REPRODUCTIVE HORMONES AND REPRODUCTIVE SUCCESS IN YELLOW BABOONS (*PAPIO CYNOCEPHALUS*) AND YELLOW-OLIVE (*PAPIO ANUBIS*) HYBRIDS IN THE AMBOSELI ECOSYSTEM, KENYA

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2020.

DECLARATION

DECLARATION

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

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DEDICATION

This thesis is dedicated to the Almighty God for seeing me through all the steps in my education. The work is also dedicated to my beloved mother, Priscilla Oduor and my sister Mary Oduor for their financial and moral support as well as prayers that made it possible.

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ABSTRACT

Several baboon species in the genus *Papio* exhibit distinct ranges across Africa. Where those ranges meet, hybridization occurs, contributing to genetic diversity and structuring in these species. Hybridization between yellow (*P. cynocephalus*) and olive (*P. anubis*) baboons is common, though previous studies on these species reported strong effects of hybrid ancestry on males. However, information on hybrid females is lacking. The present study investigated the link between yellow-olive baboon admixture and female reproductive success in the Amboseli ecosystem in Kenya. It leveraged new data as well as multidecade data from this long-term population study. The study hypothesized that miscarriage rates, birth rates, and estrogen and progesterone levels do not differ between pure female baboons (*P. cynocephalus*) and hybrid female baboons. The main objective was to examine differences in the reproductive hormones and reproductive success of the female *P. cynocephalus* baboons and hybrids between *P. cynocephalus* and *P. anubis*. To achieve this objective, female baboons were observed for cycling, pregnancy, post implantation loss, births as well as duration of lactation. Fecal samples were collected from 48 adult females with known admixture, preserved in 95% ethanol and transported to the Reproductive Biology Unit laboratory at the University of Nairobi for further processing through evaporation of ethanol, freeze-dried and sifted. Fecal estrogen and progesterone levels were determined using enzyme immunoassay technique at the School of Biological Sciences laboratory, University of Nairobi. Long-term data were extracted from the Amboseli Baboon Project's online relational database to evaluate each subject's number of miscarriages, interbirth intervals, and multi-year steroid hormone profiles as well as several covariates (hybrid score, maternal age, maternal parity, maternal rank, group size,

habitat quality, infant sex, daily maximum temperature and daily rainfall). These data were analyzed using Generalized Linear Mixed Models and Linear Mixed Model packages in the R statistical environment. Results showed influence of hybridity on estrogen with higher levels (P=0.0155) for females with more *P. anubis* genetic background. Cycling duration was longer for females with more *P. anubis* ancestry (P=0.0215), while postpartum amenorrhea was shorter for females with more *P. anubis* ancestry (P=0.0147). However, hybridity did not predict progesterone levels, miscarriage rates, or birth rates among Amboseli female baboons, indicating that admixture does not influence these measures of reproductive biology. It is concluded that hybrid and non-hybrid female baboons experience equal reproductive success and are therefore both evolutionary fit. In addition, hybridization has likely increased genetic diversity in Amboseli although its frequency is low and may not permit hybrid speciation.

CHAPTER 1

1.0 INTRODUCTION

1.1 NATURAL HYBRIDIZATION AND REPRODUCTIVE SUCCESS.

Natural hybridization is a mechanism of evolution that operates in both animals and plants and may enhance the processes of speciation as well as the gradual change in the diversification in an ecosystem (Seehausen *et al*., 2008; Arnold, 2006; Grant *et al*., 2005; Schwarz *et al*., 2005; Arnold, 1997; Rieseberg, 1997). Evolutionary biologists have demonstrated the occurrence of speciation and methods by which hybridization operates, through their knowledge of the concepts on this subject (Tung *et al*., 2008; Hewitt, 2001). Hybridization may influence biodiversity in two ways (Seehausen *et al*., 2008). First, it can lead to multiplication of species through the introduction of various combinations of genes that confer novelty as well as improvement of the subjects' ability to undergo beneficial evolution (Seehausen *et al*., 2008; Arnold, 2006). Second, it can also reduce the numbers of species by breaking down reproductive isolation and genetic differentiation between species (Rundle and Nosil, 2005; Kirkpatrick and Ravigne, 2002). Hybridization can result in either complete non-viability of the offspring or formation of a distinct species (Seehausen *et al*., 2008; Mallet, 2007) that has a mosaic genome composed of both parental lineages (Zinner *et al*., 2011). However, the outcome of hybridization in a population depends on the population's demography, genetic history and prevailing ecological factors where hybridization process takes place (Seehausen *et al*., 2008). Certain conditions support the generation of new species through hybridization; these conditions include the existence of unoccupied niches, strong selection

directed by ecological factors, excellent fertility in hybrids, and the occurrence of hybrids and parental species in separate habitats (Seehausen *et al*., 2008).

Reproductive success in animals can refer to the number of offspring left by an organism in all subsequent generations or viewed in terms of the number of offspring produced regardless of whether the offspring survived to reproductive age (Lloyd and Rosa, 1989). Hybridization can induce variations in the reproductive success between parental populations, and in the hybrid population relative to the parental forms (Burke and Arnold, 2001). For example, hybridization occur between blue monkeys (*Cercopithecus mitis*) and red-tailed monkeys (*C. ascanius*) in the forests of East Africa, (Detwiler, 2002). In Gombe National Park (Tanzania), hybrids arising from the two monkey species make up a sizable proportion of breeding individuals in the population (Detwiler, 2002). This demonstrates that factors that might hinder gene flow between the two species are not strong, given the varied hybrid phenotypes observed in this region and their breeding success (Detwiler, 2002). Reproductive success following hybridization at Awash National Park in Ethiopia is another example. Here, two species of baboons (*Papio anubis* and *Papio hamadryas*) hybridize. Male *P. hamadryas* baboons hold one-male units and exhibit sustained, intense interest in adult females, regardless of their reproductive state. On the other hand, anubis baboons (Bergman *et al*., 2008) live in multi-male, multi-female groups where males compete for ovulating females; despite the behavioral differences, the two taxa interbreed successfully to form a hybrid zone. The hybrid scores based on their intermediate behavior showed that hybrids have the highest reproductive success, though hybrid males were disadvantaged in groups where they were the minority or where female hybrids were few or absent.

In the present study, hybridization and its influence on reproductive hormones and reproductive success was studied among Amboseli baboon populations comprising of yellow baboon (*P. cynocephalus*) and those with yellow-anubis baboon admixture/ hybrids with the aim to understand the possible differences in the evolutionary fitness between the yellow and hybrid baboons. Specifically, this study compared relative reproductive success of yellow baboons and hybrids in terms of birth rates, miscarriage rates and reproductive hormone profiles. The main research question was "do hybrid female baboons have reproductive advantage over non-hybrid females"? An earlier study on Amboseli baboons showed that male hybrid baboons have higher reproductive success than non-hybrid yellow males (Charpentier *et al*., 2008), as determined by courtship rates and paternity testing; but whether this is also true for females is an important gap in the scientific knowledge of this species.

1.2 RESEARCH OBJECTIVES

1.2.1 GENERAL OBJECTIVE

To examine differences in the reproductive hormones and reproductive success of the female *P. cynocephalus* baboons and yellow-olive hybrids.

1.2.2 SPECIFIC OBJECTIVES

The specific objectives were to:

i. Estimate variation in spontaneous miscarriages among female baboons with different degrees of genetic admixture using external signs of blood stained perineum in baboons initially presumed pregnant and with presence of dead fetuses and existing miscarriage data.

ii. Investigate variation in birth rates among female baboons with varying degree of genetic admixture as well as non-hybrid females using observed live birth records and secondary birth data.

iii. Evaluate estrogen and progesterone hormone profiles among female baboons in relation to their degree of admixture through fecal sample assays and through use of existing secondary hormonal profile data.

1.3 HYPOTHESIS

1.3.1 NULL HYPOTHESES.

i. Miscarriage rates do not differ between pure female baboons (*P. cynocephalus*) and female hybrid baboons in Amboseli baboon population.

ii. Birth rates are the same between pure female baboons (*P. cynocephalus*) and female hybrid baboons in the Amboseli baboon population.

iii. There is no difference in estrogen and progesterone levels between pure female baboons (*P. cynocephalus*) and female hybrid baboons in Amboseli baboon population.

1.4 JUSTIFICATION

It is envisaged that this research will contribute valuable scientific knowledge for researchers involved in primate studies, especially with regard to hybrid reproductive performance *vis-a-vis* the parental species. Though extensive research work has been done on male hybrid baboons reproductive performance in Amboseli, there is a paucity of such work on the female hybrid reproductive performance. A large amount of data on male and female baboons' reproductive and

demographic events has been collected and stored in the Amboseli Baboon Research Project database. This study was authorized to retrieve female data from this database to augment the first-hand data for this study. Together, these data made it possible to expound on possible hybrid speciation and biodiversity in the study subjects.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 HYBRIDIZATION INSTANCES AND CONCEPTS IN RELATION TO REPRODUCTIVE SUCCESS.

Hybridization may facilitate the evolutionary diversification in animals (Charpentier *et al*., 2012; Arnold, 2006; Schwarz *et al*., 2005; Grant *et al*., 2005; Arnold, 1997) and plants (Rieseberg, 1997), including ecological diversity as well as the origin of new species (Charpentier *et al*., 2012; Grant *et al*., 2005; Schwarz *et al*., 2005). There are existing models that predict its outcomes (Harrison, 1986) and may explain hybridization episodes. The bounded hybrid superiority model explains the pattern by which hybrids are more reproductively fit than their parents (Charpentier *et al*., 2012; Endler, 1977; Moore, 1977). In contrast, the Mosaic model (Howard, 1986) explains the production of less fit individuals relative to their parental populations. This pattern can be due to unbalanced genetic introgression from one taxon to another. The tension zone model explains that the hybrid zone experiences selection against hybrid genetic material and dispersal of parental genes into the hybrid zone, and this stabilizes such zones (Barton, 1979). This means that hybrids can perform better, in equal measures, or less than parental forms (Arnold and Hodges, 1995). Conversely, according to Wilson (1965), hybridization processes and outcomes are only transient and might lead to intensification of hindrances to reproduction and amalgamation of populations engaging in hybridization episodes.

Through introduction of new genetic variation and gene combinations, hybridization may influence the prospects of evolution in the parental population, the hybrid population, or in all of the

interacting populations (Tung *et al*., 2008; Rieseberg *et al*., 2003). This might bring effects arising from evolution, which are dependent on the fitness of admixed individuals, genetic separation of parental species, and inter-species mating rates (Tung *et al*., 2008). High fitness in hybrids is an indication that hybrids are superior, while less fit hybrids portray selection against hybrids in a tension zone (Barton, 2001). This is beneficial in balancing hybrid species zone ranges and prevent the impacts of continuous hybridization between populations (Tung *et al*., 2008; Barton, 2001; Grant and Grant, 1992). If hybrid and parental species are both fit and reproductively successful, hybrids might be a manifestation of ongoing species unification (Tung *et al*., 2008; Salzburger *et al*., 2002; Rhymer and Simberloff, 1996).

Hybridization in wild primates has been studied in various species (Charpentier *et al*., 2012; Schilasi *et al*., 2007; Arnold and Mayer, 2006; Detwiler *et al.*, 2005; Bynum, 2002; Supriatna *et al*., 1992). It commonly occurs during wild primate divergence (Arnold and Mayer, 2006; Arnold, 1997) in their contact zones (Charpentier *et al*., 2012). Primate dispersal can involve males only, females only, or both sexes; in turn, these patterns determine the occurrence of hybridization and its processes (Pusey and Packer, 1987). In these events, it has been found that females associate more with males of similar phenotypic characteristics (Charpentier *et al*., 2012; Bergman and Beehner, 2003). Similar findings (Insel and Shapiro, 1992; Fuentes and Dewsbury, 1984; Foltz, 1981) support that the evolution of mate preference occurs rapidly in taxa during divergence (Charpentier *et al*., 2012; Alberts, 1999; Alberts and Altman, 1995). Alteration in male partner preferences as well as in dispersal behavior determines the frequency and individuals that take part in hybridization, which together determine hybridization's evolutionary impact (Charpentier *et al*., 2012).

Hybridization is well studied and characterized in African baboon *Papio* genus (Charpentier *et al*., 2012; Beehner and Bergman, 2006; Samuels and Altman, 1986). This genus is a preferred model for investigating forces that maintain taxonomic integrity in the face of genetic admixture (Zinner *et al*., 2013; Tung *et al*., 2012; Alberts and Altman, 2001). The genus *Papio* is composed of six species that live mainly in allopatry and possess distinguishing morphological features (Tung *et al*., 2012; Jolly, 1993). These species have distinctive behavioral characteristics and are vastly spread across Africa (Tung *et al*., 2012). They include: anubis baboon (*P. anubis*), yellow baboon (*P. cynocephalus*), kinda baboon (*P. kindae*), hamadryas baboon (*P. hamadryas*), chacma baboon (*P. ursinus*) and guinea baboon (*P. papio*) (Jolly, 1993). The *P. anubis* (also known as olive baboon), *P. cynocephalus*, *P. anubis*, *P. kindae* and *P.ursinus* are known to live in large multi-male, multi-female social groups with male biased dispersal, while *P. hamadryas* and *P. papio* baboons live in one-male, multi-female groups with nested multi-level social groups, where females are the dispersing sex (Fischer *et al*., 2017).

In Amboseli, hybridization between yellow and olive baboons was first observed in the 1980s (Charpentier *et al*., 2012; Samuels and Altman, 1986) but it may have a long history in the region (Wall *et al*., 2016). The gradual increase in *P. anubis* genetic composition in Amboseli has been monitored closely from that time (Charpentier *et al*., 2012; Tung *et al*., 2008). The occasionally observed immigration of *P. anubis* baboons into the *P. cynocephalus* inhabited Amboseli has resulted in a population that is largely composed of *P. cynocephalus* baboons with a sizable and continuously increasing fraction of hybrid baboons and a smaller number of individuals with greater *P. anubis* ancestry than *P. cynocephalus* ancestry (Tung *et al*., 2012; Alberts and Altman, 2001). However, *P. cynocephalus* and *P. anubis* baboons are known to occupy different

geographical ranges with *P. cynocephalus* to the southern and eastern regions, while *P. anubis* to the northern and western regions of Amboseli National Park (Tung *et al*., 2008; Jolly, 1993). The two populations may only exist together within a hybrid zone that runs narrowly between those ranges (Tung *et al*., 2012; Tung *et al*., 2008; Newman *et al.*, 2004; Kingdon, 1997; Jolly, 1993). These two species are easily differentiated by their phenotypic characteristics: *P. anubis* baboons are robust in build with darker fur, while *P. cynocephalus* baboons are gracile and yellow and hybrids show intermediate characteristics (Alberts and Altman, 2001). The two species mate and produce fertile hybrid offspring, (Tung *et al*., 2012).

Animal life history behaviors are subject to genetic influence (Tung *et al*., 2012; Charpentier *et al*., 2012). Therefore, the combination of diverse genes in the admixtures may project varying behavioral characteristics in the hybrid animals (Tung *et al*., 2012). Baboons with high *P. anubis* genetic ancestry in Amboseli mature earlier than baboons with *P. cynocephalus* genetic backgrounds (Tung *et al*., 2012; Ackermann, 2006). The effect of admixture is more pronounced in males compared to same-age group females; the more *P. anubis*-like males are characterized by younger ages at dispersal compared to *P. cynocephalus* baboons (Charpentier *et al*., 2008; Alberts and Altman, 2001). The courtship activity rates in more *P. anubis* male baboons is higher than that in the more *P. cynocephalus* baboons (Charpentier *et al*., 2008). In female baboons, lifetime reproductive performance is subject to genetic factors, environmental quality, and female position in the hierarchy of dominance (Altman and Alberts, 2003; Altman *et al.*, 1988). A female's ability to reproduce is subject to gradual variation with age of the specific female baboons (Tung *et al*., 2012; Beehner *et al*., 2006), and infants' growth as well as survival rate depend on the female's previous reproductive events (Altman and Alberts, 2005). A female's dominance rank influences

reproduction in many ways. High-ranking females are more successful in raising surviving offspring, reach menarche earlier, and start reproducing earlier than low-ranking females (Charpentier *et al*., 2012; Altman and Alberts, 2005; Altman *et al*., 1988).

2.2 REPRODUCTIVE EVENTS IN BABOONS.

Female baboons manifest their first signs of sexual maturity at a median age of 4.5 years (Altman *et al*, 1977), with alterations in the perineal sex skin occurring prior to the first menstrual bleeding (Castracane *et al*., 1981). The menstrual cycle of baboons varies among species, with *P. anubis* female baboons averaging 38 days (Packer *et al*., 1998), while that of *P. cynocephalus* averaging 32 days (Wasser, 1996). Menstrual bleeding lasts about three days on average (Gesquiere *et al*., 2007; Packer *et al*., 1998). There are seven characteristic phases of baboon menstrual cycle, with every phase having unique cell types and compositions, including basal cells, parabasal cells, superficial cells and intermediate cells (Hendrickx, 1971). Among the seven phases, follicular phase is characterized by high estrogen hormone levels (Gesquiere *et al*., 2018; Gesquiere *et al*., 2007), and is externally manifested by turgescence of the perineal sexual skin (Hendrickx, 1971; Altman, 1970). The largest sexual skin swelling size occurs after two weeks of gradual increase in the swelling process, lasts for an average of 5 days (Wildt *et al*., 1977), and coincides with ovulation (Gesquiere *et al.*, 2018). This period is characterized by bright red, shiny and highly enlarged sexual skin (Wildt *et al*., 1977) and intensive mating activity (Gesquiere *et al*., 2007). Swelling is followed by a gradual deturgescence of the sexual skin, which is observable for an average of 6 days (Stevens, 1997). This marks the luteal phase of the reproductive cycle, when estrogen levels decrease and progesterone levels increase in anticipation for conception and subsequent maintenance of pregnancy (Gesquiere *et al.*, 2007). During the swelling regression, the sexual skin

turns gradually from grayish-red to dull, loosening, flattening, and wrinkling in the process, which may be ensued by peeling off the epithelial parts (Hendrickx, 1971; Gesquiere *et al*., 2018). Menstrual bleeding in absence of conception follows swelling regression (Gesquiere *et al.,* 2018; Gesquiere *et al*., 2007). Female baboons with delayed menstruation after maximal sexual skin swelling are presumed to have conceived (Altman *et al.*, 1988; Altman *et al*, 1970). Pregnancy occurs at an average of 6 years of age (*P. cynocephalus*) (Rhine *et al*., 2000; Altman *et al*, 1988; Altman *et al.*, 1977). The gestation period ranges between 22 and 27 weeks (Altman *et al*., 1977).

A recent study by Gesquiere *et al*. (2018) fully mapped the inter-birth interval and its component phases and factors causing its variability between female baboons and reported a gestation period (157 to 194 days), roughly equal to that which was earlier recorded (Altman *et al*., 1977). Cycling periods for various females range from 18 to 590 days, while postpartum amenorrhea ranges between 71 and 635 days. The total inter-birth interval averages 22 months following birth of a healthy infant that survives for at least 52 weeks (Gesquiere *et al*., 2018; Altman *et al*., 1977). The interbirth interval is under the influence of prevailing conditions of the habitat, such as drought, and the body physiology of the female and infant survival (Altman *et al*., 1977). It has, however, been demonstrated that the interbirth interval is brief in colonies that are under human control (Altman *et al*., 1977). The probability to conceive increases with age in baboons and then progressively wanes, with an average of five surviving offspring during the reproductive lifetime (Altman *et al*., 1977). These species under present study can breed, mate, and give birth throughout the year; hence seasonal breeding does not hinder cross species mating and subsequent birth of offspring.

2.3 ROLE OF ESTROGEN AND PROGESTERONE IN BABOON REPRODUCTION.

Estrogen is responsible for the development of perineal sexual skin swelling (Gesquiere *et al*, 2018; Gesquiere *et al.*, 2007; Gillman, 1942), which in turn plays role in sexual interest among males (Gesquiere *et al.*, 2018; O'Neill *et al.*, 2004; Snowdon, 2004; Singh and Bronstad, 2001). Sex skin development is also significant in female sexual behavior (Engelhardt *et al*., 2005; O'Neill *et al*., 2004). During early pregnancy, estrogen initiates the synthesis of progesterone in the corpus luteum and utero-placental circulation of blood (Pepe and Albrecht, 1995). Progesterone is responsible for myometrium quiescence and subsequent embryo implantation (Pepe and Albrecht, 1995). The role of the corpus luteum in progesterone production is later taken over by the placenta from approximately days 20–25 of baboon pregnancy (Castracane and Goldzieher, 1986). Placental progesterone production is necessary in maintenance pregnancy to term (Pepe and Albrecht, 1995).

Experimental findings have shown that suppression of circulating maternal estrogen reduces chances of gestation by half in a population of baboons (Albrecht *et al*., 2000). A C-19 steroid (androgen) produced by the adrenal cortex of the fetus and conveyed to the placenta is a critically important substrate for the formation of estrogen, and this process is solely characteristic to primate pregnancy within animal kingdom (Novy, 1977). There is therefore a relatively linear increase in estrogens with advancing gestation in most primate species (baboons: Albrecht and Townsley, 1978; humans: Tulchinsky et al., 1972). This means that pregnancy estrogens are higher in primates than cycling and postpartum amenorrhea estrogens. Compromises in the feto-placental unit in the course of gestation may lead to impairment of the process of estrogen synthesis (Beehner *et al*., 2006; Novy, 1977). Surgical removal of the fetus while retaining the placenta *in utero* leads to 32.5% decrease in maternal progesterone levels, while estrogen declines to extremely low values

(Albrecht and Pepe, 1985). The biosynthesis of steroids by the ovary and the resulting hormone concentrations are influenced by stressors facing the pregnant animal (Beehner *et al*., 2006; De Catanzaro and Macuiren, 1992). Hence, it is important to model these stressors and predictor variables to understand the degree to which the female's environment alters her reproductive hormone levels.

2.4 BABOON POST IMPLANTATION MISCARRIAGES.

Post implantation miscarriage (fetal loss) is common in baboons and is mainly due to inadequate nutrition, resulting from low food availability during long, intense dry seasons. Stress from heat and low resources determines the likelihood of cycling, chances of conception, live births, and offspring survival (Beehner *et al*., 2006). The effects of nutritional deficiency on pregnancy have been demonstrated in common marmosets, where inadequate food leads to a gradual drop in blood cortisol, fetal death, and the termination of pregnancy (Tardif *et al*., 2005). Studies by Beehner *et al*. (2006) on ecology of conception and fetal loss of wild baboons showed that reproductive failure is brought about by prevailing ecological conditions, especially drought, culminating in fewer cycles, fewer conceptions, and high rates of fetal death *in utero*. Miscarriage is likely to double in baboons if conception occurs immediately after the drought conditions. It has been found that intense heat prior to estrus can also lead to a loss of the conceptus (Beehner *et al*., 2006). Females in smaller social groups are less affected by drought conditions compared to those in larger groups, probably because of feeding competition in large groups. Generally, the events surrounding female reproduction are influenced by a deficit between ecological conditions prevailing around the conception period and demography of the group.

Studies have shown that fetal loss leads to a decrease in maternal fecal estrogen concentrations from the gestation stage when the loss occurs (Beehner *et al*., 2006; Tardif *et al*., 2005). Fetal death is also accompanied by an abnormal decrease in progesterone concentrations in mothers (Albrecht and Pepe, 1990). A study in Amboseli has approximated a fetal loss rate of 91 losses out of 656 pregnancies, which is 13.9% (Beehner *et al*., 2006). This fetal loss rate is high compared to that reported from other studies of wild *P. cynocephalus*, which estimate 10% pregnancy loss (Wasser, 1995), and wild *P. anubis*, which estimate 9.6% loss (Packer *et al.*, 1995). Understanding fetal loss rates and the physiological changes following fetal loss, such as decline in steroid hormone levels, are important for this study as it tries to model role of hormonal profiles on miscarriage rates.

2.5 FACTORS THAT AFFECT REPRODUCTIVE SUCCESS.

An organism's evolutionary fitness is determined by their lifetime reproductive success, and variation in lifetime reproductive success between individuals lends an insight into evolutionary processes (Falconer and Mackay, 1996). The reproductive success of wild female primates is under the influence of both social and ecological factors alongside female innate attributes. Among ecological factors are daily temperature and rainfall. Rainfall and food availability are positively correlated. Food availability has been shown to limit female reproductive success through its influence on chances of ovulation (Emery and Wrangham, 2008). Food availability has also been shown to hamper conception in *Macaca assamensis* (Heesen *et al*., 2013) and *Presbytis entellus* (Koenig *et al*., 1997). High conceptions in baboons and their close relatives have been associated with food availability owing to plenty of rainfall (Dunbar 1980). Food availability has been shown to influence pregnancy outcome in *Callithrix jacchus* (Tardif *et al*., 2004) and *Papio hamadryas* (Antonow-Schlorke *et al.,* 2011). Food availability has a positive correlation with reproductive

success in females of many primate species (*P. cynocephalus*: Altman and Alberts, 2003; *Pongo pygmaeus*: Knott, 2001; *Macaca fascicularis*: Van Noordwijk and van Schaik, 1999). In order to avoid a negative energy balance, female baboons first meet their basic metabolic requirements for maintenance before channeling energy to reproduction. Reproductive output is thus dependent on the maternal body mass and condition. Prolonged high ambient temperatures suppress spermatogenesis in mature males, reducing the number of fertilizations in hotter seasons (Setchell, 1998). In sexually mature females, heat stress has been shown to suppress ovulation and conception (Bronson, 1989; Baumgartner and Chrisman, 1987). High heat stress during gestation terminates the pregnancy, with most of its impacts on embryo survival occurring prior to implantation; thus maternal exposure to high temperatures increases fetal loss rates (Bronson, 1989).

Certain social factors impose their effects on female reproductive success. Among the social factors, intra-group competition has been shown to influence reproductive success in primates (Chapman *et al*., 2012; Altman and Alberts, 2003; Rhine *et al.*, 1988; Wasser and Starling, 1988; Bulger and Hamilton, 1987). The main cost is elevated within-group feeding competition. Larger groups forage over long distances to meet the energetic requirements by individuals in the group (Bronikowski and Altman, 1996; Chapman *et al*., 1995), coupled with increased intra-group feeding competitions that results from direct contests over food resources and indirect effects of quick food depletion (Silk, 2007; Snaith and Chapman, 2007). Group size and high competition also contribute to increased time spent foraging (Bronikowski and Altman, 1996). High intra-group competition negatively influences female reproductive success in several primate species (baboons: Altman and Alberts 2003; *Alouatta pigra*: Van Belle and Estrada, 2008). Group composition as well affects reproductive success, where more females attract extra group males

thus leading to frequent male-male competition for mates and group take over by the extra group males. The males who successfully take over such groups induce high infanticide (Cheney *et al*., 2004; Treves and Chapman, 1996), hence lowering female reproductive success. Groups with more males, who are aggressive enough to counteract takeover attempts, have higher female reproductive success (*Cebus capucinus*: Fedigan and Jack, 2011; mantled howler monkeys: Ryan *et al*., 2008). Maternal rank also affects female reproductive success as a social factor. Dominant females have priority of access to food and hence higher reproductive success compared to low ranking females (Pusey, 2012). High rank may favor the production of more daughters due to higher maternal investment on their daughter's reproductive success compared to sons (Simpson and Simpson, 1982; Altman, 1980).

Maternal parity, age status, and the specified female's experience in rearing infants also contribute to reproductive success (Altman and Alberts, 2005; Silk, 1990). Age brings variation in reproductive success based on tradeoffs between investment in growth and reproduction (Setchell *et al*., 2002). Younger and primiparous mothers who have not achieved full body size or mastered skills required for efficient foraging during infant care recover slowly from the energetic demands of pregnancy, and their first-born infants experience higher mortality compared to later-born infants (Altman and Alberts, 2005; Altman *et al*., 1988). Old females may be experiencing reproductive senescence, and exhibit less regular cycles and higher rates of conceptive failure with hormone profiles that are significantly different from that of younger females (baboons, Papio spp.: Altman and Alberts, 2003; Packer *et al*., 1998; lions, Panthera leo; Packer *et al*., 1998; tamarins, *Saguinus spp*.: Tardif and Ziegler, 1992). The effect of parity, however, is short lived and attenuates

after first birth such that effects of parity are seen when comparing primiparous to multiparous mothers (Altman and Alberts, 2005).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 STUDY AREA.

This study was carried out in Amboseli National Park located in an ancient lake basin at the base of Mount Kilimanjaro between Kenya and Tanzania (Fig. 1). Amboseli has a latitude of 2^0 40´S, longitude of 37^0 15´E and altitude of 1100 meters above sea level. This region is a semi-arid short grass savannah ecosystem that experiences highly seasonal low rainfall (Gesquiere *et al.*, 2018; Alberts *et al.*, 2005). The area experiences about 5 months of dry season spanning from June to October (Alberts *et al.*, 2005), which is followed by a 7-month wetter season whose rainfall is extremely variable and unpredictable across months (Gesquiere *et al.*, 2018). Generally, rainfall is highly variable from one year to the next, ranging between 141 mm and 757 mm (Alberts *et al.*, 2005; Altman *et al.*, 2002).

Ambient temperature is less variable compared to rainfall with a monthly average temperature of 33.1^{o}C, while daily maximum can go above 40^{o}C in the shade, which is likely to be lower than the perceived temperature in the open fields (Gesquiere *et al.*, 2011). The present study focused on wild female yellow baboons (*Papio cynocephalus*) and hybrids between *P. cynocephalus* and *P. anubis*. The Amboseli baboon has been under study for close to 5 decades, as earlier reported (Alberts and Altman, 2012; Altman and Alberts, 2003). All the animals in study groups are individually known and monitored on near-daily basis for demographic and life history events.

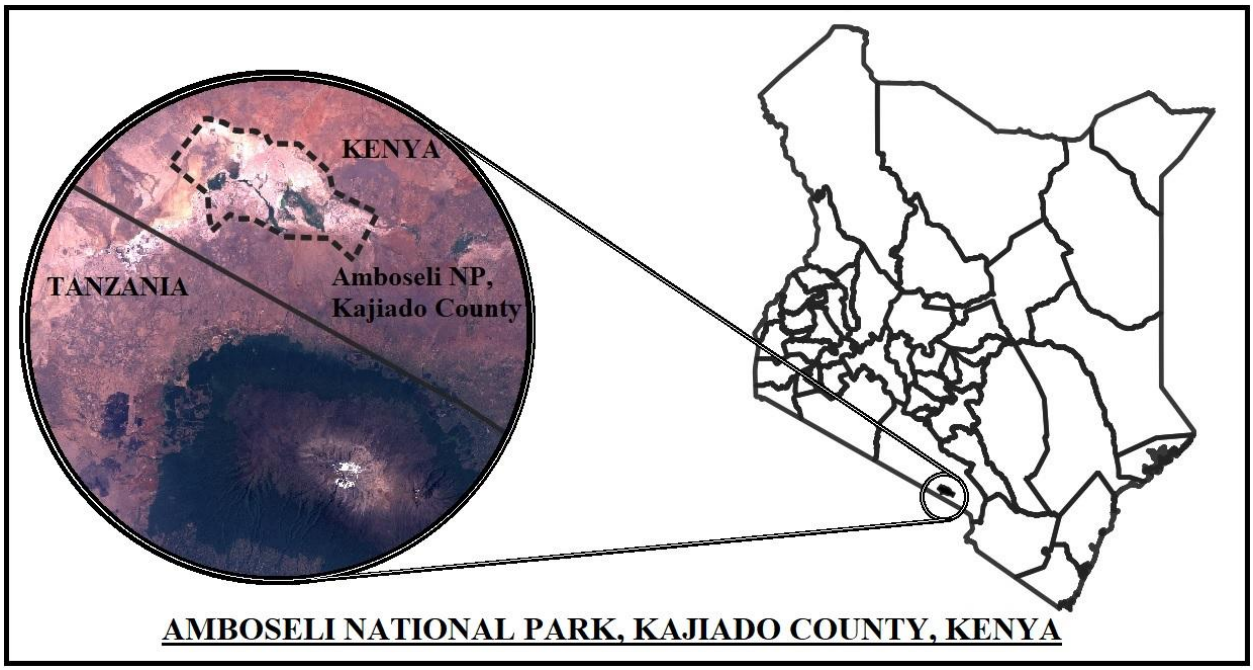

Fig 1: Map of Kenya showing the location of Amboseli National Park.

3.2 GENETICS AND REPRODUCTIVE SCORING OF BABOON

3.2.1 GENETIC SCORES OF BABOONS

Phenotypic appearance alone is not sufficient to unambiguously classify the species or hybrid status of the female baboons, given the nature of genetic exchange that takes place between the *P. cynocephalus*, immigrant *P. anubis* baboons, and the hybrid baboons. Some animals are hard to classify as *P. cynocephalus* or hybrids based on the phenotypic appearance only. The phenotypic scores vary from *P. cynocephalus* to more *P. cynocephalus* hybrids, intermediate hybrids to more *P. anubis* hybrids, with no pure *P. anubis* baboons. There was, therefore, a need for genetic scoring. This has been done using the Amboseli Baboon Research Project (ABRP) genotype data, generated by collaborating researchers (e.g. Tung *et al.*, 2008). Study individuals were genotyped at 14

microsatellite loci and the resulting genotype data were used to generate individual genetic hybrid scores using admixture analysis implemented in the program structure 2.0 (Falush *et al.* 2003). Baboons were assigned genetic scores such that individuals with lower 90% credible intervals greater than 0.05 (hybrid lower confidence) were considered hybrids while those with the lower confidence interval less than 0.05 were considered to be pure *P. cynocephalus* baboons.

3.2.2 REPRODUCTIVE SCORES OF BABOONS AND OTHER VARIABLES.

Female baboons were observed in the field; through tracking of baboons continuously from 6.30 a.m. to 11.30 a.m. on a morning shift day and from 1.30 p.m. to 6.30 pm on the afternoon shift from Monday to Friday while on Saturdays only morning shift was considered. Baboons were followed unobtrusively without interference with their daily activities including grooming, feeding, copulations, and their journey to water points. Other variables such maternal parity, age, dominance rank were also collected through observation, group size data were collected through census while daily rainfall and daily maximum temperature were collected at the camp near baboon home ranges using rain gauge and thermometer respectively.

1) *Ovarian cyclicity*. During this surveillance period, females were scored for reproductive state and ovarian cyclicity as manifested by the sexual skin swelling and changes to the paracallosal skin (Gesquiere *et al.,* 2007). For ovarian cyclicity, females were scored by the sexual skin state (whether or not the sex skin was turgescent, deturgescent, or flat) and size on a scale of 0 (T0, skin is flat) to 10 (T10, very large swelling), (Fig. 2). Various females were at different stages of ovarian cycle as observed from the size of the swelling based on the scale above.

2) *Pregnancy determination*. Baboons were observed for sign of pregnancy that is pink paracallosal skin (Altman, 1973), (Fig.3).

3) *Miscarriage assessment*. Pregnant female baboons show Pink paracallosal skin 3–4 weeks after conception (Alberts *et al*., 2016; Altman, 1973) which is the pregnancy sign. However, in cases of presumed miscarriage, this sign of pregnancy was lost and sometimes blood was observed on the female's perineum. After a successful gestation and parturition, the sex skin gradually becomes black in color (Altman *et al.*, 1970) and the mother is seen with an infant.

4) *Lactation state*. Lactation was observed and determined by the sight of a suckling infant (Fig. 4).

In total, 17 female baboons were scored as pregnant, 31 cycling, 18 lactating, 2 miscarried and 1 menstrual bleeding.

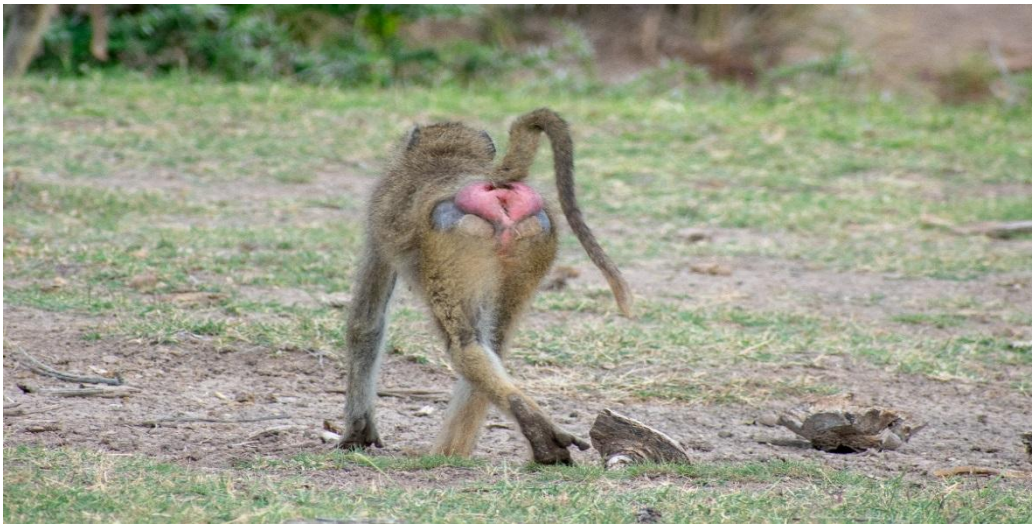

Fig 2: Sexual skin swelling (T5) in cycling female baboons (*P. cynocephalus*) in Amboseli National Park, Kenya. Photo: Emily Nonnamaker

Fig.3: The pink paracallosal skin (pregnancy sign) in *P. cynocephalus* baboons in Amboseli National Park, Kenya. Photo: Emily Nonnamaker**.**

Fig 4. A female lactating baboon (*P. cynocephalus*) carrying her infant on her back in Amboseli National Park, Kenya. Photo: Emily Nonnamaker.

3.4 FECAL SAMPLE COLLECTION, PRESERVATION AND PROCESSING

The procedures described below for fecal sample collection, preservation, storage and extraction were performed in accordance with those described previously by Lynch *et al* (2003) and Khan *et al* (2002) and referred to as the Altman laboratory procedures.

3.4.1 SAMPLE COLLECTION

Fecal samples were collected from 48 free-living female baboons in Amboseli for purposes of determining hormonal variations during cycling, pregnancy and postpartum amenorrhea. Known baboons were randomly observed in the process of defecation and freshly dropped fecal samples were carefully collected using a wooden stirrer within minutes following defecation, placed in a small waxed paper cup, mixed, and aliquots placed in plastic 20 ml vials pre-filled with 95% ethanol in a ratio of 2.5:1 of ethanol to feces. The plastic vials were labeled at both the top and side for baboon identity, sampling date, and time of collection using alcohol resistant marker.

3.4.2 SAMPLE PRESERVATION AND FINAL TRANSPORTATION

Samples were stored in charcoal evaporation refrigerator at approximately 20^{o}C in Amboseli for up to 2 weeks and thereafter transported to University of Nairobi, Reproductive Biology Unit (RBU) laboratory for further processing.

3.5 LABORATORY SAMPLE PROCESSING AND PRESERVATION

3.5.1 ETHANOL EVAPORATION

At the Reproductive Biology Unit laboratory, the caps were removed and the vials placed in a fume hood chamber for ethanol evaporation, during which, the samples were stirred at intervals of 2 h to ensure complete and uniform drying of samples. The water content of the fecal sample was high

for a few samples; therefore, it took 48 h for complete ethanol evaporation and drying of samples. Samples were crunchy when stirred on the second day of drying, which was an indicator of complete drying. One sample, however, took a little longer to completely dry. Thereafter, sample vials were re-capped and stored at -20^{0}C (Fig. 5) until freeze-drying.

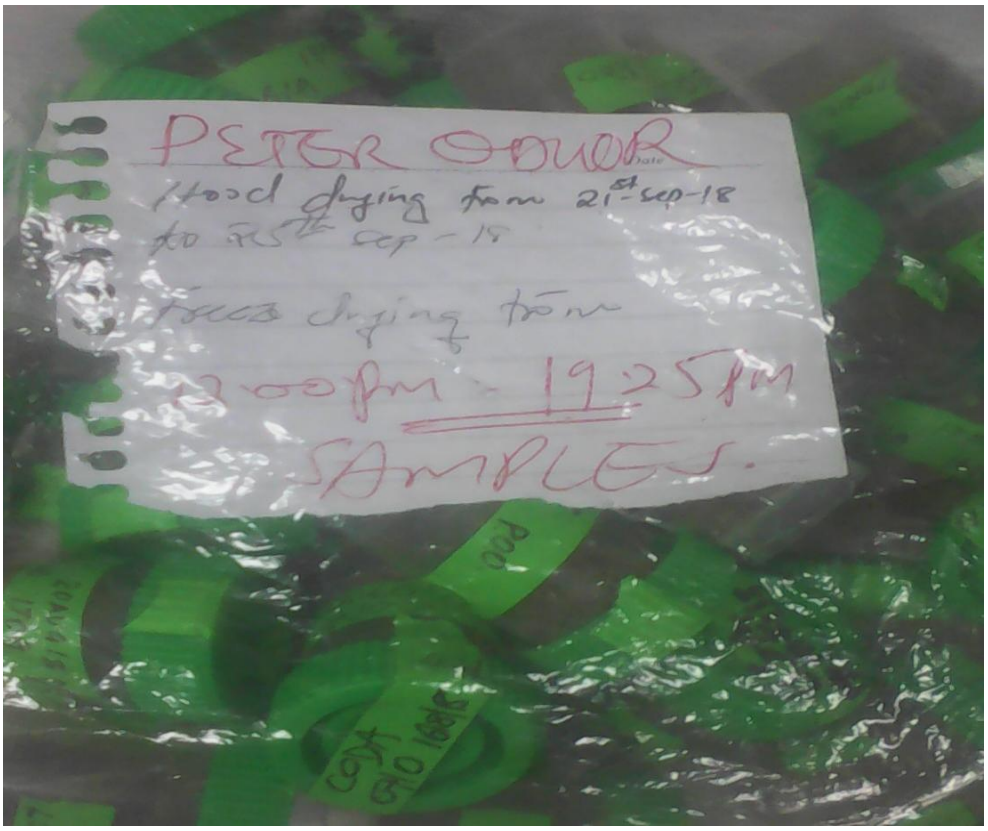

Fig 5: Hood-dried samples in labelled plastic vials at the Reproductive Biology Unit (RBU) laboratory, University of Nairobi. Photo: Peter Oduor.

3.5.2 FREEZE DRYING OF SAMPLES

The vial caps were removed and 7 cm^2 pieces of cotton gauze fastened over the vials using rubber bands, followed by loading into freeze-drying flasks and thereafter in a freezer to cool down to -

20^0C for 30 min (Fig. 6). The cold samples were freeze-dried for 8 h at -50^0C and vacuum of 30 millitorrs. After freeze-drying, the samples were offloaded and cotton gauze pieces were removed and discarded, and the sample vials were re-capped. The freeze-dried samples were stored at -20^0C prior to sifting.

Fig 6: Samples in freeze-drying flasks mounted onto the freeze-drier at RBU. Photo: Peter Oduor.

3.5.3 SAMPLE SIFTING AND WEIGHING

The freeze-dried fecal samples were sifted through a mesh tea strainer of approximately 40 mesh on a 10.16 cm by 10.16 cm diagonally folded weighing paper. Each sample was strained one at a time using the tube brim of the empty vial to break fecal samples while sifting through strainer

mesh under humid area to keep airborne fecal dust at minimum. Very dry and fine fecal powder was collected during each sample straining and transferred back into the collection plastic tubes for storage at -20^{0}C. Between each sample, the strainer was cleaned using 70% ethanol to avoid contamination. Samples were weighed on diagonally creased weighing paper placed on a high precision weighing balance (Denver instruments Co. A-250, made in United States). Fecal samples (0.2 g) were weighed and weight recorded on a data sheet. The weighed samples were placed into 16 x 100 mm test tubes identified by baboon name using alcohol resistant marker. Tubes were capped loosely and placed on a rack. Weighed samples in tubes were stored at -20^{0}C before extraction. The empty vials and weighing paper were discarded in the biohazard waste bin.

3.5.4 METHANOL EXTRACTION

Into each sample tube, 2 ml of 90% methanol was added using repeater pipette. The tubes were capped tightly and placed on multi-pulse vortex machine and vortexed for 30 min at motor speed of 70 and pulse of 60. The sample-methanol mixture was centrifuged at 2300 rpm for 20 min at 25^{0}C and thereafter the supernatant aliquoted into 1.5 ml Eppendorf tubes. The supernatant was centrifuged at 3200 rpm for 15 min. The final supernatant was transferred into 2 ml microcentrifuge tubes, closed using O-ring caps to avoid evaporation and subsequently stored at -20^{0}C.

3.6 SEX STEROID HORMONE ASSAYS.

Fecal estrogen and progesterone were analyzed by enzyme immunoassays (EIA) following the manufacturer's guidelines and procedures. In this study, the quantitative determination of reproductive hormones was performed using an EIA test kit sourced from MP Biomedicals,

diagnostic division Orangeburg (United States), NY 10962-1294, and catalog numbers 07BC-1111 and 07BC-1113 for estrogen and progesterone respectively.

3.6.1 PRINCIPLE OF THE ENZYME IMMUNOASSAY TEST

This analytical test detects the presence of a ligand in a liquid sample using antibodies. The antigen (hormone) from the sample is attached on the inside surface of the wells of a microtitre plate coated with an antibody, and then a second, specific antibody, linked to an enzyme, is added into the wells to bind the antigen, which in this case is the hormone. Substrate is added and the ensuing competitive reactions produce a detectable color change that is then read in the ELISA reader at a given wavelength. The intensity of the final color is directly proportional to the amount of unknown antigen (hormone) in the test solution.

3.6.2 ASSAY PROCEDURES FOR ESTRADIOL

Reagents were allowed to equilibrate to approximately 25˚C, followed by careful swirling to mix thoroughly without foaming before use. Goat anti-rabbit immunoglobulin G (primary antibody)-coated wells of each EIA plate were assigned as follows: 76 wells for samples, 10 wells for the Estradiol reference standards (0, 10, 30, 100, 300 and 1000 pg/ml), 8 wells for the Estradiol quality controls and blank (2 wells). Twenty- five (25) µl of each sample, standards and controls were dispensed into designated microtitre-wells in duplicates. One hundred (100) µl of Estradiol-HRP conjugate reagent was dispensed into each micro-well, followed by 50 µl of rabbit anti-Estradiol reagent (the secondary antibody). The micro-well contents were thoroughly mixed for 30 seconds and thereafter incubated at room temperature for 90 minutes. The microtitre-wells were rinsed 5 times using distilled water and flicked at each rinse, and thereafter 100 µl of TMB reagent dispensed

into each well and mixed gently for 10 seconds. The microtitre-well contents were incubated at room temperature for 20 minutes and the reaction stopped by dispensing 100 µl of stop solution (1N HCL) into each micro-well. Micro-well contents were mixed gently until all the blue color changed to yellow. The optical density was measured spectrophotometrically at 450 nm using microplate well reader within 15 minutes of processing and estradiol standard curve generated (Fig 9).

3.6.3 ASSAY PROCEDURES FOR PROGESTERONE

Reagents were allowed to equilibrate to approximately 25°C, followed by careful swirling to mix thoroughly without foaming before use. Goat anti-rabbit immunoglobulin G (primary antibody)-coated wells on each EIA plate were assigned as follows: 76 wells for samples, 10 wells for the progesterone reference standards (0, 0.5, 3.0, 10, 25 and 50 ng/ml). Eight wells for progesterone quality controls and 2 wells for blanks. Twenty-five (25) µl of each sample, standards and controls were dispensed into designated microtitre-wells in duplicates. One hundred (100) µl of Progesterone-HRP conjugate reagent was dispensed into each microtitre-well followed by 50 µl of rabbit anti-Progesterone reagent (the secondary antibody). The microtitre-well contents were thoroughly mixed for 30 seconds and thereafter incubated at room temperature for 90 minutes. The microtitre-wells were rinsed 5 times using distilled water and flicked at each rinse, and 100 µl of TMB reagent was dispensed into each well and mixed gently for 10 seconds. The micro-well contents were incubated at room temperature for 20 minutes and the reaction stopped by dispensing 100 µl of stop solution (1N HCL) into each micro-well. Micro-well contents were mixed gently for 30 seconds until all the blue color changed to yellow. The optical density was measured

spectrophotometrically at 450 nm using microplate well reader within 15 minutes of processing and progesterone standard curve generated (Fig 10).

3.7 DATA RETRIEVAL

In addition to original data collection, the study was augmented with long-term reproductive and demographic data comprising of births, miscarriages and steroid profiles of baboons from seven study groups, which were retrieved from the Amboseli baboon project's online relational database, "Babase" (Alberts and Altman, 1995; Alberts *et al*., 1996, 2003; Altman and Alberts, 2003), See the website http://www. princeton.edu/~baboon for a complete bibliography and the Baboon Project Monitoring Guide for additional details on the data collection protocols (Altman *et al*., 1988; Pereira., 1988; Shopland., 1987). Babase is housed on a virtual server hosted by the VMWare Server Hosting facility at Duke's Office of Information Technology. The reproductive and demographic data for all females with corresponding morphological or genetic hybrid scores were retrieved from Babase using Standard Query Language (SQL) by the permission of the Amboseli baboon research leaders. The data retrieved consisted of pregnancy outcomes, interbirth intervals and hormonal profiles as the dependent variables, with maternal rank, maternal age, infant sex, group size, maternal parity, hybrid scores, habitat quality, daily rainfall, and daily maximum temperature serving as the independent variables.

3.8 SAMPLE SIZE

The present study analyzed firsthand and multi-decade data consisting of eight response variables and nine predictor variables. The multi-decade data retrieved from ABRP database was summarized in Table 1 below. The table consists of response variables for each objective as

miscarriages, births, interbirth intervals (IBIs), pregnancies (Pgs), cycles, postpartum amenorrhea (PPAs), estrogen (E_2) and progesterone (P_4). Table 1 also contains the total number of females and total number of observations for objectives 1 and 2, and the number of samples used in statistical modeling for part of objective 3. In addition, enzyme immunoassay data from the 48 fecal samples were used in objective 3.

TABLE 2. A summary of the data used in the present study

	Objective 1		**Objective 2**				**Objective 3**	
Response variable	Miscarriages	Births	IBIs	Pgs	cycles	PPAs	E_2	P_4
Number of females	217	217	188	188	188	188	204	204
Total number of observations/samples	173	1020	705	705	699	699	14139	10447

3.9 STATISTICAL METHODS

3.9.1 MISCARRIAGE RATES: TESTING THE INFLUENCE OF GENETIC AND ECOLOGICAL FACTORS

To test the influence of genetic hybridization on spontaneous miscarriage as well as other factors known to influence miscarriage, a Generalized Linear Mixed effects Model (GLMM) with a binomial error structure and a Logit link function was applied. Individual animal identity (the female's identity code) was used as a random factor. The dependent variable, pregnancy outcome, was coded with miscarriage represented by 1 and live birth represented by 0. The independent/predictor variables were hybrid score (genetic ancestry score for the female, ranging

from 0 to 1), parity (a number indicating whether this is the female's first, second, third etc. offspring), group size (the number of adult females living in the female's social group on the day of conception), habitat quality (the habitat quality score high or low), daily rainfall (the average daily rainfall in the 6 months before the female conceived), daily maximum temperature (the average maximum temperature in the 6 months before the female conceived), dominance rank (the female's dominance rank on the day of conception), and maternal age (the age of the female on the conception date).

Statistical analyses were performed using the glmmTMB package (Mollie E. Brooks, Kasper Kristensen, Koen J. van Benthem *et al*. 2017) in the R statistical environment, version 3.6.1 (2019-07-05) -- "Action of the Toes" Copyright (C) 2019 The R Foundation for Statistical Computing Platform: x86_64-w64-mingw32/x64 (64-bit), (R Core Team, 2019). The full model and all possible reduced models were performed, and the best model selected using Akaike Information Criteria (AIC) with the MuMIn package (Kamil Barton, 2019) using R software.

3.9.2 BIRTH RATE VARIATION: TESTING THE INFLUENCE OF GENETIC, MATERNAL AND ECOLOGICAL FACTORS

For this objective, this study tested predictors of interbirth intervals in the Amboseli baboons and various independent variables. The independent variables were hybrid score (genetic ancestry score for the female, ranging from 0 to 1), group size (number of adult females in the female's group on the day the offspring that started the IBI was born), daily rainfall (mean daily rainfall during the IBI) and daily maximum temperature (average daily maximum temperature during the IBI). Maternal factors were maternal rank (female's ordinal dominance rank in the month the offspring

that initiated the IBI was born), maternal age (female age on the day the infant that started the IBI was born), primiparity (if the offspring that started the IBI was the female's first offspring), infant sex (sex of the infant that initiated the IBI) and habitat quality (low or high).

The dependent variables were, interbirth interval duration (duration in days between the birth of an offspring that survived for at least 1 year, and the birth of the subsequent live offspring), postpartum amenorrhea duration (days spent in postpartum amenorrhea during this IBI), pregnancy duration (days spent in pregnancy during this IBI) and cycling duration (days spent in ovarian cycling during this IBI). Individual ID as a random effect was included to control for differences in the number of observations between female subjects. This was achieved using the statistical package glmmTMB (Mollie E. Brooks, Kasper Kristensen, Koen J. van Benthem *et al*. 2017) with Poisson errors and a log link function. Models were run with all the above covariates and Akaike Information Criteria (AIC) and stepwise model reduction implemented in the R statistical package MuMIn (Kamil Barton, 2019) were used to select the best models.

3.9.3 FEMALE REPRODUCTIVE HORMONES: TESTING THE INFLUENCE OF GENETIC, MATERNAL AND ECOLOGICAL FACTORS.

This study tested the influence of hybridity, ecological factors, and maternal factors on estrogen and progesterone levels in Amboseli female baboons. The independent variables were hybrid score (genetic ancestry score for the female, ranging from 0 to 1), ecological factors such as daily rainfall (average daily rainfall based on 30 days prior to date of sample collection), daily maximum temperature (average maximum daily temperature based on 30 days prior to date of sample collection), group size (number of adult females present in maternal group on day of sample collection), maternal factors such as maternal rank (maternal dominance rank on day of sample

collection), maternal age (maternal age on day of sample collection), parity (cardinality of the pregnancy on the date of sample collection) and reproductive state (reproductive state of the female on the date of the sample collection, pregnant, cycling or postpartum amenorrhea).

The dependent variables were estrogen (log transformed Estrogen concentration) and progesterone (log transformed Progesterone concentration). Linear mixed models (LMM) were performed using log (estrogen) and log (progesterone) as response variables and individual animal identity (the female's identity code) as a random effect. This was achieved using the statistical package LME4. For each hormone, a model with all the above covariates were run and Akaike Information Criteria and stepwise model reduction as implemented in R. MuMIn package (Kamil Barton, 2019) was thereafter used to select the best model. The Enzyme immunoassay data obtained from the 48 fecal samples were used to generate the error bar graph plots (Fig 7 and 8). The raw hormonal data were organized by individual name and reproductive phase and plotted using the ggplot2 and doBy packages in R version 3.6.1.

CHAPTER 4

4.0 RESULTS

4.1 THE INFLUENCE OF GENETIC, MATERNAL, AND ECOLOGICAL FACTORS ON MISCARRIAGE RATES IN BABOONS.

GLMMs testing all possible combinations of predictor variables indicated that none of the models had stronger statistical support than the other because their delta AIC values were all less than 2 (Table 2). The analyses thus did not find any statistically significant predictors of variation in miscarriage rates among baboons including the hybrid score (Table 3).

TABLE 2: ALL GLMMS AND THEIR COVARIATES, AICC VALUES, AND MODEL WEIGHTS FOR PREDICTING MISCARRIAGES IN BABOONS.

Model components and covariates	AICc	Delta(δ) AICc	Weight
1. Random component + Intercept + group size + parity	990	0	0.065
2. Random component + Intercept + group size	990.2	0.2	0.059
3. Random component + Intercept + parity	991.5	1.43	0.032
4. Random component + Intercept	991.6	1.6	0.029
5. Random component + Intercept + group size + habitat quality + parity	991.7	1.69	0.028
6. Random component + Intercept + daily rainfall + group size + parity	991.7	1.7	0.028
7.Random component + Intercept + daily temperature + group size + parity	991.7	1.72	0.027
8. Random component + Intercept + daily rainfall + group size	991.9	1.9	0.025
9. Random component + Intercept + daily temperature + group size	992	1.95	0.024
10. Random component + Intercept + group size + hybrid score + parity	992	1.97	0.024
11. Random component + Intercept + group size + habitat quality	992	2	0.024

TABLE 3: PROBABILITY VALUES OF PARAMETER ESTIMATES FOR THE FULL MODEL AND THE BEST MODEL PREDICTING THE INCIDENCE OF MISCARRIAGE IN BABOONS

A. FULL MODEL PREDICTING MISCARRIAGE RATES

Covariate	Estimate	Std. Error	Z	P
Intercept	-0.82113	1.718901	-0.478	0.6329
Hybrid score	-0.06556	0.326606	-0.201	0.8409
Group size	-0.0351	0.019862	-1.767	0.0772
Habitat quality (low vs high)	0.176089	0.309659	0.569	0.5696
Dominance rank	-0.00156	0.014512	-0.108	0.9142
Parity	0.009	0.027518	0.327	0.7436
Daily maximum temperature	-0.01646	0.050564	-0.326	0.7448
Daily rainfall	-0.06595	0.121177	-0.544	0.5862

B. BEST SUPPORTED MODEL PREDICTING MISCARRIAGE RATES

Model	Model covariates	Estimate	Std. Error	Z	P
1	Intercept	-1.456	0.235	-6.198	<0.001
	Group size	-0.035	0.019	-1.835	0.067
	Parity	0.009	0.027	0.313	0.754

4.2 VARIATION IN BABOON BIRTH RATES: THE INFLUENCE OF GENETIC, MATERNAL AND ECOLOGICAL FACTORS

The results of multiple models, including their AIC values are listed in Table 4. The GLMM results revealed that interbirth interval duration was longer when females were primiparous, when females had low social rank status, lived in low quality habitat, and when daily temperatures were high. Similarly, inter-birth intervals were longer when females were older and when females were living in large social groups. Hybrid score, infant sex and daily rainfall did not significantly predict the duration of interbirth intervals as shown in the full model (Table 5).

TABLE 4: ALL GLMMS AND THEIR COVARIATES, AICC VALUES, AND MODEL WEIGHTS FOR PREDICTING INTERBIRTH INTERVAL DURATION IN BABOONS.

Model	Model covariates	AICc	Delta(δ) AICc	Weight
1	Random effect + Intercept + Daily temperature + Group Size + Habitat quality + Maternal age + Primiparity + Maternal rank	13612	0	0.186
2	Random effect + Intercept + Daily temperature + Group Size + Habitat quality + Infant sex + Maternal age + Primiparity + Maternal rank	13612.5	0.55	0.141
3	Random effect + Intercept + Daily rainfall + Daily temperature + Group Size + Habitat quality + Maternal age + Primiparity + Maternal rank	13613.3	1.31	0.096
4	Random effect + Intercept + Daily temperature + Habitat quality + Maternal age + Primiparity + Maternal rank	13613.6	1.64	0.082
5	Random effect + Intercept + Daily temperature + Group Size + Habitat quality + Hybrid score + Maternal age + Primiparity + Maternal rank	13613.8	1.77	0.077
6	Random effect + Intercept + Daily rainfall + Daily temperature + Group Size + Habitat quality + Infant sex + Maternal age + Primiparity + Maternal rank	13613.9	1.87	0.073

TABLE 5: PROBABILITY VALUES OF PARAMETER ESTIMATES FOR THE FULL MODEL AND THE BEST MODEL PREDICTING INTERBIRTH INTERVAL DURATION IN BABOONS

A. FULL MODEL FOR INTER-BIRTH INTERVAL

Covariate	Estimate	Std. Error	Z	P
Intercept	5.5391	0.0629	88.09	<0.0001
Hybrid score	-0.0248	0.0462	-0.54	0.5918
Primiparity (true vs false)	0.0927	0.0054	17.21	<0.0001
Maternal rank	0.0075	0.0007	11.34	<0.0001
Daily rainfall	0.0057	0.0067	0.86	0.3913
Daily temperature	0.0227	0.0017	13.17	<0.0001
Group size	0.0009	0.0005	1.96	0.0498
Habitat quality (low vs high)	0.1960	0.0127	15.38	<0.0001
Maternal age	0.0091	0.0006	14.26	<0.0001
Infant sex (male vs female)	0.0042	0.0035	1.23	0.2192

B. BEST, SIMPLEST MODEL FOR INTER-BIRTH INTERVAL.

Covariate	Estimate	Std. Error	Z	P
(Intercept)	5.5359	0.0613	90.28	<0.0001
Primiparity (true vs false)	0.0927	0.0054	17.24	<0.0001
Maternal rank	0.0074	0.0007	11.31	<0.0001
Daily temperature	0.0229	0.0017	13.3	<0.0001
Group size	0.0009	0.0005	1.92	0.055
Habitat quality (low vs high)	0.1952	0.0127	15.34	<0.0001
Maternal age	0.0091	0.0006	14.31	<0.0001

The model selection procedures for postpartum amenorrhea duration yielded three equally supported models based on AIC (Table 6). GLMM results revealed that postpartum amenorrhea duration was shorter when females had higher hybrid score (i.e. more anubis ancestry), when females had high social rank status and longer when females were primiparous, when daily rainfall and daily temperature were high, when females were living in a low-quality habitat, and when females were older. Infant sex and group size were non-significant as shown by the full model (Table 7).

TABLE 6: ALL GLMMS AND THEIR COVARIATES, AICC VALUES, AND MODEL WEIGHTS FOR PREDICTING POSTPARTUM AMENORRHEA DURATION IN BABOONS.

Model	Model covariates	df	AICc	Delta(δ) AICc	Weight
1	Random effect + Intercept + Daily rainfall + Daily temperature + Habitat quality + Hybrid score + Maternal age + Primiparity + Maternal rank	9	12162.1	0	0.24
2	Random effect + Intercept + Daily rainfall + Daily temperature + Habitat quality + Hybrid score + Infant sex + Maternal age + Primiparity + Maternal rank	10	12162.4	0.21	0.216
3	Random effect + Intercept + Daily temperature + Habitat quality + Hybrid score + Maternal age + Primiparity + Maternal rank	8	12163.9	1.79	0.098

TABLE 7: PROBABILITY VALUES OF PARAMETER ESTIMATES FOR THE FULL MODEL AND THE BEST MODEL PREDICTING POSTPARTUM AMENORRHEA DURATION IN BABOONS.

A. FULL MODEL FOR DURATION OF POSTPARTUM AMENORRHEA

Covariate	Estimate	Std. Error	Z	P
Intercept	4.7645	0.0903	52.77	<0.0001
Hybrid score	-0.1713	0.0700	-2.45	0.0144
Primiparity (true vs false)	0.0820	0.0077	10.65	<0.0001
Rank	0.0089	0.0009	9.61	<0.0001
Daily rainfall	0.0187	0.0095	1.98	0.0483
Daily temperature	0.0241	0.0025	9.73	<0.0001
Group size	0.0001	0.0006	0.18	0.8569
Habitat quality (low vs high)	0.2686	0.0178	15.12	<0.0001
Maternal age	0.0159	0.0009	17.7	<0.0001
Infant sex (male vs female)	0.0067	0.0049	1.37	0.1716

B. BEST, SIMPLEST MODEL FOR THE DURATION OF POSTPARTUM AMENORRHEA (MODEL 1 IN TABLE 5)

Covariate	Estimate	Std. Error	Z	P
Intercept	4.7673	0.0902	52.83	<0.0001
Hybrid score	-0.1708	0.0700	-2.44	0.0147
Primiparity (true vs false)	0.0816	0.0077	10.62	<0.0001
Maternal rank	0.0090	0.0009	9.82	<0.0001
Daily rainfall	0.0185	0.0095	1.96	0.05
Daily temperature	0.0242	0.0025	9.76	<0.0001
Habitat quality (low vs high)	0.2676	0.0176	15.22	<0.0001
Maternal age	0.0159	0.0009	17.82	<0.0001

One model was supported based on AIC for the cycling duration (Table 8). The GLMM results for cycling duration indicated that cycling duration was longer when females had higher hybrid score (i.e. more anubis ancestry), were primiparous, when daily temperature was high, when females were living in large social groups, had low social rank status, when females were living in low

quality habitat, and when the infant that initiated the cycle was male. Maternal age and daily rainfall were non-significant as shown in the full model (Table 9).

TABLE 8: THE GLMM AND ITS COVARIATES, AICC VALUE, AND MODEL WEIGHT FOR PREDICTING CYCLING DURATION IN BABOONS.

Model	Model covariates	df	AICc	Delta(δ) AICc	Model weight
1	Random effect + Intercept + Daily temperature + Group Size + Habitat quality + Hybrid score + Infant sex + Primiparity + Maternal rank	9	24478	0	0.44

TABLE 9: PROBABILITY VALUES OF PARAMETER ESTIMATES FOR THE FULL MODEL AND THE BEST MODEL PREDICTING CYCLING DURATION IN BABOONS.

A. FULL MODEL FOR CYCLING DURATION

Covariate	Estimate	Std. Error	Z	P
Intercept	2.7637	0.1429	19.339	<0.0001
Hybrid score	0.2986	0.1297	2.303	0.0213
Primiparity (true vs false)	0.1969	0.0114	17.239	<0.0001
Maternal rank	0.0164	0.0015	10.809	<0.0001
Daily rainfall	-0.0020	0.0150	-0.136	0.8918
Daily temperature	0.0534	0.0038	13.918	<0.0001
Group size	0.0039	0.0010	3.877	0.0001
Habitat quality (low vs high)	0.3285	0.0290	11.341	<0.0001
Maternal age	0.0002	0.0014	0.168	0.8663
Infant sex (Male vs female)	0.0246	0.0076	3.224	0.0013

B. BEST, SIMPLEST MODEL FOR CYCLING DURATION (MODEL 1 IN TABLE 8).

Covariate	Estimate	Std. Error	Z	P
Intercept	2.7727	0.1293	21.44	<0.0001
Hybrid score	0.2982	0.1296	2.3	0.02145
Primiparity (true vs false)	0.1959	0.0100	19.655	<0.0001
Maternal rank	0.0165	0.0015	11.072	<0.0001
Daily temperature	0.0532	0.0036	14.947	<0.0001
Group size	0.0039	0.0010	3.918	0.0001
Habitat quality (low vs high)	0.3270	0.0271	12.062	<0.0001
Infant sex (male vs female)	0.0245	0.0076	3.218	0.00129

The model selection procedures for pregnancy duration resulted in 10 equally supported models according to their AIC values (Table 10). The resulting GLMMs revealed that pregnancy duration was longer when females were living in low quality habitat. All other independent variables were non-significant; see full model and best model (Table 11).

TABLE 10: ALL GLMMS AND THEIR COVARIATES, AICC VALUES, AND MODEL WEIGHTS FOR PREDICTING PREGNANCY DURATION IN BABOONS.

Model	Model covariates	df	AICc	Delta(δ) AICc	Weight
1	Random effect + Intercept + Daily rainfall + Habitat quality	4	5109.9	0	0.048
2	Random effect + Intercept + Habitat quality	3	5110.1	0.24	0.043
3	Random effect + Intercept + Daily rainfall + Habitat quality + Primiparity	5	5111.5	1.6	0.022
4	Random effect + Intercept + Habitat quality + Primiparity	4	5111.6	1.77	0.02
5	Random effect + Intercept + Daily rainfall + Daily temperature + Habitat quality	5	5111.7	1.79	0.02
6	Random effect + Intercept + Daily temperature + Habitat quality	4	5111.7	1.79	0.02
7	Random effect + Intercept + Daily rainfall + Habitat quality + Maternal age	5	5111.7	1.82	0.019
8	Random effect + Intercept + Daily rainfall + Habitat quality + Maternal rank	5	5111.7	1.87	0.019
9	Random effect + Intercept + Daily rainfall + Group Size + Habitat quality	5	5111.8	1.93	0.018
10	Random effect + Intercept + Daily rainfall + Habitat quality + Hybrid score	5	5111.8	1.95	0.018

TABLE 11: PROBABILITY VALUES OF PARAMETER ESTIMATES FOR THE FULL MODEL AND THE BEST MODEL PREDICTING PREGNANCY DURATION IN BABOONS.

A. FULL MODEL FOR PREGNANCY DURATION

Covariates	Estimate	Std. Error	Z	P
Intercept	5.2270	0.0743	70.35	<0.0001
Hybrid score	-0.0033	0.0107	-0.31	0.7575
Primiparity (true vs false)	-0.0043	0.0089	-0.49	0.6257
Maternal rank	0.0002	0.0005	0.34	0.7364
Daily rainfall	-0.0159	0.0114	-1.39	0.1644
Daily temperature	-0.0009	0.0021	-0.41	0.6833
Group size	0.0001	0.0007	0.11	0.9127
Habitat quality (low vs high)	-0.0323	0.0114	-2.84	0.0045
Maternal age	0.0000	0.0009	0.05	0.9574
Infant sex (male vs female)	-0.0004	0.0057	-0.07	0.9405

B. BEST, SIMPLEST MODEL FOR PREGNANCY DURATION (MODEL 1 IN TABLE 9)

Covariates	Estimate	Std. Error	Z	P
Intercept	5.2001	0.0108	482	<0.0001
Daily rainfall	-0.0169	0.0113	-1.5	0.1323
Habitat quality (low vs high)	-0.0311	0.0110	-2.8	0.0045

4.3 FEMALE REPRODUCTIVE HORMONES: THE INFLUENCE OF GENETIC, MATERNAL AND ECOLOGICAL FACTORS

Table 12 shows the top four equally supported models based on their AIC as obtained from the model selection procedures. Estrogen concentrations in female baboons were low when females were living in large social groups, were in postpartum amenorrhea, and when females had higher parity. Estrogen concentrations were high when females had high hybrid scores (i.e. more anubis

hybrids), when daily rainfall and daily temperature were high, and when females were pregnant, as shown by the full model and best model (Table 13).

TABLE 12: ALL LMMS AND THEIR COVARIATES, AICC VALUES, AND MODEL WEIGHTS FOR PREDICTING ESTROGEN CONCENTRATIONS IN BABOONS.

Model	Model covariates	df	AICc	Delta(δ) AICc	Weight
1	Random effect + Intercept + Reproductive status + Daily rainfall + Daily temperature + Group size + Hybrid score + Daily temperature + Parity	11	25236.4	0	0.255
2	Random effect + Intercept + Reproductive status + Daily rainfall + Daily temperature + Group size + Hybrid score + Parity	10	25237	0.59	0.19
3	Random effect + Intercept + Reproductive status + Daily rainfall + Daily temperature + Group size + Hybrid score + Daily temperature + Maternal rank + Parity	12	25238.4	1.94	0.097
4	Random effect + Intercept + Reproductive status + Daily rainfall + Group size + Hybrid score + Daily temperature + Parity	10	25238.4	1.96	0.096

TABLE 13: PROBABILITY VALUES OF PARAMETER ESTIMATES FOR THE FULL MODEL AND THE BEST MODEL PREDICTING ESTROGEN CONCENTRATIONS IN BABOONS.

A. FULL MODEL FOR ESTROGEN CONCENTRATIONS

Covariate	Estimate	Std. Error	Z	P
Intercept	3.687247	0.189686	19.44	< 0.0001
Hybrid score	0.175656	0.072413	2.43	0.0153
Parity	-0.03259	0.014783	-2.2	0.0275
Reproductive state (postpartum amenorrhea vs cycling phase)	-0.4253	0.023293	-18.26	< 0.0001
Reproductive state (pregnancy vs cycling phase)	2.132386	0.024067	88.6	< 0.0001
Maternal rank	-0.00065	0.002498	-0.26	0.7947
Group size	-0.02339	0.002324	-10.07	< 0.0001
Maternal age	0.015733	0.009813	1.6	0.1089
Daily rainfall	0.027027	0.006425	4.21	< 0.0001
Daily maximum temperature	0.01099	0.005501	2	0.0458

B. BEST, SIMPLEST MODEL FOR ESTROGEN (MODEL 1 IN TABLE 11).

Covariate	Estimate	Std. Error	Z	P
Intercept	3.684261	0.189355	19.46	< 0.0001
Hybrid score	0.175131	0.072368	2.42	0.0155
Parity	-0.03253	0.014789	-2.2	0.0279
Reproductive state (postpartum amenorrhea vs cycling phase)	-0.42521	0.023293	-18.25	< 0.0001
Reproductive state (pregnancy vs cycling phase)	2.132552	0.02406	88.64	< 0.0001
Group size	-0.02349	0.002293	-10.24	< 0.0001
Maternal age	0.015651	0.009811	1.6	0.1107
Daily rainfall	0.02704	0.006425	4.21	< 0.0001
Daily maximum temperature	0.010945	0.005498	1.99	0.0465

Top two equally supported models and their covariates predicting progesterone concentration are indicated in Table 14. Progesterone concentrations were low when females were living in large social groups, when females had higher parity, and when they were in postpartum amenorrhea. Progesterone concentrations were high when females were pregnant, when females were older and when they had high rank status. Hybrid score, daily rainfall and daily maximum temperature did not influence progesterone levels as shown by the full model and best model (Table 15).

TABLE 14: ALL LMMS AND THEIR COVARIATES, AICC VALUES, AND MODEL WEIGHTS FOR PREDICTING PROGESTERONE CONCENTRATIONS IN BABOONS.

Model	Model covariates	df	AICc	Delta(δ) AICc	Weight
1	Random effect + Intercept + Reproductive status + Daily rainfall + Daily temperature + Group size + Maternal age + Maternal rank + Parity	11	13832.6	0	0.446
2	Random effect + Intercept + Reproductive status + Daily rainfall + Group size + Maternal age + maternal rank + Parity	10	13834.1	1.45	0.216

TABLE 15: PROBABILITY VALUES OF PARAMETER ESTIMATES FOR THE FULL MODEL AND THE BEST MODEL PREDICTING PROGESTERONE CONCENTRATIONS IN BABOONS.

A. FULL MODEL FOR PROGESTERONE CONCENTRATIONS

Covariate	Estimate	Std. Error	Z	P
Intercept	5.829313	0.168764	34.54	< 0.0001
Hybrid score	-0.00027	0.062006	0	0.9965
Parity	-0.04915	0.012361	-3.98	< 0.0001
Reproductive state (postpartum amenorrhea vs cycling phase)	-0.19866	0.019167	-10.37	< 0.0001
Reproductive state (pregnancy vs cycling phase)	1.549398	0.020528	75.48	< 0.0001
Maternal rank	0.005328	0.002113	2.52	0.0117
Group size	-0.02688	0.001794	-14.98	< 0.0001
Maternal age	0.03345	0.008173	4.09	< 0.0001
Daily rainfall	0.000716	0.005291	0.14	0.8923
Daily maximum temperature	0.009068	0.004879	1.86	0.0631

B. BEST, SIMPLEST MODEL FOR PROGESTERONE CONCENTRATIONS

Covariate	Estimate	Std. Error	Z	P
Intercept	5.829222	0.167463	34.81	< 0.0001
Parity	-0.04916	0.01223	-4.02	< 0.0001
Reproductive state (postpartum amenorrhea vs cycling phase)	-0.19867	0.019145	-10.38	< 0.0001
Reproductive state (pregnancy vs cycling phase)	1.5494	0.020526	75.48	< 0.0001
Maternal age	0.033455	0.008083	4.14	< 0.0001
Maternal rank	0.005327	0.00211	2.52	0.0116
Group size	-0.02688	0.00179	-15.01	< 0.0001
Daily rainfall	0.000716	0.00529	0.14	0.8923
Daily maximum temperature	0.009068	0.004878	1.86	0.063

The firsthand estrogen and progesterone data from the Enzyme immunoassays as presented by the error bar graphs showed that estrogen and progesterone levels were subject to variation with the reproductive phase during which the fecal samples were collected. Both estrogen and progesterone

levels were low during postpartum amenorrhea and increased during cycling with an increasing trend into the gestation period when the two hormones peaked (Fig. 7 and 8).

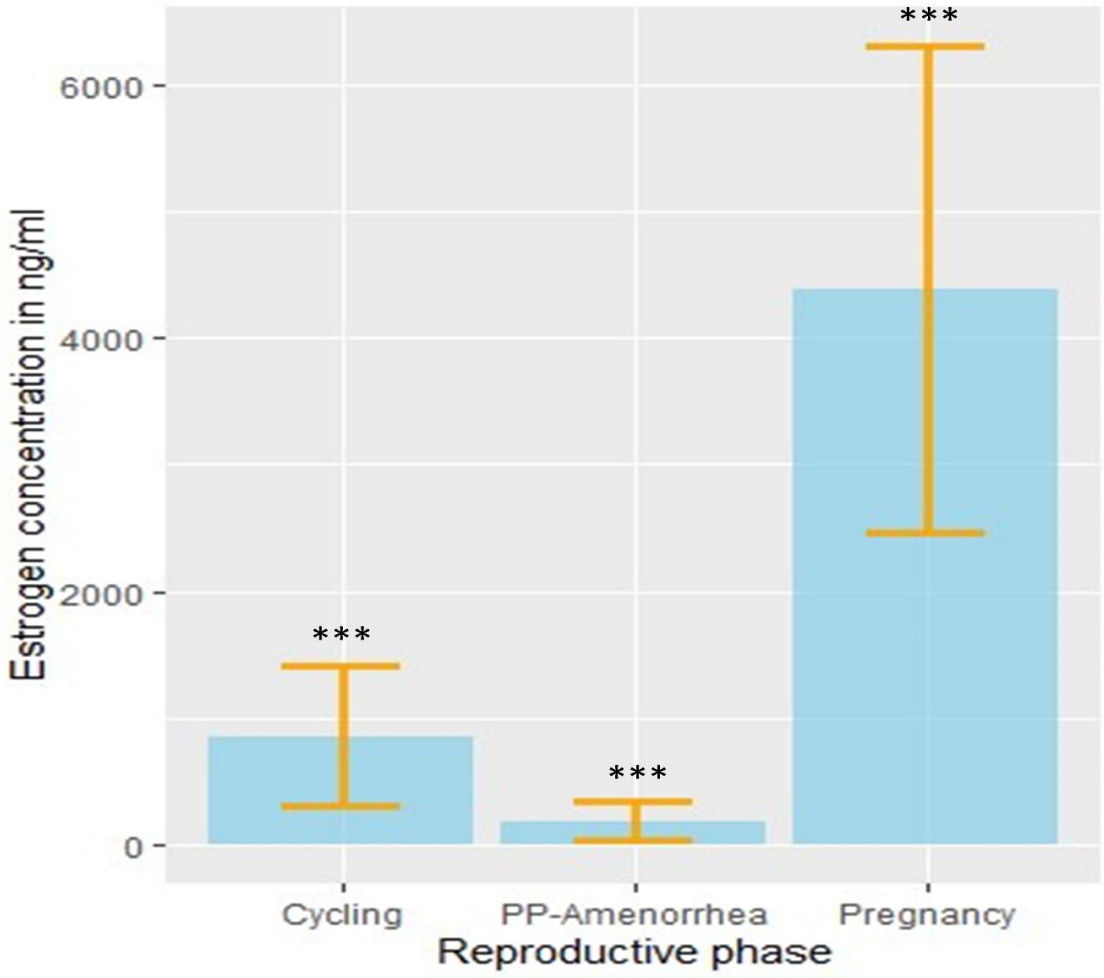

Fig 7: The changes in estrogen concentrations during cycling, postpartum amenorrhea and pregnancy for 48 fecal samples from Amboseli baboons. The y-axis represents the concentration of estrogen (ng/ml) while the x-axis represents the reproductive phase (cycling, postpartum amenorrhea and pregnancy). Estrogen levels were significantly different ($P \leq 0.05$) during cycling, postpartum amenorrhea and pregnancy.

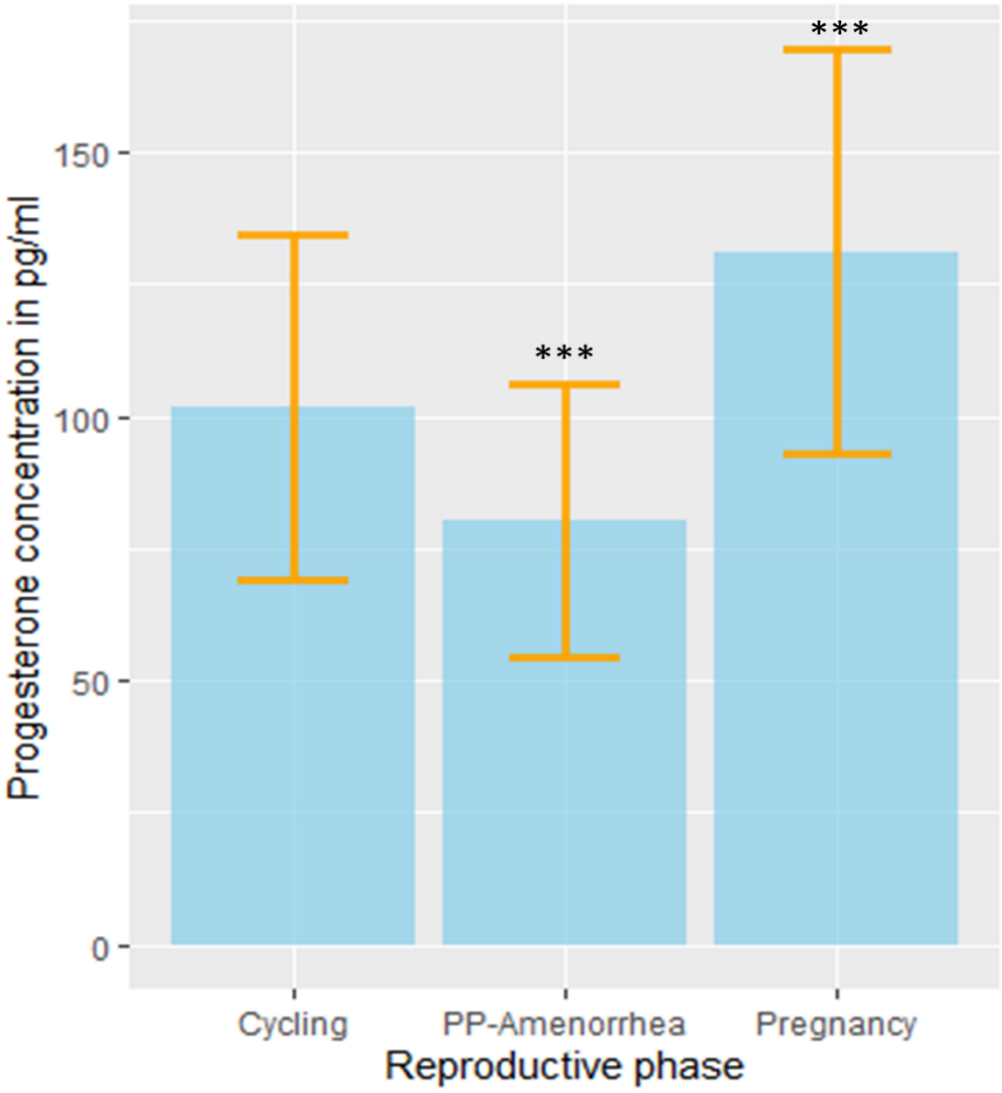

Fig 8: The trends in progesterone levels during cycling, postpartum amenorrhea, and pregnancy for 48 fecal samples from Amboseli baboons. The y-axis represents the concentration of progesterone hormone (pg/ml) while x-axis represents the reproductive phase (cycling, postpartum amenorrhea and pregnancy). Progesterone levels were significantly different ($P \leq 0.05$) during postpartum amenorrhea and pregnancy.

CHAPTER 5

5.0 DISCUSSION

The main objective of this study was to examine differences in female reproductive hormones and reproductive success in hybrid baboons, relative to females with low or no admixture. To achieve this, three specific objectives were carried out. These objectives were to estimate, for females with different degrees of genetic admixture, rates of spontaneous miscarriage, variation in birth rates, and estrogen and progesterone hormone profiles.

First, this study did not find any relationship between miscarriage rates and genetic ancestry in the Amboseli baboons; hybridization thus does not influence miscarriage rates among Amboseli baboons. Fetal loss rates were equal between the yellow female baboons and the hybrid baboons.

Second, there was no genetic influence on the Amboseli baboon birth rates as estimated by the total interbirth interval. Similarly, the hybrid score of the animals did not influence duration of pregnancy, which is one component of inter-birth interval. However, the other two phases of interbirth interval, namely: postpartum amenorrhea and cycling duration, were related to female genetic ancestry. Specifically, the duration of cycling was shorter for baboons with a more *P. cynocephalus* genetic background and longer for baboons with a more *P. anubis* genetic background. In contrast, the duration of postpartum amenorrhea was shorter for animals with a more *P. anubis* genetic background compared to animals with a more *P. cynocephalus* genetic background. Together, these effects may have neutralized each other, and as a result, they did not translate to meaningful differences in the interbirth intervals between yellow and hybrid females. As such, the study found that ancestry played no statistically significant role in birth rate determination in female baboons from Amboseli.

Third, female reproductive steroids were partly related to animals' genetic ancestry. Estrogen levels were higher in animals with a more *P. anubis* genetic background compared to animals with a more *P. cynocephalus* genetic background. However, progesterone levels did not differ significantly between *P. cynocephalus* females and hybrid females.

Together, these results indicate that neither parental baboons nor hybrid baboons in Amboseli are reproductively advantaged or disadvantaged more than the other is. The main difference in reproductive events such as shorter postpartum amenorrhea in hybrids with a more *P. anubis* genetic background, shorter duration of cycling in a more *P. cynocephalus* animals or higher estrogen levels in a more *P. anubis* animals did not alter the pattern of reproductive success between hybrids and their parental forms. However, some previous studies on various baboon populations reported that reproductive success is higher in hybrids than in non-hybrid animals (Bergman *et al*., 2008; Detwiler, 2002). Yet others reported that hybrids have lower reproductive success compared to non-hybrid animals (Melanie *et al*., 2007).

By measuring female reproductive success as a function of hybrid ancestry, it is possible to determine whether hybrids have a reproductive advantage (Burke and Arnold, 2001). It has been argued that hybrids can perform better, in equal measure, or less than parental forms (Arnold and Hodges, 1995). The present study has demonstrated that Amboseli baboon hybrids perform just like their parental forms under similar ecological conditions. Any differences in reproductive success between hybrids and parental forms in the Amboseli baboon population are driven, therefore, by other genetic factors or variation in the environment. Group size influenced inter-birth intervals and estrogen and progesterone levels. Baboons living in large social groups

experienced longer interbirth intervals and lower estrogen and progesterone levels, although miscarriage rates were not influenced by group size.

Reproductive success thus depends on ability to handle stress arising from such factors as within group competition, with high intra-group competition being a barrier to reproductive success (Chapman *et al*., 2012; Van Belle and Estrada, 2008; Silk, 2007; Snaith and Chapman, 2007; Altman and Alberts, 2003). High rainfall seasons were accompanied by an increasing estrogen levels, but with no significant influence on miscarriage rates, interbirth intervals and progesterone levels. Environmental factors are known to determine the habitat quality; high rainfall favors food availability, which act as the main driver of reproductive success in such a social animal population. Studies have shown that rainfall and food availability are positively correlated and influence chances of ovulation and conception (Heesen *et al*., 2013), with high conceptions during wet seasons (Dunbar, 1980). Food availability also influences pregnancy outcome (Antonow-Schlorke *et al.,* 2011) and lactation success (Tardif *et al*., 2001). Hence, rainfall and food availability, contrary to the findings of this study, are known to play key roles in reproductive success in females (Altman and Alberts, 2003; Knott, 2001).

High daily temperatures had a negative relationship with reproductive success in both hybrids and non-hybrid females. Specifically, interbirth intervals were longer when daily temperatures were high. Previous studies showed that high ambient temperatures influence reproductive success of most male mammals with scrotal testes (Setchell, 1998). Prolonged high temperatures suppress spermatogenesis and reduce the number of fertilizations. In females, heat stress has been shown to suppress ovulation, conception (Bronson, 1989; Baumgartner and Chrisman 1987), and increase

fetal loss rates (Bronson, 1989). The present study, however, did not find significant effects of temperature on baboon miscarriage rates and steroid hormone profiles, which, mirrored results obtained by Beehner et al. (2006).

Despite the lack of meaningful differences in reproductive performance linked to hybrid ancestry, the present research did find patterns of variation in reproductive success with ecological factors, maternal factors, and infant sex. Higher reproductive success was associated with higher parity (Gesquiere *et al*., 2018; Altman and Alberts, 2005; Silk, 1990), high quality habitat (Gesquiere *et al.*, 2018), low temperatures (Beehner *et al.*, 2006), lower maternal age (Nishida *et al*., 2003), smaller social groups (Gesquiere *et al.*, 2018; Altman and Alberts, 2003) and high maternal rank (Gesquiere *et al*., 2018; Tung *et al*., 2012; Cheney *et al*., 2004; Wasser *et al*., 2004; Altman and Alberts, 2003).

Infant sex as a predictor variable did not show any relationship with interbirth intervals or estrogen and progesterone levels. However, previous studies indicated that mothers favor the offspring sex that provides higher reproductive benefits, which can result in biased sex ratios at birth (Schino, 2004). Reproductive success had a relationship with maternal age: old females experienced longer interbirth intervals and progesterone levels also tended to increase with increase in maternal age, but age did not influence miscarriage rates and estrogen levels. The effect of maternal age on reproductive success is seen among younger, middle aged and older females. Younger females are still investing in their own growth (Setchell *et al*., 2002), while older females might be experiencing first stages of reproductive senescence, characterized by less regular cycles and higher conceptive failure rates as well as low hormone profiles (Altman and Alberts, 2003; Packer *et al*., 1998; Tardif

and Ziegler, 1992). Thus, peak reproductive success is seen in females in their middle ages (Beehner *et al*., 2006). Maternal parity influenced birth rates and reproductive hormone profiles. Estrogen and progesterone levels decreased with increasing maternal parity, while interbirth intervals were longer for primiparous females. These results demonstrate that primiparous females experience higher steroid hormone profiles compared to multiparous females who, however, experience accelerated reproduction (Altman and Alberts, 2005; Altman *et al*., 1988).

The fact that this study did not find an effect of hybrid score on female reproductive success in this population is not unexpected since previous studies in this population found that admixture has stronger effects in male than female baboons (Charpentier *et al.*, 2008; Alberts and Altman, 2001). The most prominent findings of this study are that, hybridity in Amboseli baboons influences cycling duration, postpartum amenorrhea duration, and female baboon estrogen levels. As such, the present study's first and second hypotheses were supported, that miscarriage and birth rates are the same between pure female baboons and female hybrid baboons in Amboseli baboon population. The third hypothesis, that there is no difference in estrogen and progesterone levels between pure female baboons (*P. cynocephalus*) and female hybrid baboons in Amboseli baboon population, was rejected for estrogen hormone and accepted for the progesterone hormone.

5.1 CONCLUSION AND RECOMMENDATIONS.

Amboseli female baboons' reproductive success was not related to the individual genetic ancestry. Hybridity influenced the duration of cycling, duration of postpartum amenorrhea and estrogen levels, but not the length of pregnancy, overall interbirth intervals, or progesterone levels. In terms of reproductive hormones, hybridization between yellow and anubis baboons has resulted in more superior hybrids with higher estrogen levels than pure female baboons, however progesterone levels remained the same between hybrids and pure female baboons. Hybridization does not influence miscarriage rates or birth rates between hybrids and pure female baboons thus both pure and hybrid females were equally able to conceive and were reproductively successful. The reproductive variability between hybrids and their parental forms was driven by innate maternal factors, infant sex, and ecological factors. In this baboon population, hybridization does not appear to influence reproductive performance between parental forms and hybrid forms. Hybridization between *P. cynocephalus* and *P. anubis* in Amboseli thus generates genetic diversity and is of evolutionary significance as the two inter-breeding species are both evolutionarily fit, adapted to the environment and are able to produce viable hybrid offspring. These offspring are in turn able to mate and produce reproductively fit offspring at a rate equal to that of the parental forms. Despite few or no genetic barriers to hybrid reproduction, hybridization rates in Amboseli remains low and may not currently contribute to hybrid speciation. The present study recommends that future research in this area aim to understand why hybridity influenced cycling and postpartum amenorrhea duration in the opposite directions between pure and hybrid females, thereby neutralizing hybridity effect on interbirth interval and thus reproductive success.

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APPENDICES

APPENDIX 1

1.1 GENERAL ABBREVIATIONS

ABRP	Amboseli Baboon Research Project
ANOVA	Analysis of variance.
S.Q.L	Structured Query Language.
UON	University of Nairobi.
BABASE	Amboseli Baboon Database (individual-based database)
GLMM	Generalized Linear Mixed Models
LMM	Linear Mixed Models
RIA	Radioimmunoassay
RBU	Reproductive Biology Unit
MP Biomedical	Worldwide corporation manufacturing laboratory diagnostic chemicals and equipment.
IgG	Immunoglobulin G
HRP	Horse-radish peroxidase enzyme
TMB	3, 3', 5, 5'- Tetramethylbenzidine

1N HCL	Hydrochloric Acid of 1 Normality.
EIA	Enzyme immunoassay
SQL	Standard query language
glmmTMB	Generalized Linear Mixed Model Template Model Builder
lmer	Linear Mixed effect function in lme4 package in R statistics.
lme4	Linear Mixed effect Model package in R using Eigen an S4
R	A language and environment for statistical computing and graphics.

1.2 UNIT ABBREVIATIONS.

M.A.S.L	Meters above sea level.
RPM	Revolutions per minute.
S.E.M	Standard error of mean.
%	Per cent
g	Gram
^{o}C	Degree Celsius
h	Hour
min	Minutes
ml	Milliliter

mm	Millimeter
nm	Nanometer
pg/ml	Picogram per milliliter
ng/ml	Nanogram per milliliter
cm	centimeters

APPENDIX 2

2.0 MATERIALS AND REAGENTS FOR ASSAYS.

2.1 PROGESTERONE ASSAYS.

2.1.1 WORKING PROGESTERONE-HRP CONJUGATE PREPARATION.

Working progesterone-HRP conjugate reagent was prepared by adding 0.1 ml of progesterone-HRP conjugate concentrate (11x) to 1ml of progesterone-HRP conjugate diluent (1:10 dilution) and mixed well. The total progesterone-HRP conjugate concentrate was 1.3 ml while its diluent was 13 ml, which is in ratio of 1:10. The whole content of the progesterone-HRP conjugate concentrate was mixed with the whole content of the diluent. The other materials and reagents for progesterone assays were provided within the kit and ready to use and included:

Goat Anti-Rabbit IgG-microtiter wells, 96 wells in the plates.

Progesterone reference standards in the following concentrations: 0, 0.5, 3.0, 10, 25 and 50ng/ml in liquid forms of 0.5 ml each ready to use.

Rabbit anti-progesterone reagent 7 ml, pink in color.

Progesterone controls 1 and 2, 0.5 ml liquids ready to use.

TMB reagent for one-step use, 11 ml.

Stop solution (1N HCL), 11 ml.

2.1.2 ESTRADIOL ASSAYS.

The materials and reagents for estradiol assays were packed within the kit and were ready to use and included:

96-well plates coated with Goat Anti-Rabbit IgG.

Estradiol reference standards: 0, 10, 30, 100, 300 and 1000 pg/ml in liquid form each 0.5 ml.

Rabbit Anti-Estradiol reagent pink in color, 7 ml.

Estradiol-HRP conjugate reagent blue in color, 12 ml.

Estradiol controls 1 and 2, 0.5 ml each and ready to use.

One-step TMB reagent, 11 ml.

Stop solution (1N HCL), 11 ml.

The kits and respective reagents were imported.

2.2 MATERIALS NOT PROVIDED WITH THE KIT BUT REQUIRED.

For both progesterone and Estradiol assays, these materials were required and were bought locally:

Microplate reader.

Linear-linear graph papers.

Vortex mixer equivalent.

Absorbent papers.

Distilled water.

Precission pipettes: 25, 50, 100, 200 microliters and 1.0 ml.

Disposable pipette tips.

2.3 ESTRADIOL STANDARD CURVES

The standard curve for estradiol was obtained by assaying the estradiol standard solutions within each assay batch.

The X-axis units are in log form while Y-axis units are in logit. The curve has a slope of -1.666, intercept=2.547. IC 50=33.823, R^2=0.992 and dilution factor 1.

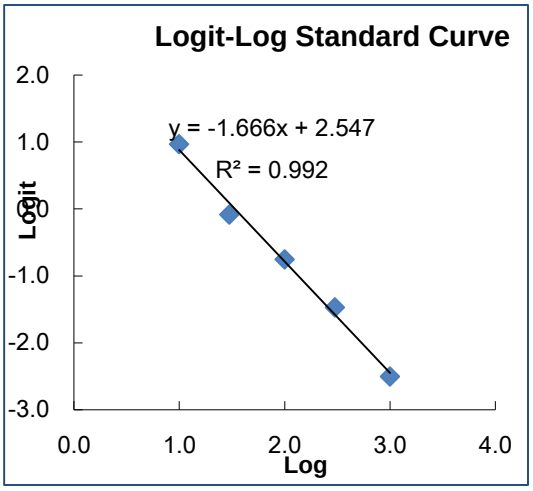

Fig 9: Estradiol standard curve.

2.4 PROGESTERONE STANDARD CURVES

The standard curve for estradiol was obtained by assaying the progesterone standard solutions within each assay batch. The X-axis units are in log form while Y-axis units are in logit. The curve has a slope of -1.560, intercept=0.686. IC 50=2.753, R^2=0.997 and dilution factor 1.

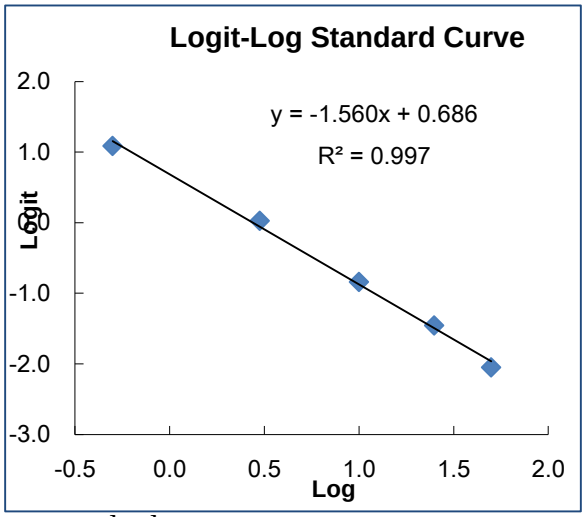

Fig 10: Progesterone standard curve.