting 19±5 days was clearly marked from the follicular phase. The latter phase had P_4 levels ranging between 10-30 nmol/l with nadir of 3.6±1.4 nmol/l, which lasted 23.4±9.2 days. Figs 6(vi) shows the P_4 profiles of three of the five animals, between days 20-80 after VO. From this figure of the representative animals, a similar pattern of high P_4 levels of 40-60 nmol/l are seen decreasing between days 20-35 after VO. Low levels between 10-20 nmol/l are then observed around days 40-50 after VO, thereafter rising around days 60-80 to levels similar to those shown in the previous peak.

The E_2 concentrations showed baseline levels of between 100-500 pmol/l. Peak levels of over 1000

pmol/l with a mean of 969.8±265.2pmol/l were seen at the end of each phase of the cycle (mostly just before VO). The profiles of this hormone are best correlated with the P_4 levels taken at the same time in each animal.

Two combined graphs (figs 6 vii, viii) of the five non-pregnant animals, obtained by plotting the means of all the animals at two different times of the year, show low P_4 levels from the time just before the vaginal orifice opens (3-5 days before VO) to about day 20 after VO. Fig 6(ix) was obtained by plotting one of these graphs (fig 6 viii) with the corresponding E_2 concentration. High P_4 levels were maintained between day 20-35 and then these decreased to remain low between days 35-45 after VO.

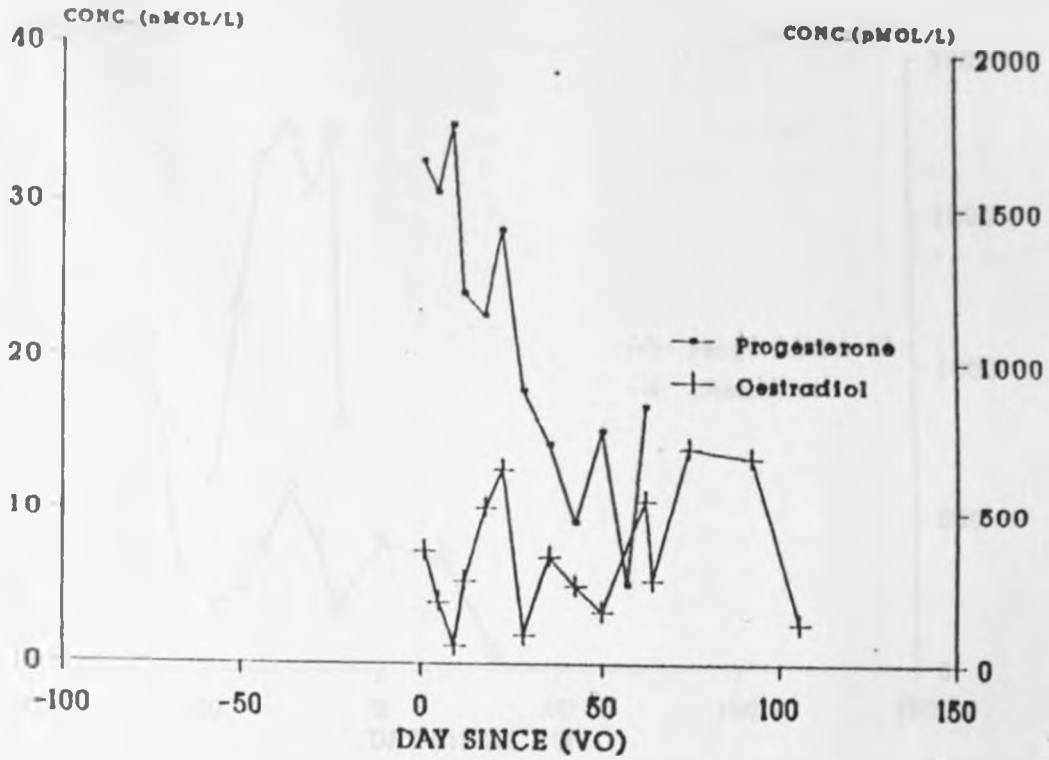


fig 6 (i).

Fig 6. Progesterone (nmol/l) and oestradiol (pmol/l) levels during the oestrous cycles of non-induced non-pregnant bush-babies. They were standardised from the first day of membrane regression (VO). Fig 6(i) shows the levels in Gal 62.

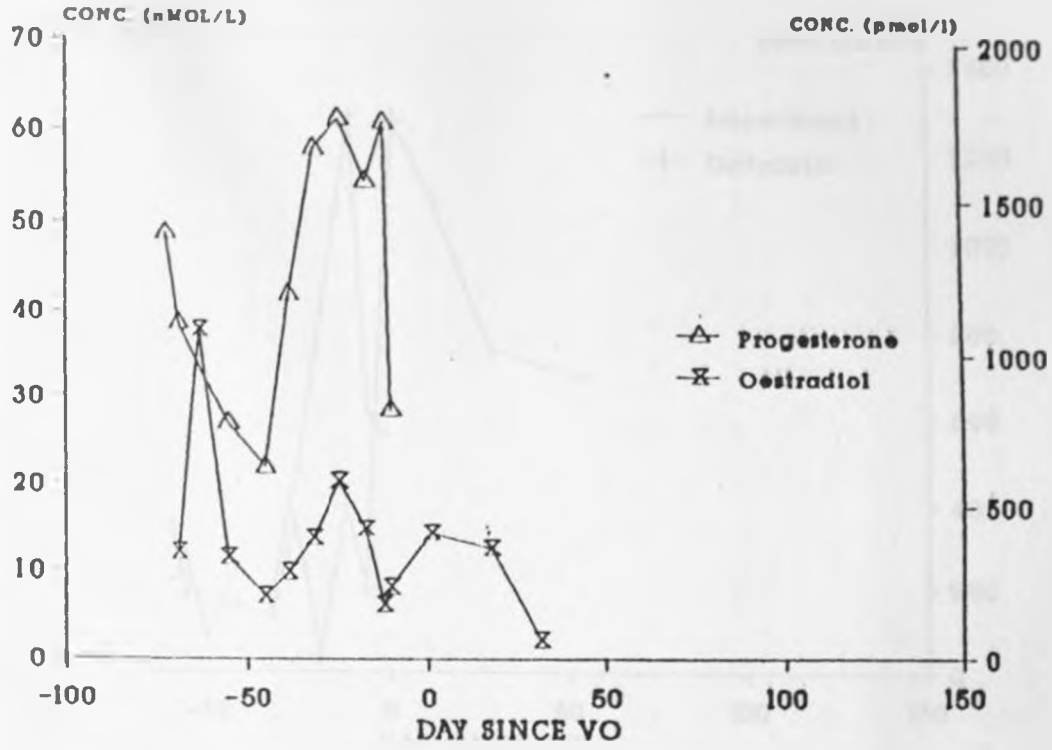


Fig 6(ii). Progesterone and oestradiol levels during the oestrous cycle of Gal 109, standardised from the first day of membrane regression (VO).

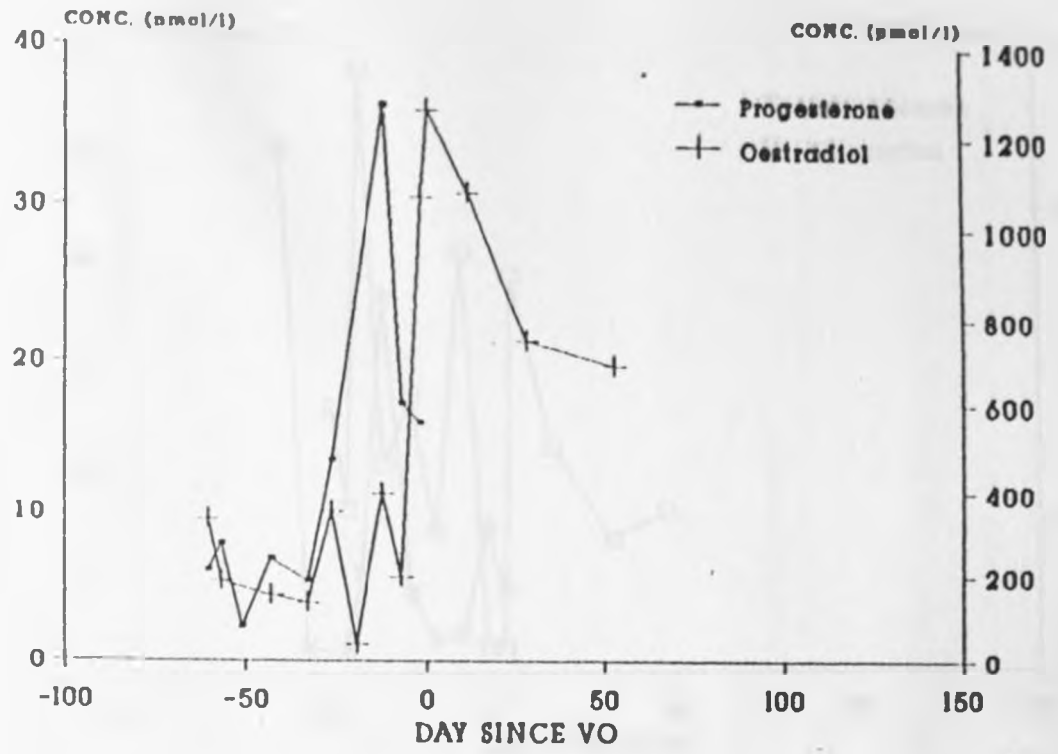


Fig 6(iii). Progesterone and oestradiol levels during the oestrous cycle of Gal 113, standardised from the first day of membrane regression (VO).

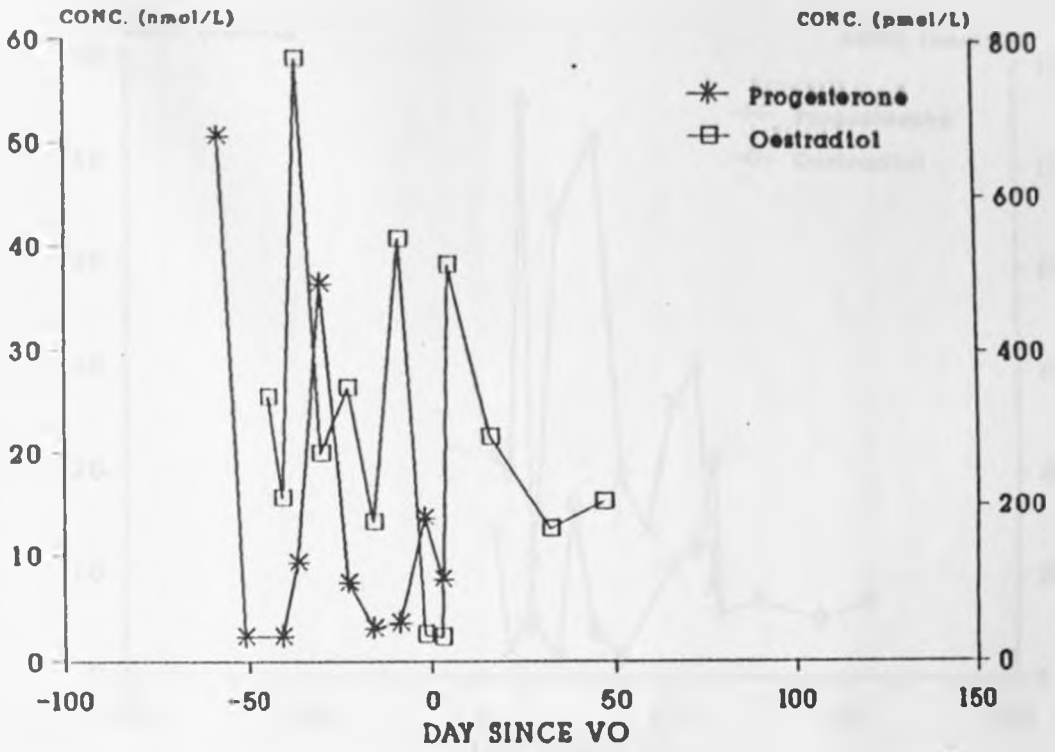


Fig 6(iv). Progesterone and oestradiol levels during the oestrous cycle of Gal 128, standardised from the first day of membrane regression (VO).

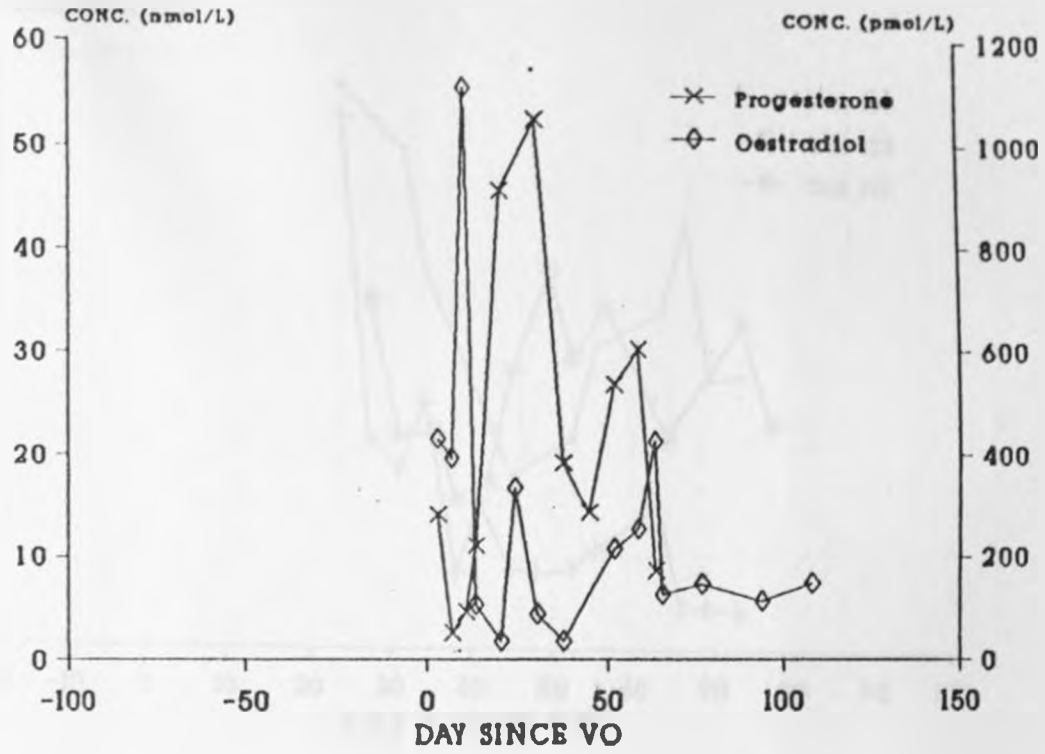


Fig 6(v). Progesterone and oestradiol levels during the oestrous cycle of Gal 145, standardised from the first day of membrane regression (VO).

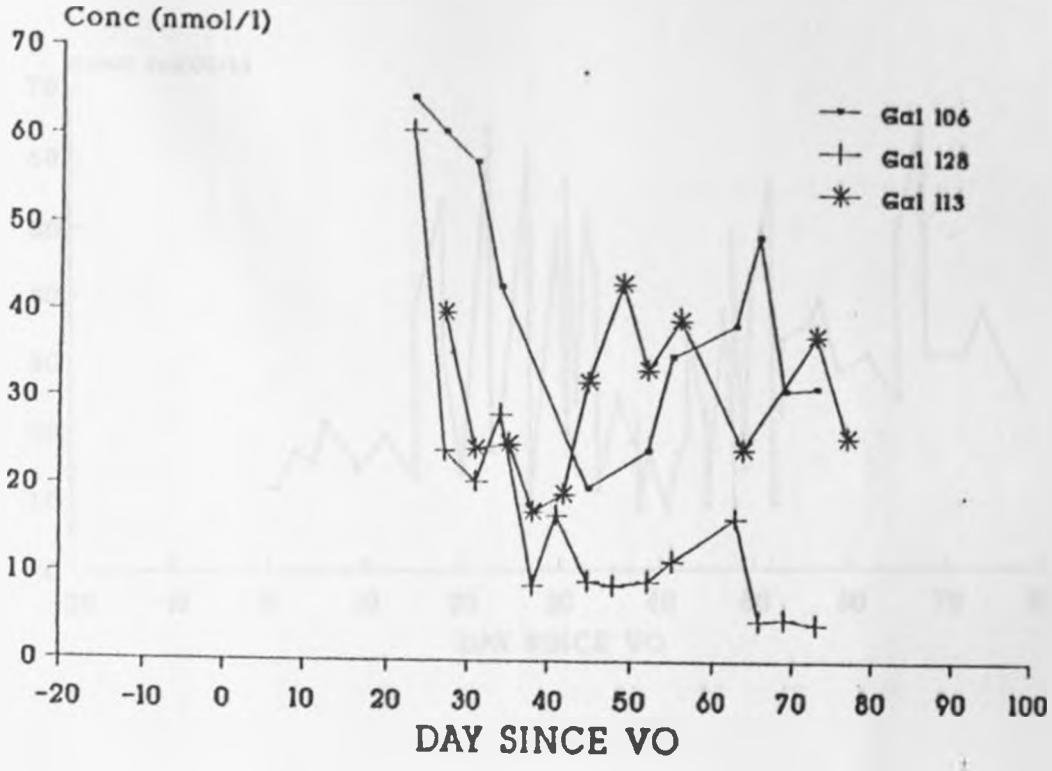


Fig 6 (vi) Progesterone levels of three of the five bush-babies, plotted together. The pattern in these representative animals show a similar decline between day 30-40 after VO, and a consequent increase thereafter. The nadir levels represent the follicular phase of a second cycle although no VO was noted at the end of the preceeding luteal phase.

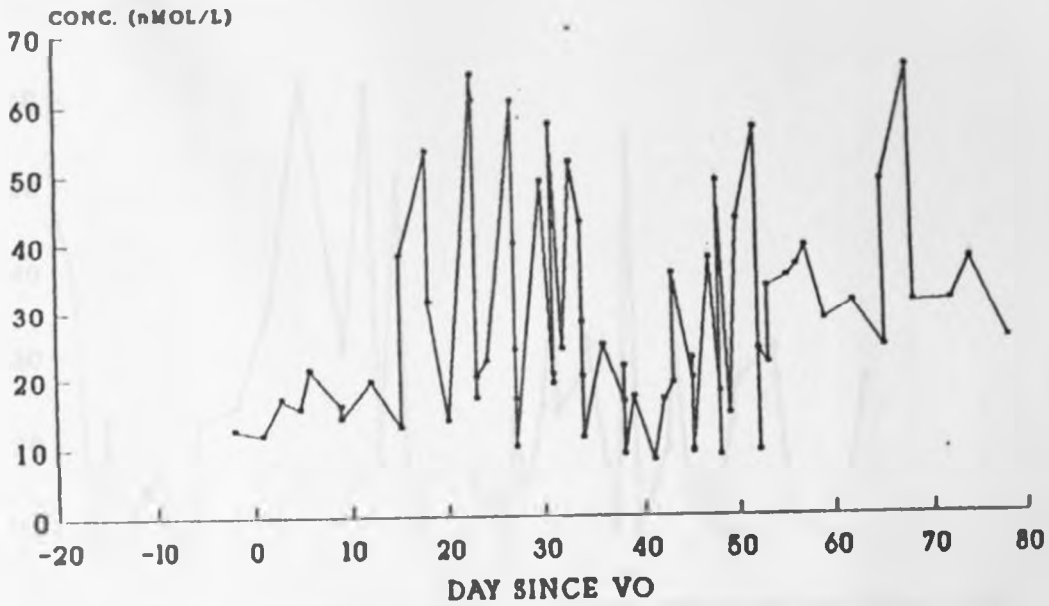


Fig 6 (vii) Mean levels of plasma progesterone from all the five animals. The samples were collected between February and April, 1991.

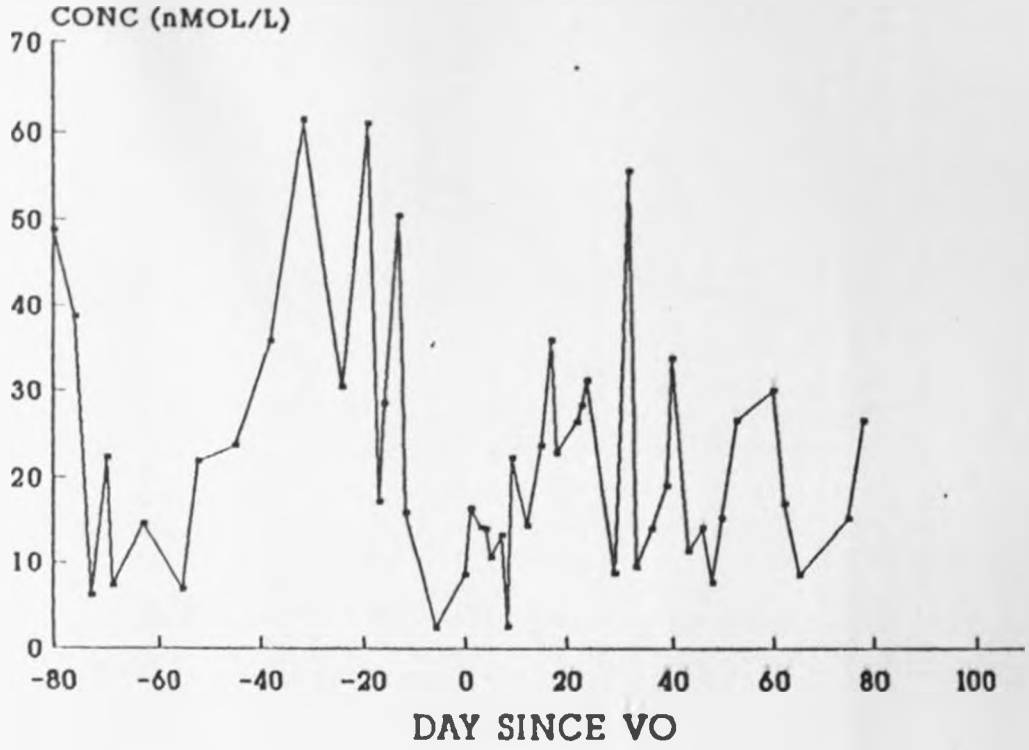
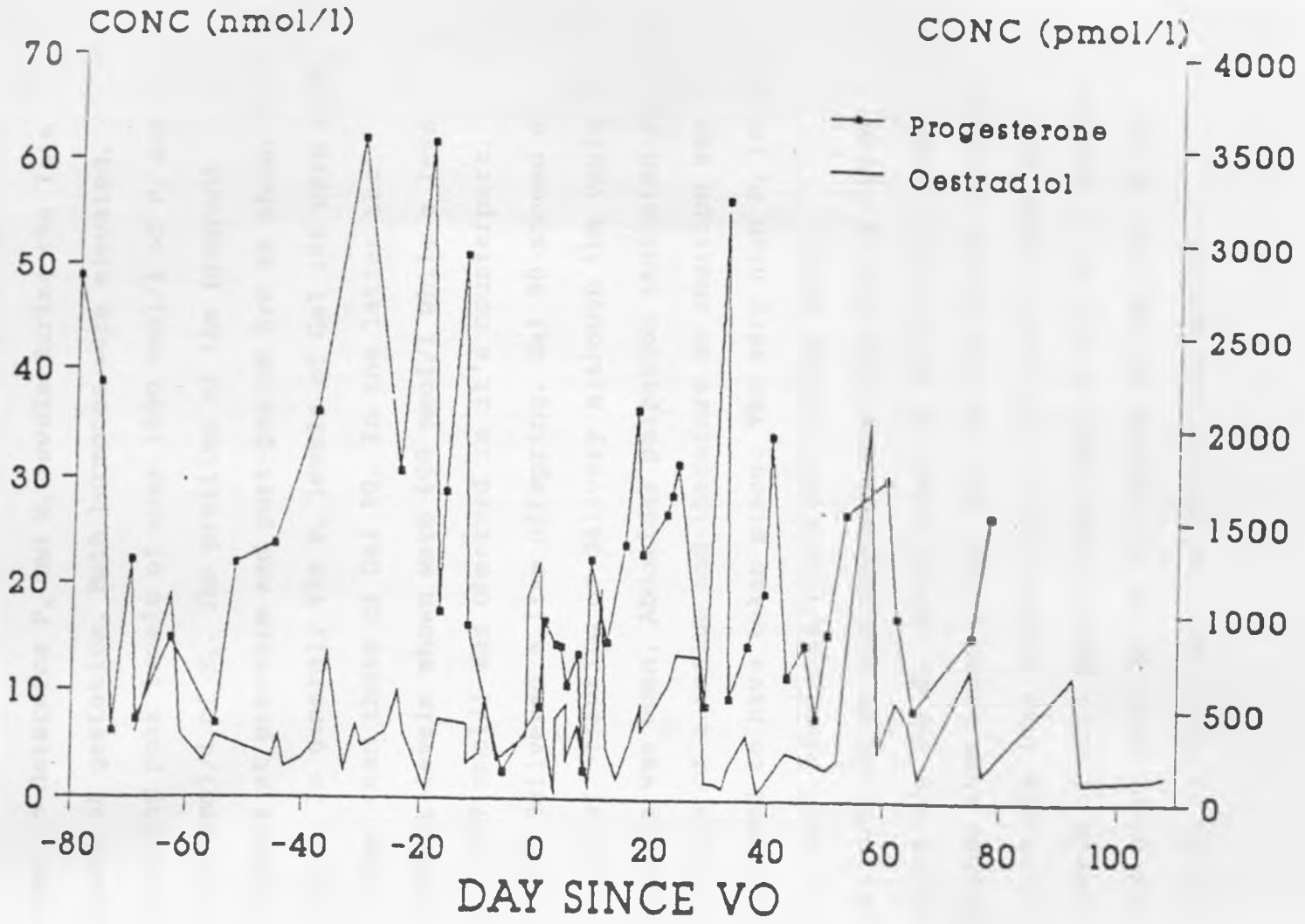


Fig 6 (viii) Mean levels of plasma progesterone from all the five animals. The samples were collected between Oct-Dec. 1990.

Fig 6 (ix) Combined progesterone and oestradiol profile of all the five animals plotted from their mean values.


CONC (nmol/l)
CONC (pmol/l)
Progesterone
Oestradiol
70
60
50
40
30
20
10
0
4000
3500
3000
2500
2000
1500
1000
500
0
-80
-60
-40
-20
0
20
40
60
80
100
DAY SINCE VO

3.3.2 Hormonal profiles during pregnancy

The two pregnant animals (Gal 90 & 106) were used to determine P_4 and E_2 levels during the late stage of gestation. Both hormones were elevated, reaching peak levels of over 1000 nmol/l of P_4 and 3000 pmol/l of E_2. The profiles of the pregnant animals at pre-term and post-partum are as shown in fig 7. In general, the P_4 levels of Gal 106 were much higher than those of Gal 90. In the latter the highest levels shown were 600 nmol/l while a level of 1257 nmol/l was observed in it's counterpart, which delivered a live offspring. Gal 90 showed no signs of offspring or delivery although the vaginal orifice was open. Abdominal palpation indicated an absence of a foetus and therefore an abortion was presumed to have taken place. The very high P_4 levels declined drastically to nadir levels after parturition or the presumed abortion (VO in these animals). The E_2 levels shown by Gal 90 were much higher than those of Gal 106. In the latter maximum concentrations reached were 1787 pmol/l while peak levels of 3113 pmol/l were seen in Gal 90. A decline was also noted in the E_2 hormone at the time of VO, though in this case the decline was gradual.

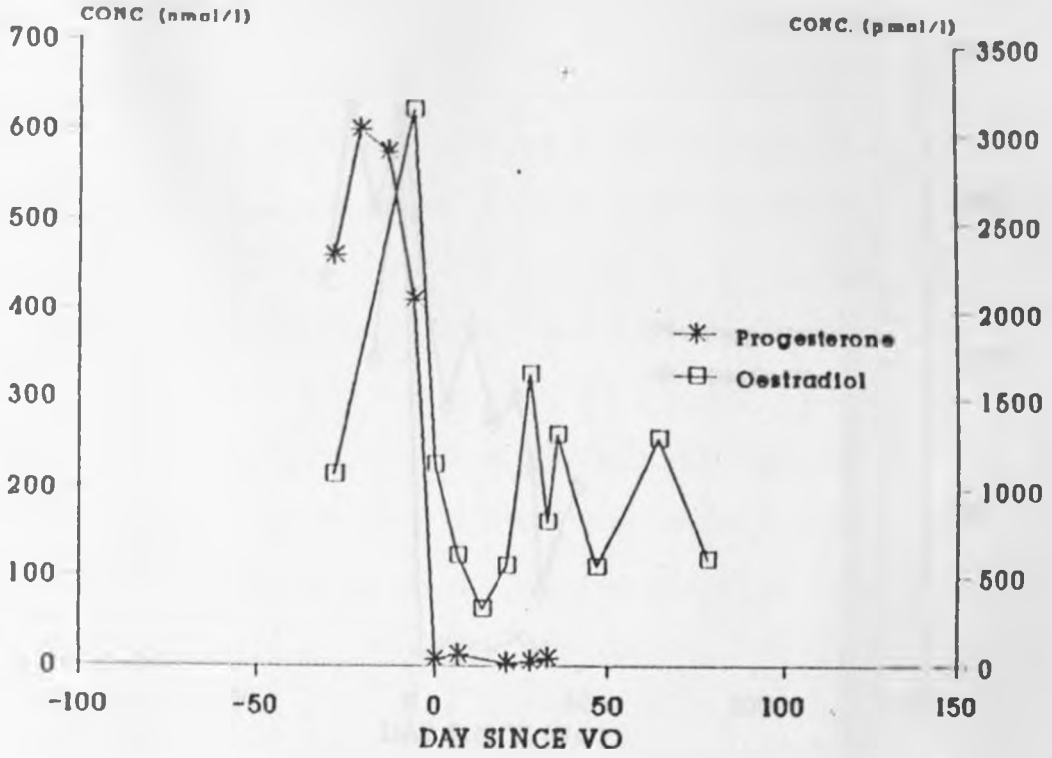


Fig 7 (i) Progesterone and oestradiol levels during late pregnancy (Gal 90). Membrane regression (VO) in this case was a result of either resorption or an abortion since there was no evidence of delivery.

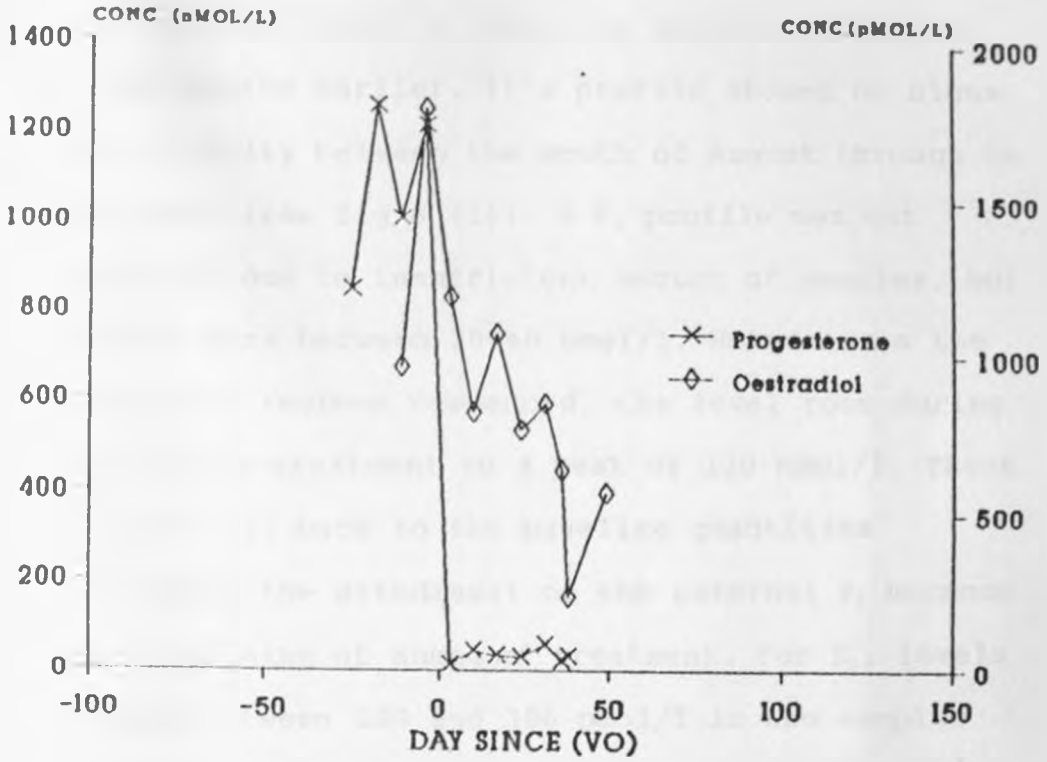


Fig 7 (ii) Progesterone and oestradiol levels during late pregnancy (Gal 106). Membrane regression (VO) in this case was a result of parturition. A live offspring was delivered on 5/11/1990.

3.3.3 Profiles of induced animals

Two of the animals used in this part were WC while the other two were CB. Of the WC animals, Gal 163 had been used in the pilot project in August, three months earlier. It's profile showed no signs of cyclicity between the month of August through to November (see fig 8 (i)). A P_4 profile was not obtained due to insufficient amount of samples, but levels were between 20-40 nmol/l. However, as the induction regimen commenced, the level rose during the P_4 pre-treatment to a peak of 120 nmol/l. These levels fell back to the baseline quantities following the withdrawal of the external P_4 hormone and beginning of Humegon® treatment. For E_2, levels ranged between 100 and 300 pmol/l in the samples collected between August and December 1991, apart from the time the animal was under experimental induction. Levels of up to 7000 pmol/l were attained following hCG injection in the pilot project, whereas a value just below 3000 pmol/l only was seen after the second induction treatment in the main project. VO in this animal was only observed after the induction treatment, following a dose of hCG. The amounts decreased through the period of October, November and increased drastically to about 4000 pmol/l in the month of December when the animal was induced once more. These increasing levels were

observed four days after the first dose of Humegon® was given.

The results shown by this animal was typical of the profiles obtained for the induced animals. Profiles of the induced animals are represented in fig 8. Here, the mean peak values of 211.5 ± 67 nmol/l P_4 and 7575 ± 4000 pmol/l E_2 were attained. E_2 peaks were observed 1-2 days before VO (a day before or on the day of hCG administration). In all the induced animals, the value was higher than those obtained at pre-VO in the non-pregnant animals, but was similar to pre-term levels in one pregnant animal (Gal 90).

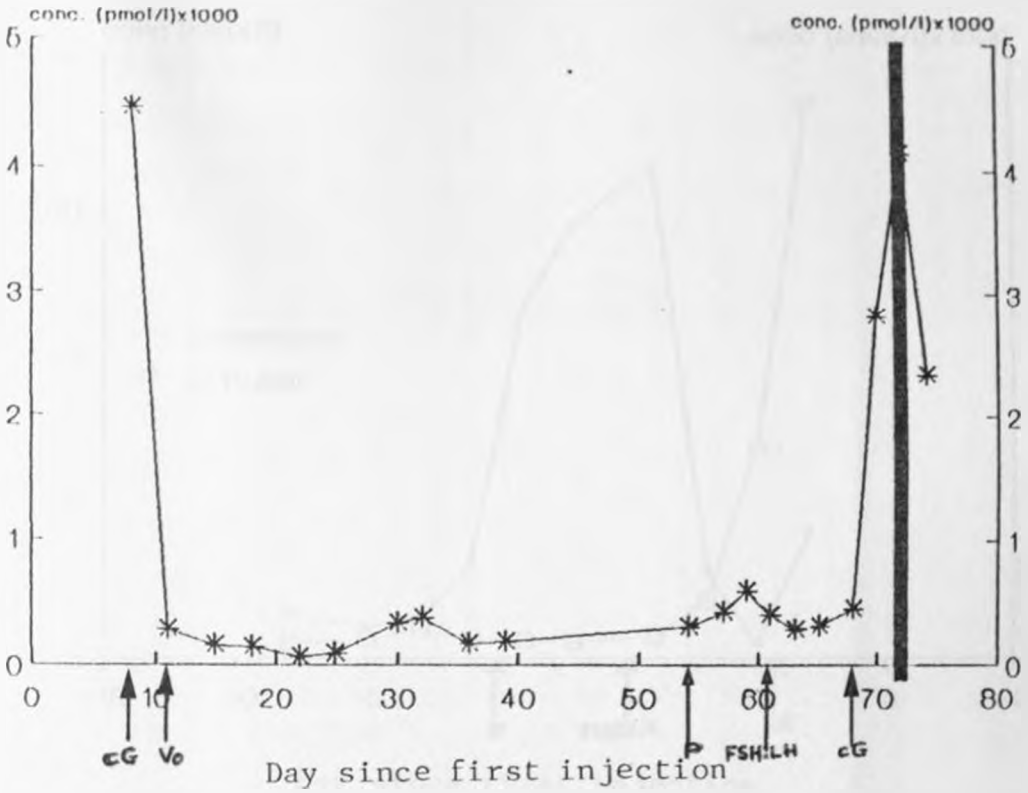


Fig 8 (i) Oestradiol profile after two induction treatments (pg 39), in Gal 163. Relatively low levels are seen between the two peaks observed after hCG injections, although two phases of an increase are noted. The arrows show the day and the hormone injected.The solid vertical bar represents the second VO following induction.

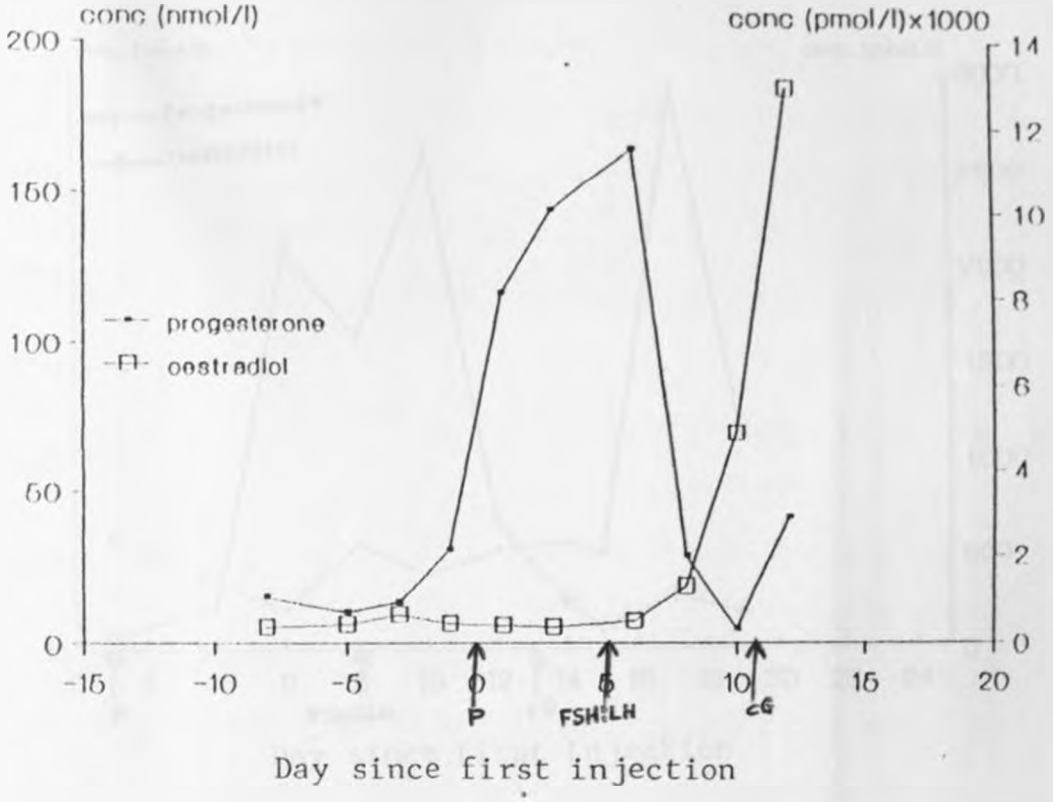


Fig 8 (ii) Progesterone and oestradiol levels during oestrus induction procedure (Gal 109). VO was noted on treatment day 12 (after the second hCG injection)

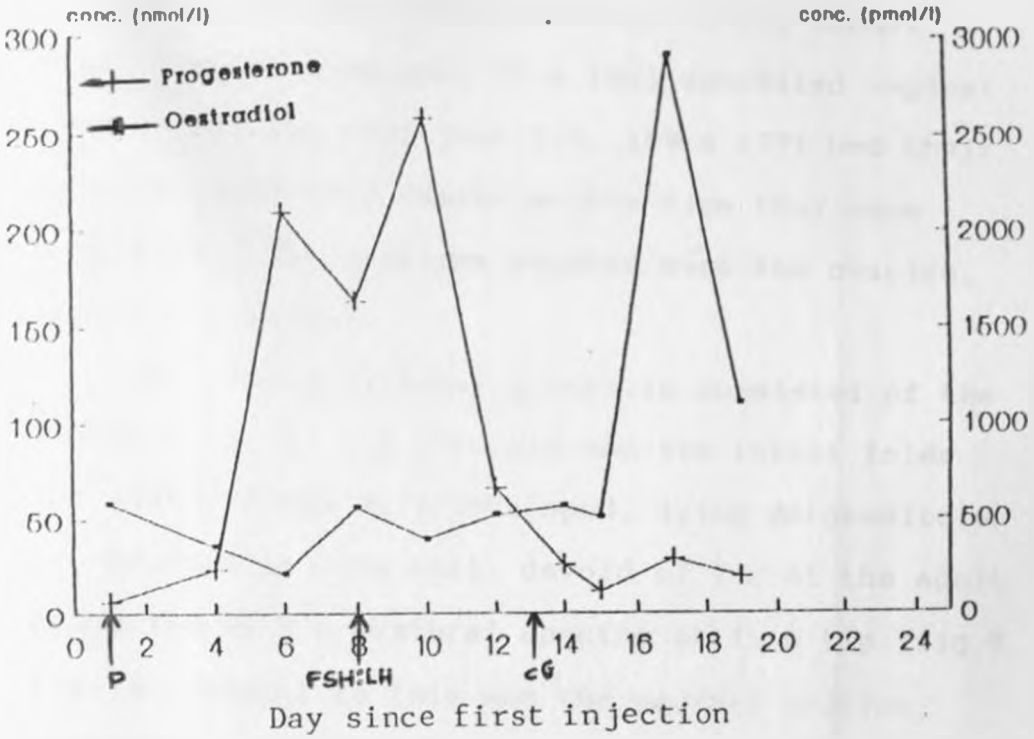


Fig 8 (iii) Progesterone and oestradiol levels during oestrus induction procedure (Gal 145). VO was noted on treatment day 15 (after the second hCG injection)

3.4 General morphology of the reproductive tract

3.4.1 Morphology of the normal tract

The five animals that did not undergo any manipulations with exogeneous hormones (table 3) were used to study the morphology of the normal tract. Two of them (Gal 90 & 106) exhibited vaginal oestrus but the rest (Gal 113, 169 & 177) had their orifice completely sealed at the time they were sacrificed. The sections studied were the ovaries, uterus and vagina.

The female external genitalia consisted of the vulvar orifice, the clitoris and the labial folds. The clitoris was well-developed, lying perpendicular to the ventral body wall, devoid of fur at the adult stage and with a urethral opening at it's tip (fig 9 i & ii). Caudal to this was the vaginal orifice, which was covered by an external membrane at all times except during oestrus. Labial folds on either side of the vaginal orifice were clear-marked.

In the adult male, the testes were permanently descended (fig 10). The colour of the scrotal skin varied from whitish in the young adult that has just lost it's scrotal hair to a deep pink to violet in the fully mature adult.

Fig 9(i) External genitalia of a female bush-baby with the orifice closed (Gal 113). Note the labial folds (Lf), the large pendulous clitoris (Cl) and its perpendicular postion to the ventral body wall. A membrane completely seals the vaginal opening (Vo).

Fig 9 (ii) External genitalia of a female bush-baby with the orifice open (Gal 109). Note the watery mucous secretion (M) commonly seen at mid- to late-oestrus.

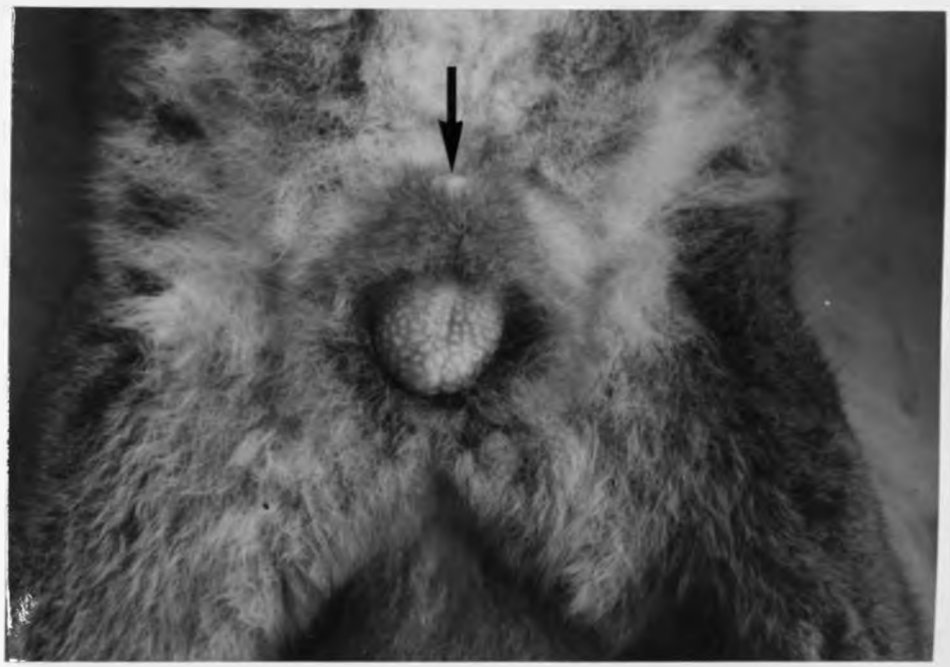


Fig 10 External genitalia of a male Bush-baby. The penis (P) lies in a similar position as the clitoris in the female (fig 9). The scrotal skin is devoid of fur and is in a similar position as the female labial folds.

The female reproductive tract comprised of paired ovaries, paired fallopian tubes or oviducts, a bicornuate uterus and the vagina, which opens externally into the vaginal orifice. The tract of Gal 169 showed the least development when compared with other non-induced, non-oestrus female tracts. It was relatively small, having the smallest ovaries, uterus and, judging from the appearance and size of the external genitalia, showed minimum degree of maturation into adult. In contrast, the tract of Gal 113 was characteristic of a mature adult. The external genitalia had a prominently large clitoris and similarly, large labial folds with conspicous glands as compared to those of Gal 169. The tract of Gal 177, appeared more similar to Gal 113 than Gal 169, from size and appearance. All these animals were in the same reproductive state (VO-0).

The ovaries, in the bush-baby, were completely enclosed within an ovarian bursa which was continuous the the mesovarium in the caudal regions of the abdominal cavity (fig 11). To gain access to the ovary, the encapsulating bursa had to be removed, and the ovary examined. Grossly it was an ovoid structure that appeared compact with a smooth surface when quiescent, or lobulated with protruding follicles when active (fig 12). The left and right

ovaries did not show significant difference in size ($n=8$, $P< 0.05$) and on average the ovary of a mature adult bush-baby measured 0.87 cm long X 0.42 cm deep (table 6).

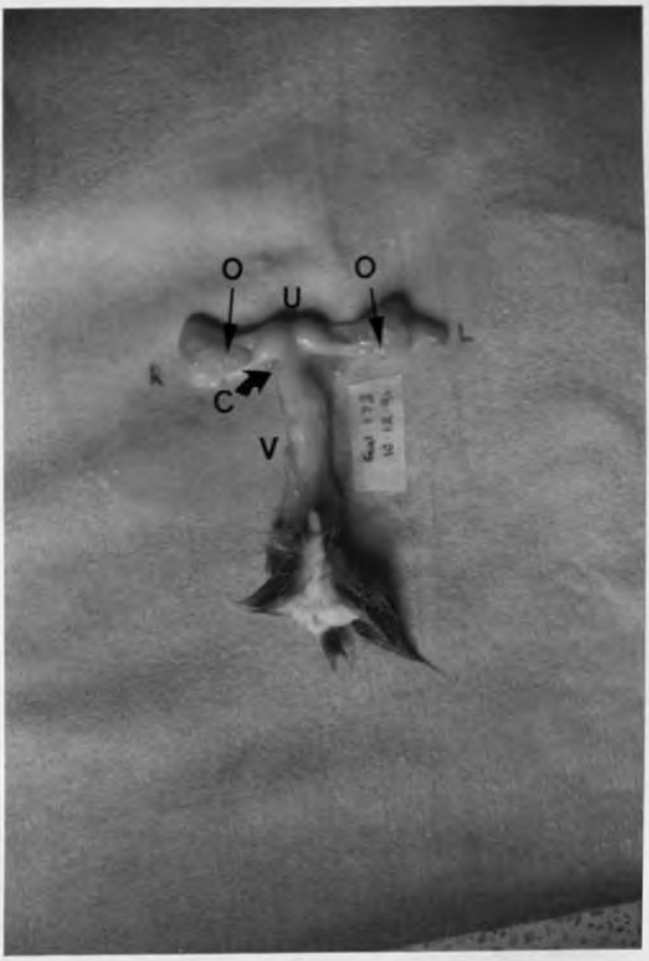


Fig 11 Gross appearance of the reproductive tract (Gal 173). Note the ovaries (O) enclosed in the ovarian bursa (B), the bicornuate uterus (U), the cervix (C), and the vagina (V). The external genitalia comprised of the clitoris, labial folds and the vaginal orifice is also shown.

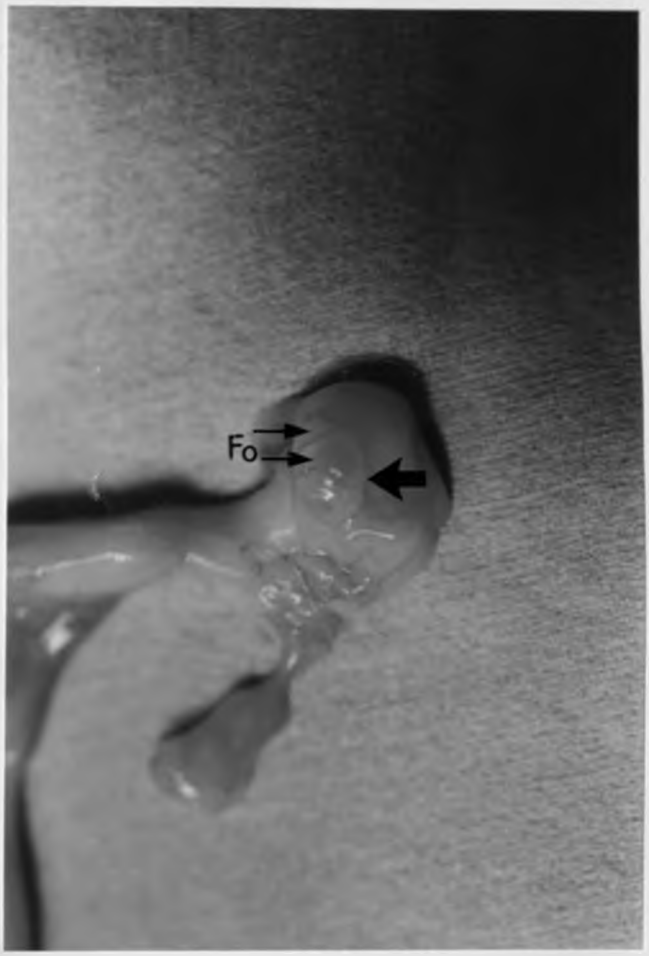


Fig 12 (i) A close up photograph of the left ovary of Gal 113, (arrow) during the quiescent stage. Only a few follicles (Fo) are seen protruding from the ovarian surface giving the ovary a smooth appearance.

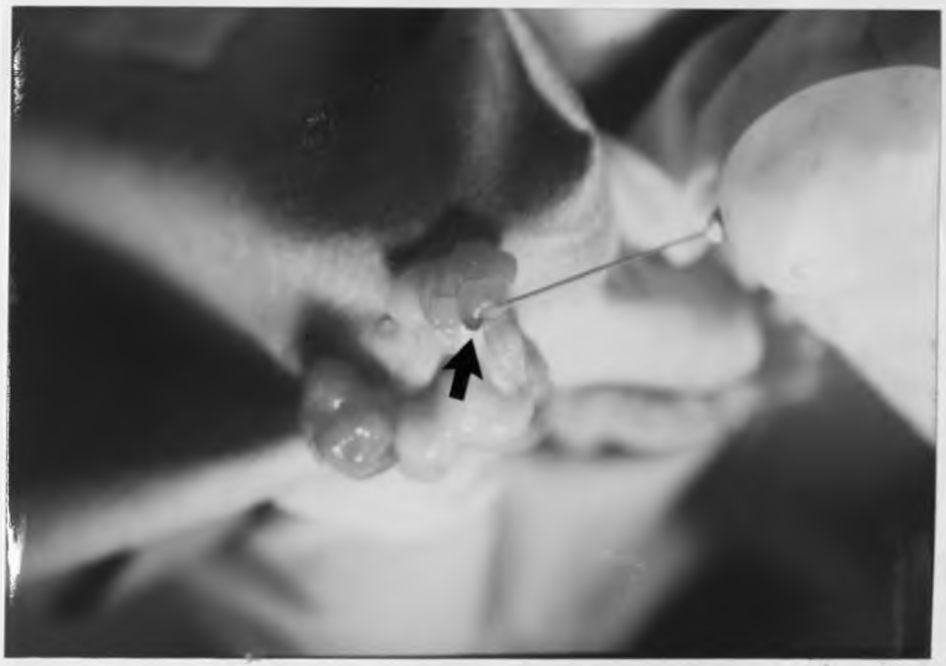


Fig 12 (ii) Gross picture of the ovaries of Gal 163, showing the active stage. Several follicles project from the ovarian surface giving it a lobular appearance. Note the haemorrhagic point(arrow) indicating a recent ovulation.

Table 6 Measurements (cm) of some parameters of the reproductive tract.

(i) Group 1

Animal	90	106[b]	113	169	177	x±s.d
Parameter						
Day since VO	12	6	69	unknown		
Tract length[a]	5.9		7.0	4.3	6	5.8±1.1
Vaginal diam	0.5		0.6	0.35	0.4	0.5±0.1
Cervical diam	0.95		0.5	0.32	0.5	0.6±0.3
Ovarian length						
Right	0.7		0.9	0.7	0.9	0.8±0.1
Left	0.8		0.7	0.5	0.9	0.7±0.2
Follicles:						
Right M	-		-	-	2	
O	-		-	-	-	
CL	-		1	-	-	
Left M	*		-	-	3	
O	*		-	1	-	
CL	*		-	-	-	

[a] Tract length was measured from the uterine inter-cornual to vulval region.
[b] measurements not taken.
* Ovary was cystic and therefore not used in the analysis.
(M) Mature
(O) Ovulated
(CL) Corpus luteum

Table 6 (cont) Measurements (cm) some parameters of the reproductive tract.

(ii) Group 2

Animal Parameter	109	145	163	173	x±s.d
Day since VO	2	2	2	5	
Tract length*	7.5	8	6.8	7.5	7.5±0.5
Vaginal diam	1.1	0.82	1.02	0.8	0.9±0.2
Cervical diam	1.4	0.8	0.8	0.82	0.96±0.3
Ovarian length					
Right	0.9	1.2	1.1	1.0	1.0 ±0.1
Left	1.0	1.0	1.0	1.0	1.0
Follicles:					
Right M	-	-	-	6	
O	-	-	1	-	
CL	-	-	1	-	
Left M	-	-	3	3	
O	-	-	-	-	
CL	-	-	1	-	

Key as in page 76

Ovarian activity was gauged from the presence of numerous preantral and antral follicles, or corpora lutea. When active, large antral follicles caused local bulging of the ovarian surface and depending on their level of development and number, gave the ovary a lobulated surface (fig 12(ii)).

Histologically, the ovarian surface was entirely covered by a germinal epithelium that was made up of cuboidal cells (fig 13). The epithelium formed clefts that penetrated the underlying tunica albuginea, giving rise to sub-surface crypts. Beneath the tunica albuginea, an inner medulla and an outer cortical region were identified, However, the distinct demarcation of the two regions was obscured by the infiltration of developing follicles into both regions of the ovary.

The cortex was made up of follicles in different stages of development. Germinal cords were also seen scattered in the cortical region of the ovaries. In general, the ovaries of the non-induced group were characterized by mainly developing preantral and a few antral follicles. The left ovaries of Gal 106 and 113, and the right ovary of Gal 90 consisted of a few small preantral follicles embedded in the mainly interstitial tissue. Gal 169 ovary had numerous large atretic follicles, while

the ovaries of Gal 177 had several developing antral follicles and a few large atretic ones. The right and left ovaries of Gal 106 and Gal 90 respectively, were cystic with the ovarian parenchyma largely destroyed by the cysts. Several atretic follicles, tiny developing ones and no signs of a corpus luteum were seen in the non-cystic ovaries of the two oestrus females (Gal 90 & 106). Neither a corpus luteum nor an ovulating follicle was seen in the ovaries of the non-induced group.

The uterus was a bicornuate structure. Histologically it consisted of three layers; an outer perimetrium, middle myometrium and an inner endometrium. The appearance of the endometrium largely depended on the phase of reproductive cycle at the time of sacrifice. The endometrium could further be sub-divided into an epithelial layer bordering the lumen, with low columnar cells which under certain conditions had a pseudostratified appearance. Uterine glands and blood vessels were found in the lamina propia beneath the epithelial layer. The myometrium was made up of strong inner circular and outer longitudinal muscles and surrounded endometrium. This was in turn surrounded by the outer perimetrium.

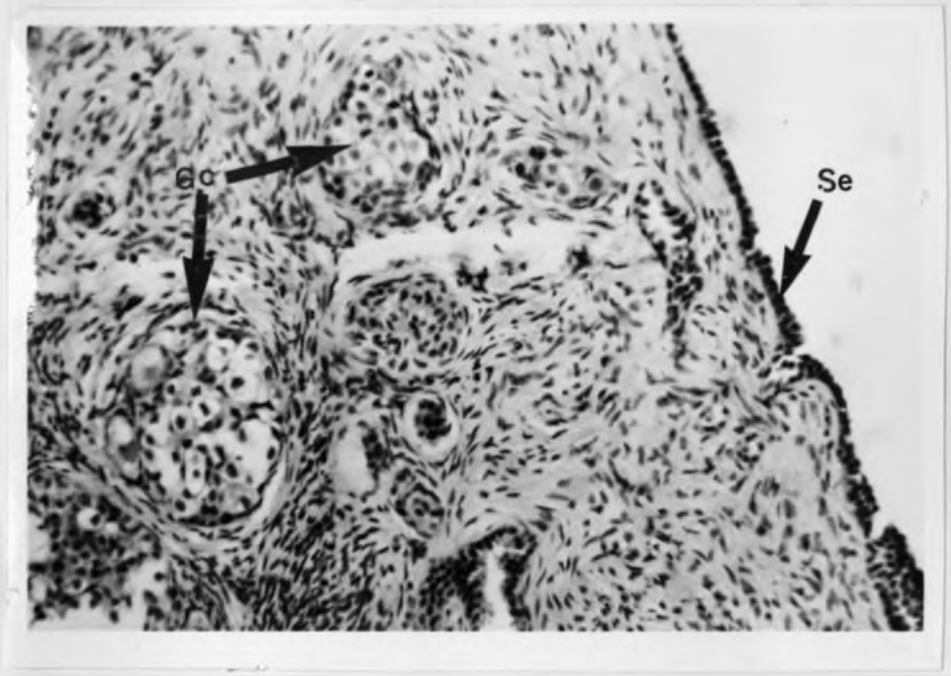


Fig 13 A Photomicrograph of part of the ovarian surface in (Gal 145). Note the surface epithelium (Se) with clefts that penetrate the tunica albuginea to form sub-surface crypts. Germinal cords (Gc) are also visible in the underlying cortical region.

(H & E, X 750)

In the two oestrus females, the uterine epithelium in Gal 90 was made up of simple columnar cells, while that of Gal 106 (fig 14) was mainly pseudstratified. Both were rich in blood vessels but in Gal 106, they were more numerous and penetrated further into the lamina functionalis. Gal 106 was also rich in uterine glands which had a pseudostratified epithelium and contained secretions in their lumen. These had a saw-tooth appearance and opened into the uterine lumen. Gal 90 had few of these glands, which appeared in both the lamina basalis and lamina functionalis, and opened into the uterine lumen. The glandular epithelium of this animal was composed of simple columnar cells which showed proliferation and a few were also vacuolated.

Among the remaining non-induced females (Gal 113,169 & 177), none appeared similar to that of Gal 106. While that of Gal 113 and 177 had similarity in appearance to that of Gal 90, the uterine epithelium in Gal 169 was simple columnar while the uterine glands indicated neither proliferation nor secetory activity. In this animal the glands were more numerous in the lamina basalis.

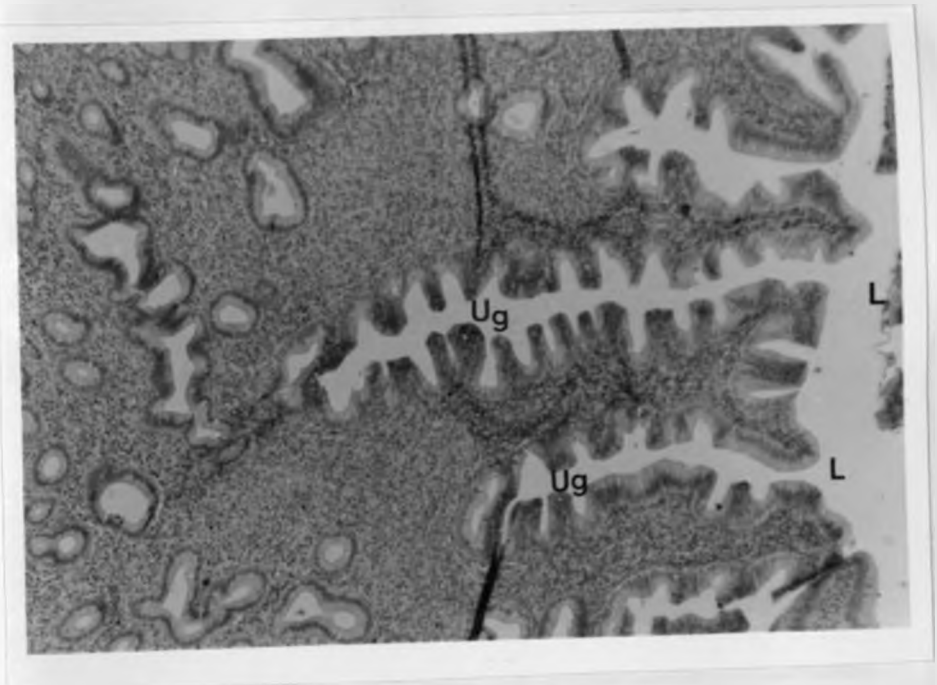


Fig 14 (i) A photomicrograph of a part of the uterine endometrium in the late secretory phase (Gal 163). Note the numerous uterine glands (Ug) which open into the uterine lumen (L).

(H & E. X 50)

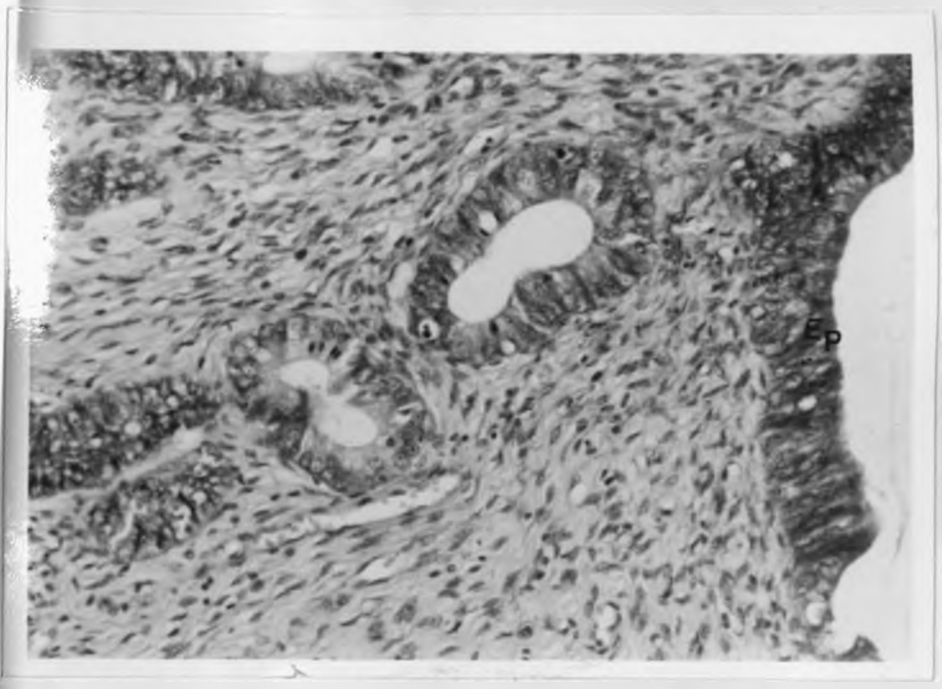


Fig 14 (ii) A close up of the uterine glands and the uterine epithelium (Ep) in Fig 14(i). The uterine glands consist of tall columnar epithelial cells, while the uterine epithelium has a pseudostratified appearance.

(H & E, X 260)

The cervix was made up of an epithelial layer consisting of tall mucus-secreting columnar cells whose oval nuclei were basally located. This layer was highly folded into deep furrows that penetrated the underlying lamina propia, giving them the appearance of branched tubular glands (fig 15). At the portion of the cervix that projected into the vagina, the simple tall columnar cells gave way to a stratified squamous non-keratinised epithelium similar to that seen in the vagina. However, in Gal 106, keratinization of this stratified layer was noted. The cervical lumen in most cases was narrowed due to contraction of the circular muscles of the cervix.

The vagina was lined by a thick stratified epithelium whose basal lamina had papillae projecting from the lamina propia beneath (fig 16). The vagina of Gal 90 and Gal 113 were identical. They had a proliferated non-keratinized epithelium with numerous papillae in its basal lamina. In Gal 169 and 177, the epithelial layer was neither proliferated nor keratinized. However, in Gal 106, epithelium was highly proliferated and had a conspicuous keratinized layer with some squamous cells peeling off the epithelial layer.

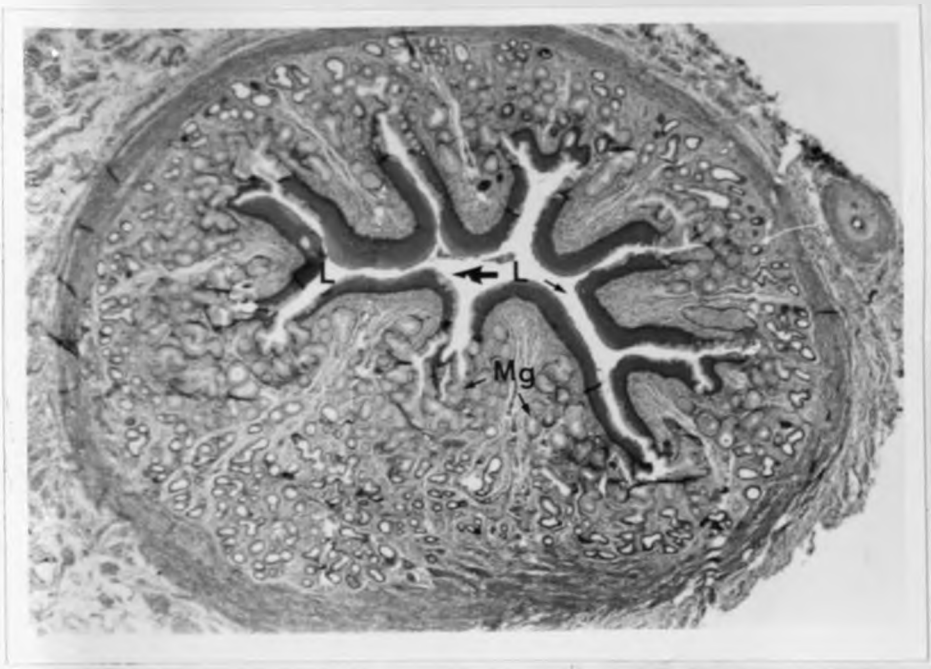


Fig 15(i) A photomicrograph of the cervix (Gal 145). The cervical lumen (L)is surrounded by a highly folded epithelial layer (arrows), forming deep tunnels that appear like glands (Mg).

(H & E, X 100)

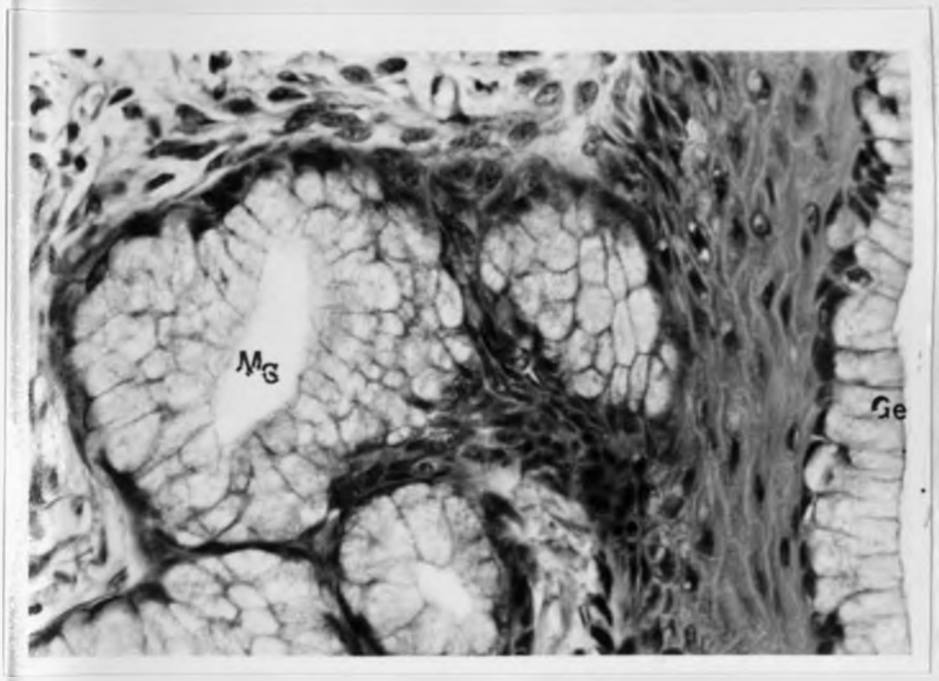


Fig 15(ii) A photomicrograph of part of the cervix in Fig 15(i). The secretory cervical epithelium penetrates the lamina propia to form "mucosal glands" (Mg). Note the glandular epithelium (Ge) above the stratified epithelium.

(H & E, X 460)

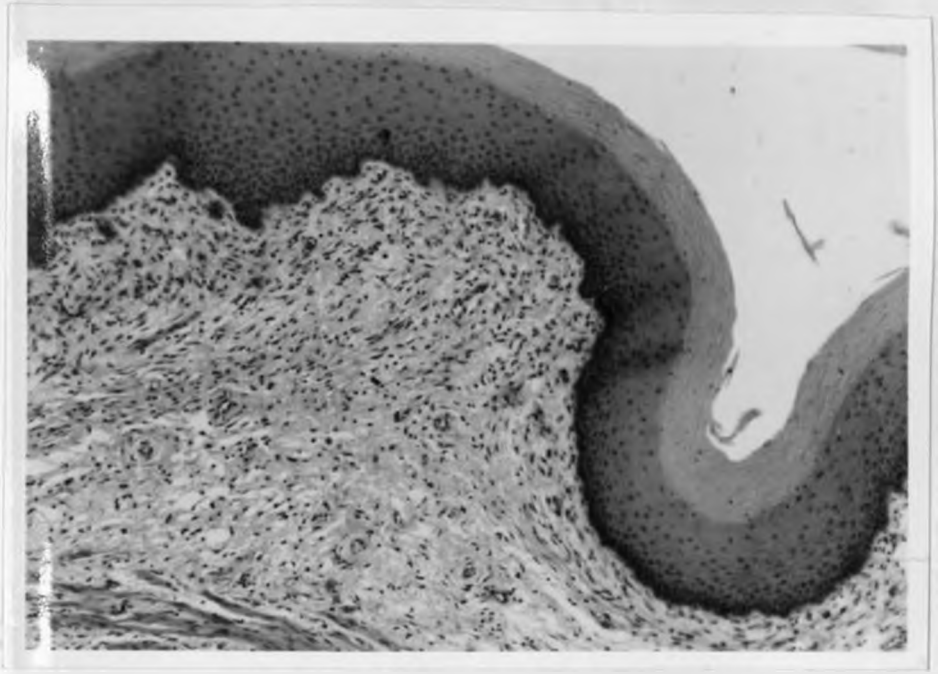


Fig 16 (i) A photomicrograph of the vagina (Gal 163), showing a keratinized stratified squamous epithelium.
(H & E. X 100)

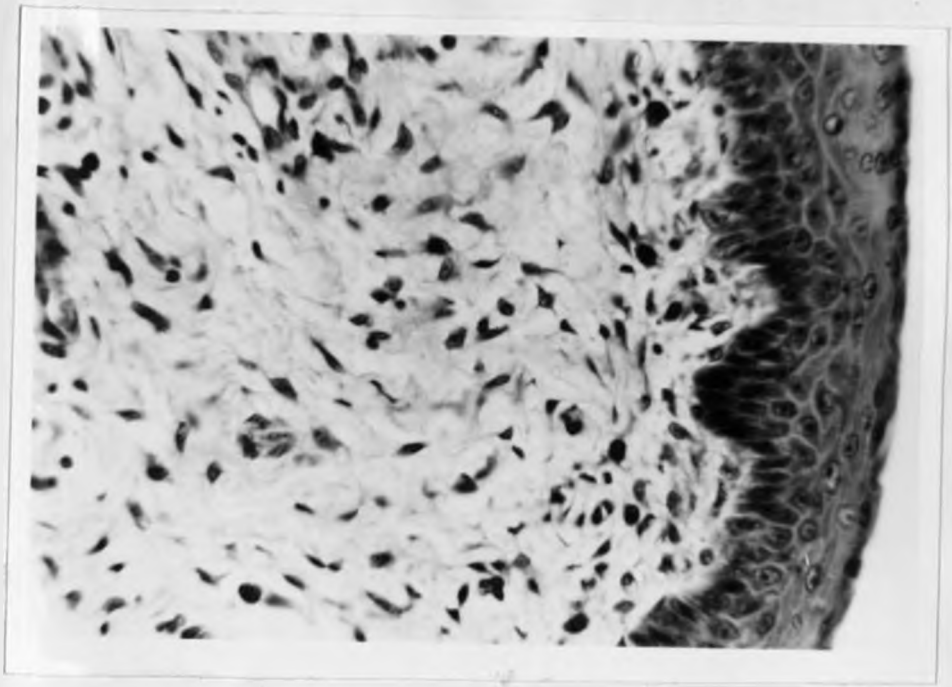


Fig 16 (ii) A photomicrograph of the vagina (Gal 90), showing a non-keratinized stratified squamous epithelium.

(H & E, X 1000)

3.4.2 Morphology of the induced tract

The external genitalia in all the induced animals exhibited VO at the time of sacrifice. Grossly, there was no significant difference (n=4, $P<0.05$) in measurements of the cervical diameter, tract length, or the ovarian length between the group 1 and 2. Although a significant difference was only observed in the vaginal diameter (n=4, $P>0.05$), the whole tract of the induced animals appeared thick-set (turtous) and firm (fig 11 & 17). All the ovaries of these animals had large transluscent follicles protruding from their surface, giving them a lobular appearance. Only Gal 163 had a ruptured haemorrhagic point on the surface of its right ovary, indicating the ovulation point, and early corpus luteum development.

Histologically, large atretic follicles infiltrated both the cortex and medulla in the ovaries of Gal 109 and 173 (fig 18). Here, several smaller follicles were also present but no corpus luteum was seen. Corpora lutea were seen in the left ovary of Gal 145, and in the right one of Gal 163 (fig 19).

Observations of the uterus showed similarity of Gal 109 to that of Gal 106, with a pseudostratifed uterine epithelium and secretory uterine glands. The uterus of Gal 163,173 and Gal 145 resembled that of

Gal 90. The uterine epithelium consisted of pseudostratified columnar cells. Most of the uterine glands were in the stratum compactum although there were some that penetrated the stratum spongiosum, extending to the lumen. Very little evidence of secretory activity was shown in these glands.

The cervix in these animals had a greatly proliferated epithelium that had relatively more secretory "glands" and secretory materials than the dioestrus females. It was also observed in Gal 145 that in some sections of the epithelium the glandular cells lay above the stratified squamous layer.

The vagina was similar in all the animals in this group. They all had a thick keratinized stratified squamuos epithelium.

Fig 17 (i) The reproductive tracts of two females on induced vaginal oestrus (Gal 109 & 163), with the tracts appearing firm and stout.

Fig 17 (ii) The reproductive tracts of two dioestrus females (Gal 113 & 169).

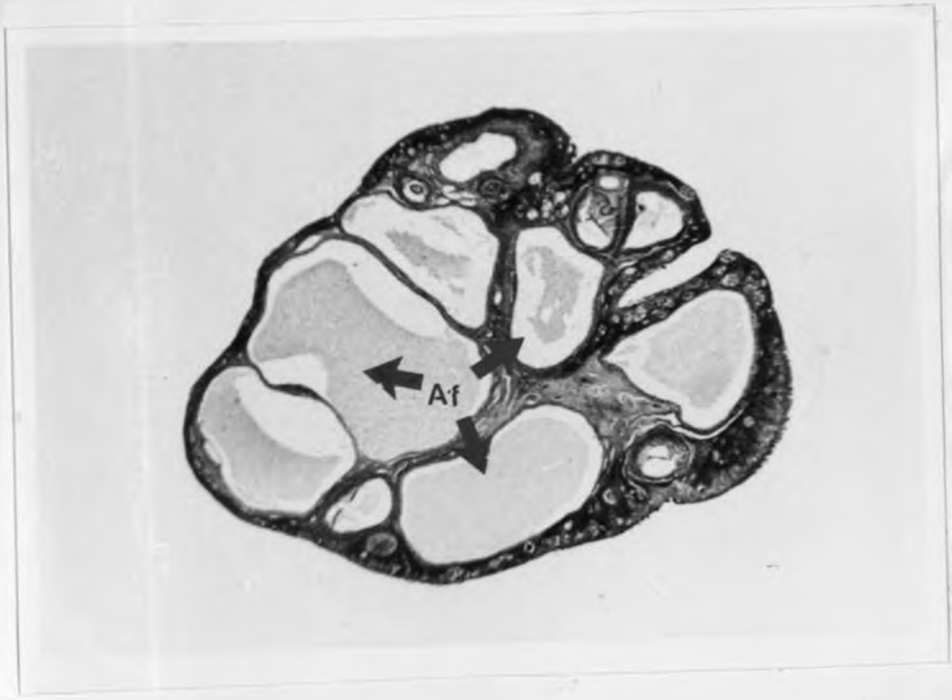


Fig 18 A photomacrograph of the ovary of an induced animal (Gal 173), showing the presence of large atretic follicles (Af) that infiltrate the cortex and medulla regions.

(H & E X 270)

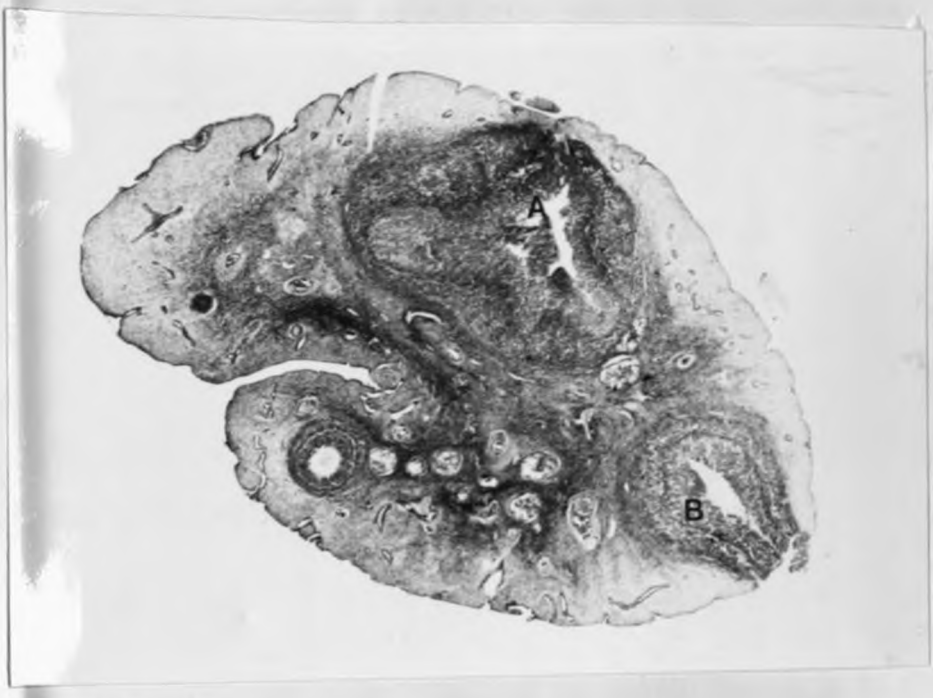


Fig 19 A photomacrograph of the ovary of an induced animal (Gal 163), showing the presence of a large corpus luteum (A) and a smaller one (B) in the same ovary.

(H & E X 300)

CHAPTER FOUR

DISCUSSION

4.1 General discussion

Reports from laboratory colonies all indicated reproductive success of the Greater Bush-babies in captivity [Valerio et al (1972 a & b), Eaton et al (1973), Hendrickx & Newman (1978), Izard & Fail (1988). These authors therefore concluded that the animals are not stressed by captive conditions. However, from examination of reproductive parameters of the animals in the present study, it is clear that they do not respond as reported in previous studies. Prior to the initiation of the study, breeding success was reported in the colony (all except one captive animal were conceived and born in captivity)[Njuguna et al (1984)]. This diminished during the experimental period, where one of the major observation was the cessation of membrane regression (absence of vaginal oestrus) in some animals, while others experienced very long dioestrus periods.

In general, the mean cycle length of animals in the colony showed a great divergence from that given for G. crassicaudatus, a close relative of G. garnettii. It also showed a divergence from other members of Galago genus, which range between 32 to

44 days. The cycle lengths of the individual animals in the present study had a very wide range (44-72 days, excluding the two that had means of over 100 days). In previous documented reports, varied lengths have been given for a given animal species, which could be credited to several factors. The lengths cited could differ due to different experimental conditions employed, the location of the study, method of cycle determination, or even the method of calculation used. In this study, uniform conditions were employed for all the animals used, and as such, a wide variation in the individual cycle lengths was not expected.

Although Hendrickx & Newman (1978) had an overall success in breeding captive animals in their study, they observed reproductive failure in some of these animals. In such cases, they suggested that failure was due to aggressive female behavior towards males, or due to aged animals. Aging in animals in this context is equivalent to menopause in humans. In addition Hendrickx & Newman (1978) described patterns similar to those seen in this colony. These were long anoestrous periods and lack of a distinct oestrus; instead meteoestrus was exhibited instead. These features were also observed in the present study; females that earlier had cycles of an average of about 40 days, had their

orifices sealed for over 200 days, and when they opened the appearance were more characteristic of metoestrus than oestrus. However, all these animals were between 5-9 years of age as compared to those of Hendrickx and Newman's study, that had a minimum of 10.5 years when they exhibited signs of aging. If the animals in this study were to represent the onset of aging, then the discrepancy in age difference in the two studies can only be attributed to the difference in species used. However, there is need to confirm this with studies on altered steroid and gonadotropin levels in addition to their changing sexual behavior at advanced ages, before any conclusive remarks can be drawn.

Aggression in this study was only observed when WC males were introduced into cages of CB females. It can therefore be suggested that failure of breeding in these situations was due to their unfriendly behavior. However, this could not explain the failure when breeding was attempted between the CB animals. Although males introduced into cages of oestrus females showed a clear indication of being attracted to the females, the females withdrew from them and did not show signs of being receptive. Male interest could be deduced from their constant approach towards the female, sniffing of the female genital region, low clicks they made as they

followed the retreating females and their continued attempts to mount the females [Eaton et al (1973)]. No mating was observed in this study and neither did vaginal swabs of the oestrus females reveal the presence of any spermatozoa.

Although Keating (1975) demonstrated that handling and repeated examination did not greatly interfere with cycling in G. senegalensis senegalensis, Darney & Frankling (1982) showed stabilization of individual cycles when handling was reduced. From the present study, stress (mainly from handling) would seem the most appropriate reason for breeding failure. In addition, the different management procedures employed in this study could also have contributed to this failure. These procedures include the uncontrolled photo and thermal conditions used here as opposed to the regulated ones in previous work. It has been demonstrated that the photo period had some effect on reproductive characteristics and breeding patterns [Doyle et al (1967; 1971), Darney & Franklin (1982), Keating (1975)].

4.2 Hormonal profiles

4.2.1 Non-pregnant, non-induced cycles

The results from the combined graph (of all the females) are suggestive of two reproductive cycles within the given period when samples were analysed, with the luteal phase of one cycle ending around day 35-40 to mark the beginning of the next cycle. Based upon the successive peaks of oestradiol at the end of each follicular phase, the mean cycle length of these animals falls between 30-40 days. This is consistent with observations made from vaginal cytology and behavioral in other species of the Greater Bush-baby [Eaton et al (1973), Hendrickx & Newman (1978)].

However, the profiles in the present study disregard the external manifestation of the cycle, since peaks were seen even with the failure of the vaginal orifice to open. Darney & Franklin (1982) showed that high E_2 concentrations were required for membrane regression. In the present study, although some E_2 peaks were observed, membrane regression was not evident and it can be suggested that a threshhold level is necessary for the event to occur.

The profiles obtained in this study were similar to those reported in two other prosimian species [Borgat et al (1977), Eaton et al (1973)].

Serum concentrations of E_2 and P_4 varied in individual animals in the colony, but a distinct follicular and luteal phase was shown in each cycle. An E_2 peak was seen at the end of the follicular phase prior to the gradual rise of circulating P_4 concentrations. The increasing P_4 concentrations were suggestive of luteal function. A second E_2 peak was also seen at the time when the P_4 declined marking the end of the luteal phase. The two phases shown here are characteristic of higher primates. However, it was also noted that one female had a P_4 peak prior to vaginal opening. High concentrations of this hormone were also seen around this period (VO ±5 days) in two other females. If vaginal opening is indicative of ovulation, then this pre-ovulatory P_4 peak seen in the present study, is an unusual feature; this peak is not evident in most higher mammals. In the guinea pigs, a similar situation has been reported [Feder *et al* (1968), Garrison & Foreman (1984)]. It would appear that this P_4 peak acted synergistically with the E_2 to cause membrane regression. A synergistic role of P_4 to E_2 was shown to cause an LH and FSH pre-ovulatory surge in castrate and postmenopausal women [Odell & Swerdloff 1968). A similar effect was also shown in rats [Swerdloff *et al* (1972)]. If the P_4 had a synergistic role in causing these pre-ovulatory LH/FSH surges,

then it is most likely that they also have a similar effect in other oestrus-related events. This would explain the high levels seen at the periovulatory period in the three bush-babies, However, it does not explain why membrane regression was observed in the other two animals that did not show this peri-ovulatory rise. Further research on the role of P_4 in membrane regression is therefore necessary before any conclusive remarks can be drawn.

4.2.2 Induced cycles

As was expected, the profiles of the ovarian steroids in the induced animals were altered as a result of introducing the exogenous hormones. Baseline levels of P_4 rose only to decrease following the withdrawal of the external source. The E_2 concentrations remained at the basal levels and only shot up when hCG was administered. Studies on steroid manipulations by exogeneous gonadotropins done on higher primates, mostly humans, showed that increasing levels of E_2 simulate the follicular phase concentrations when the ovary is stimulated. The ovarian response can therefore be monitored by measuring the concentrations of the circulating E_2. Considering that most of the E_2 in peripheral circulation is derived from the developing antral follicles, a gradual increase would be expected as

the follicular number and size increase. It is difficult to explain why the levels remain at baseline levels only to shoot up after the hCG administration. In an *in vitro* study performed by Bagavandoss & Midgley Jr. (1981), it was suggested that hCG acts by altering the permeability of granulosa cells of the pre-ovulatory follicles. When the membrane becomes more permeable, it allows the release of the hormone synthesised by the follicles, and this could explain the sudden surge when hCG is given. However, taking into account the nature of the cell membrane, steroids should pass into and out of the cells with little difficulty. It is therefore necessary to find a more suitable explanation to this surge. It is probably that the surge may be related to the mode of release. Membrane regression was evident in each of the induced animals after the E_2 surge was noted. This result is in agreement with the earlier suggestion that high E_2 concentrations are required for membrane regression in the Lesser bush-baby [Darney & Franklin (1982)].

It has been shown that hMG can cause LH surge and premature luteinization [Nader *et al* (1986)]. The luteal cells are the major source of P_4 production. The hormone can therefore be used as an index to show that ovulation has taken place, and normal luteal function ensues [Poindexter *et al*

(1983)]. The P_4 profile exhibited by one of the animals used in the pilot experiment was similar to the expected luteal phase increase, following the induction treatment. This observation further suggested that ovulation may have taken place in this animal.

It was also noted that peak concentrations of both E_2 and P_4 in the induced group were higher than in the non-induced group. This increase could be due to overstimulation of follicles in the ovaries of the induced group.

4.2.3 Profiles during pregnancy

It is known that P_4 increases as pregnancy progresses, and in man, maximum levels are attained during the third trimester [Johansson (1969)]. The sequential pattern for serum P_4 in this study was in agreement with these findings. Maximum P_4 levels were obtained just prior to parturition and the gain was a 20-fold increase over the average luteal phase peak levels of 60-70nmol/l. Concentrations of about 1270nmol/l(397.51 ng/ml) were seen in the pregnant animals. These were much higher than those shown in pregnant G.crassicaudatus of 141.7 ng/ml [Izard & Fail (1988)], or in humans of 175ng/ml [Tulchinsky et al (1972)]. The different assays used in the different studies, their sensitivity and the sample

sizes used could all have contributed to these differences. In most old world monkeys, values at pregnancy do not exceed a four-fold increase over luteal phase levels [Chambers & Hearn (1979)]. Hominids (apes and man) on the other hand show results similar to those obtained in this study. At term, chimpanzees have been shown to attain a 20-22 fold increase [Reyes et al (1975). This may be an indication that similar mechanism of synthesis or release of this hormone are employed during pregnancy in these primates, or that they originate from a common source. It could also be related to a similar function, hence the remarkable increase is directed to a common course. However, a comparative study on the mode of synthesis and release would be more conclusive.

Whereas a relatively gradual decline in plasma P_4 has been reported to occur prior to spontaneous abortion in humans [Manganiello et al (1981)], a drastic drop was seen after VO (and therefore the presumed abortion) was noted in this study. Although this hormone is necessary for the maintenance of pregnancy in mammals, the decrease seen here could not have caused the abortion but rather was a result of it.

The E_2 levels values were slightly elevated but not substantially different from those of non-

pregnant animals. The reason for this could be that the placenta, being the main source of the steroid hormones in late gestation, synthesizes more P_4 than E_2. The main oestrogen produced in pregnancy in higher primates is oestriol (E_3) even though an increase is also observed in oestrone and E_2. No data on E_3 hormone has been given for Galagos. The assay system employed in this study detected only the E_2 in circulation. It was therefore not detected if there was any alteration in the concentration of the other two oestrogens. In addition, it was shown that peak similar to a pre-ovulatory one was observed at pre-partum. The high levels of E_2 at this period were required for parturtion, and were probably of placental origin.

No additional information could be obtained on the hormonal levels at earlier stages of pregnancy. A study in this area could be carried out by taking frequent samples at progressive stages of pregnancy since the onset of gestation to term, and analyzing for the hormones.

4.3 Morphology of the normal and the induced tract

In the new-born, the external genitalia is ambiguous [Haines (1976)]. A small tuft of hair was described at the tip of the clitoris and due to the size and position, it could easily be mistaken for a penis. The labial folds shown on either side of the vaginal orifice could be confused with a male scrotum. However, unlike in the newborn male described,in the adult male the testes were permanently descended. The vagina showed a greater resemblance to lower mammals than humans. Cyclic keratinization of the vaginal epithelium, similar to that shown in guinea pigs, rats and other rodents, was seen at various times of the cycle. The uterus diverged from the simplex type seen in most higher primates including man, to a bicornuate type similar to that of some domestic animals, and characteristic of prosimian primates. Another feature unique to Lorises and Galagos is the presence of oogenetic cords in the adult ovary. These were seen in these animals, which was in agreement with documented observations on the ovary of G. senegalensis, G. crassicaudatus, G. demidovii among the Galago, and L. tardigaradus, N. potto of the other prosimians [Anand Kumar, (1974)]. The presence of sub-surface crypts seen in this study was in agreement with

those described for the mammalian ovary [Harrison & Mathews (1951)].

The effect of the inducing gonadotropins on the reproductive tract was seen in the gross and histological sections, while the steroid profiles were also altered.

The induced animals showed features that are characteristic of high oestrogenic influence. Membrane regression has been associated with high plasma oestrogens [(Darney and Franklin (1982)]. This was evident in all the induced animals and two non-induced females at the time of sacrifice. Proliferation of the vaginal and uterine epithelium was evident in the induced animals, as well as keratinization of the vaginal epithelium. Perrotta (1962) showed a similar proliferation in the vaginal epithelium of castrate rats after treatment with oestrogens. Generally, high oestrogen levels have been associated with gonadotropin induction in mammals (Compretto et al (1981), Garrison and Foreman (1984), Goldenberg et al (1973 a & b), Yun et al (1987)]. It has been shown that FSH- hCG combination given to rats resulted in massive proliferation and luteinization of granulosa and thecal cells, and corpus luteum formation (Goldenberg et al (1973 a). It has also been shown that maximum hCG uptake is observed when a corpus

luteum is present in the ovary (Goldenberg et al (1973 b). In the present study, follicular development was greatly enhanced in the induced animals. There was evidence of overstimulation of follicular development in two of the animals, where multiple follicles were obtained. The results are similar to those obtained in a study on humans [Comparetto et al (1981)]. In that study follicular morphology was correlated to plasma oestradiol. It has also been shown that hMG can cause premature luteinization [Nader et al(1986), and this could account for the corpora lutea seen in the other two induced animals. The granulosa and thecal cells are the main source of ovarian steroids. It can therefore be deduced that, following ovarian stimulation with the gonadotropins, there is massive proliferation of these cells and this leads to increased ovarian weight which inturn leads to a corresponding increase in steroidal output. This was confirmed by measuring the steroid concentration, which showed E_2 peaks during this period. The net effect of this E_2 increase was shown by the the changes that took place in the vagina, cervix and uterus of these animals. These organs are the main targets of ovarian steroids.

Excessive stimulation of the ovary by exogenous gonadotropins has been reported to cause

ovarian cysts [Schenker & Weinstein (1978)]. However, this could not have been the cause in the two animals found with cystic ovaries, since they were not subjected to the induction treatment. The ovaries that were not cystic in these animals showed a similar level of development, with many small atretic follicles and relatively only a few large ones. There was no corpus luteum in either ovaries. It is therefore apparent that the cystic ovaries had a role in suppressing the development of the non-cystic ones.

The morphology of the cervix, especially the mucosal appearance, was similar to those reported in most primates. However, one of the induced animal showed keratinization of the epithelium in the portion projecting into the vagina. In addition, tall columnar mucous secreting cells were seen above this keratinized epithelium in some regions. These observations were unusual and have not been reported in any previous work on the cervix. These features could have been caused by the high oestrogen concentrations coming from the induced ovary.

4.4 Conclusions

The first part of the study was intended to look at the basic physiology of the Brown bush-baby, by characterizing the oestradiol and progesterone hormones during the oestrus cycle and during pregnancy. The profiles obtained were to be correlated with the state of the external genitalia. The second part of the project looked at the effect of inducing gonadotropins on the oestrous cycles, hormonal levels and on the morphology of the reproductive tract.

1. The features seen in the profiles obtained showed similarity to other prosimians and simian cycles. However, a descrepancy on the role of P_4 at the periovulatory period was shown in some of the animals. This is an aspect that requires to be studied further.
   From the two representative pregnant animals, it can be deduced that prior to and at parturition, P_4 levels resemble those of obtained in G. crassicaudatus, chimpanzees and humans.

2. A correlation could be deduced between the state of the vaginal orifice, behaviour and steroidal levels. Unfriendly behaviour between

sexes was observed in anoestrus (WC) and the dioestrus (CB) females. Sexual proceptivity was seen when males were introduced into cages of females in either natural or induced oestrus. The profiles obtained, especially from the combined graphs show peak E_2 levels prior to VO, and low concentrations of both hormones at the time of VO. The pro-VO E_2 peak could have been responsible for the proceptive behaviour and membrane regression.

3. The effect of the inducing gonadotropins on the reproductive tract and hormonal profiles were evident in all the induced animals. Overstimulation ellicted the formation of multiple follicles which could have been responsible for the rise in steroidal content. Oestrogenic influence was observed in the vagina and uterus. These animals could therefore be used for research in the studies related to some aspects of ovarian hyperstimulation syndrome in humans.

In view of the results obtained in this study, under the given management and conditions, the brown bush-baby was generally a difficult animal to work with in aspects related to reproduction. It would

therefore be apt to say it is not the best choice model for human physiological research in reproduction, under the given circumstances.

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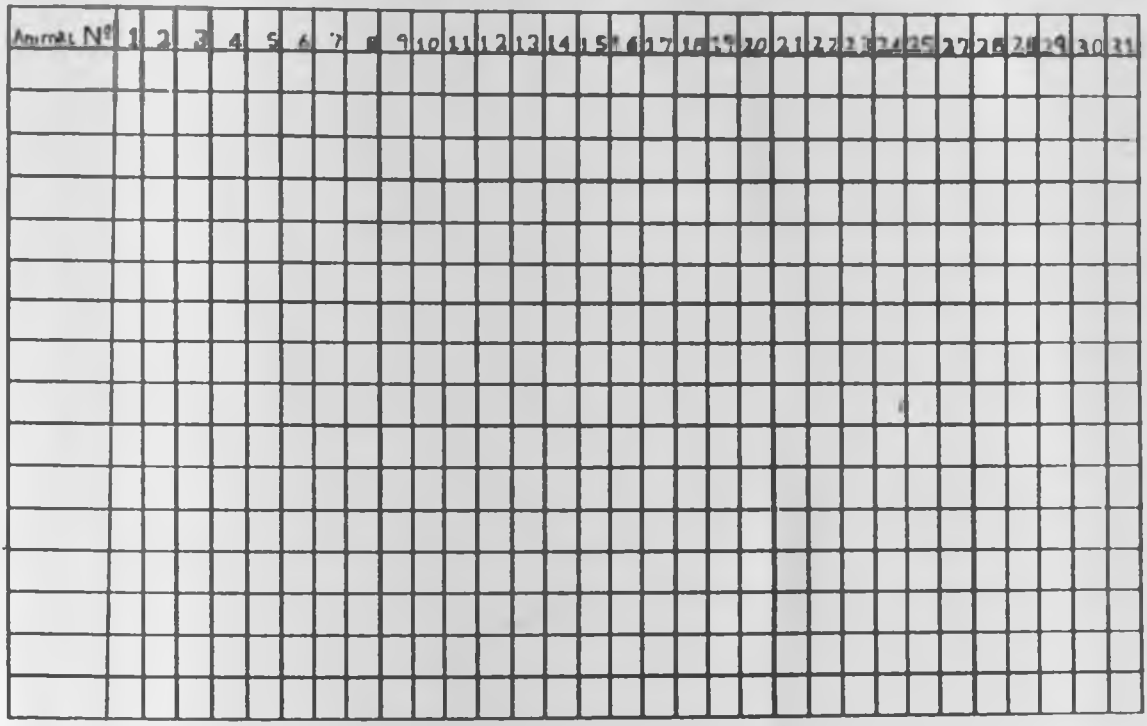
Appendix

CYCLE CHART

Reproductive cycle of the brown bush-baby

Month_____. Year_____.



Code used to determine the state of the vaginal orifice:

0- complete closure
1- pinhole opening towards opening
2- medium sized opening towards opening
3- complete opening with hyperaemia, during oestrus
4- medium sized opening towards closing
5- pinhole opening towards closing
6- complete closure on the day following vaginal oestrus
8- complete closure during pregnancy
9- complete opening at parturition.