COMPARATIVE STUDY FOR THE ANALYSIS OF THE MICROBIOTA OF THE GLANS PENIS AND THE VAGINA OF THE OLIVE BABOONS (Papio Anubis)

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Certification

The undersigned certifies that this dissertation is the work of the candidate carried out during his training in the Master of Science in Tropical and Infectious Diseases under my direct supervision

The undersigned certifies that I have read and here by recommended it for consideration by University of Nairobi, the dissertation entitled; **COMPARATIVE STUDY FOR THE ANALYSIS OF THE MICROBIOTA OF THE GLANS PENIS AND THE VAGINA OF THE OLIVE BABOONS (Papio Anubis)**

This dissertation submitted in partial fulfillment of the requirements for the award of Masters of Science-Tropical and Infectious Diseases, University of Nairobi- Institute of Tropical and Infectious Diseases (UNITID)

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I, declare that this dissertation is my original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award and is not previously or currently under copyright

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List of Acronyms

tPSA	Total Prostate Specific Antigen
ECLIA	Electrochemiluminescence Immunoassay
BPH	Benign Prostatic Hyperplasia
NHPs	Non-human primates
BV	Bacterial Vaginosis
STIs	Sexually Transmitted Infections
OxS	Oxidative Stress
DNA	Deoxyribonucleic Acid
W H O	World Health Organization
IPR	Institute of Primate Research
IRC	Institutional Review Committee
PID	Pelvic Inflammatory Disease
UTI	Urinary Tract Infections
PPROM	Preterm, Pre-labor, Rupture of Membranes
CS	Coronal Sulcus
SED	Sexually Enhanced Disease
HIV	Human Immunodeficiency virus
HSV	Herpes Simplex virus
HPV	Human Papillomavirus

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Abstract

The human reproductive system is one of the important microbial habitats that harbor different species of bacteria in variable quantities and relative proportions that are known to have important effects on health, both normal and pathological effect. The baboon models have been useful to provide detailed understanding of the human reproduction, Reproductive physiology; fertility mechanism, immunology, pathology and anatomy in humans due to their phylogenetic similarities. However little is known about the composition of the vaginal and glans penis microbial ecosystem. The knowledge of the composition of vaginal microbial and the gland penis ecosystem is essential for understanding the etiology, prevention and treatment of urinary tract infection, sexually transmitted disease and prostatitis in males.

The study was a laboratory experimental case-control study design carried out at the institute of primate research aimed at comparing the microbiota of the baboon vaginal and glans penis microbial flora. Blood, vaginal and urethral swabs samples were collected. Gram stained and cultures were done aerobically and anaerobically then scored using the Nugent criteria. Biochemical identification of the cultures was performed using the API® kits and analyzed by apiwebTM standalone identification software. The Total free serum prostate specific antigens levels in male baboon were determined using electrochemiluminescence immunoassay "ECLIA" (Roche Diagnostics).

The study was approved by the institute of primate research Ethics and Institutional Review Committee. Data was analyzed using Microsoft excel 2010 version. In this study, the baboon (Papio Anubis) vaginal and lower male genital tract microbiota are heterogenous in terms of species composition, but the vaginal is typified by a scarcity of lactobacilli unlike the women,

while the majority of the colonizing organism in both sex being staphylococci aureus more than 60% of all the animals.

The most isolated and identified bacterial species was Lactobacilli, Staphylococci, streptococcus Clostridia, Bacilli, Corynebacterium, gram-negative rods mostly *E.coli* in females only, and other species of Gram-positive rods, cocci and Candida species with c. albicans the most predominant in females but absentee in males.

CHAPTER ONE

1.0 Introduction

The composition and function of the microbiota in different body habitats plays a vital role in development, physiology, immunity and nutrition [Dethlefsen *et al.,* 2007]. The penis itself provides distinct anatomical and biochemical environments in the urethra and coronal sulcus. Studies of CS region have mostly concentrated on the etiology of balanoposthitis and the effect of circumcision on the sexual transmission of pathogens (including HIV, HSV and HPV) while studies of urethra are usually carried out for detecting sexually transmitted pathogens and mycoplasmas[Reet *et al.,* 2012]. Both sites are exposed to foreign microbial communities during sexual activities but at the same time provide a suitable micro biotope for aerobic, microaerophilic and anaerobic bacteria that form their microbiota.

Abnormal vaginal flora in women, probably due to Bacterial Vaginosis (BV), one of the most common gynecological disorder caused by an alteration in the vaginal flora in which the normally predominant lactobacilli are replaced by anaerobic Gram-negative rods belonging to the genera Prevotella, Porphyromonas and Bacteriodes; genital mycoplasmas; Gardnerella and anaerobic Peptostrprococcus species, with a consequent increase in vaginal pH, has been implicated as a cause of adverse pregnancy outcomes including amniotic fluid infection, pre-mature birth, low-birth weight infants, histologic chorioamnionitis and post-cesarean and post-abortal endometritis [Haggerty *et al.,* 2004 , Hillier *et al.,* 1992].

In addition, to ply-microbial Bacterial Vaginosis (BV) (Leppäluoto *et al.*, 2011) proposed there could be a mono-bacterial form of BV, G. Vaginalis, which may be a physiological post-coital condonation for protection of ejaculated spermatozoa, characterized by 'pure' Gardnerella flora and elevated PH as an immediate result of an incidental unprotected coital act through neutralization of the vaginal acid and replacement of lactobacillus by gardnerella flora. BV is also associated with upper-genital-tract infection, increased risk of human immunodeficiency virus-1 (HIV-1) infection [Cohen *et al.*, 1995, Schwebke *et al.*, 2009, Voravuthikunchai *et al.*, 2006] and other sexually transmitted infections (STIs) [Hillier *et al.*, 1993]. Vaginal communities of women with symptoms of BV have high numbers of strictly anaerobic bacteria, many of which are various taxa that belong to the order Clostridiales [Fredricks *et al.*, 2005, Thies *et al.*, 2007].

However the presence of these organisms in high numbers is necessary, but not sufficient to elicit the symptoms associated with BV and that differences in the complex of symptoms that become manifest are likely dictated by differences in the immune response of the host. This seems sensible given that disease results not only from the ill effects of microbial activities and products, but also from the nature and severity of the host immune response to the organisms [Zhou *et al.*, 2010]. Thus it is logical to suggest that when examining the vaginal habitat, the microbe-host immune system interaction should also be focused [Romero *et al.*, 2004].

Recent studies in women have shown that distinct and significant differences in bacterial communities occur in women. Elevated vaginal pH ≥ 4.5 and presence of bacterial communities not dominated by lactobacilli are normal in women from some ethnic groups and do not indicate a disease state [Ravel *et al.*, 2011, Zhou *et al.*, 2007].

Non-human primates (NHPs) are useful models for studying human reproduction, Reproductive physiology; fertility mechanism, immunology, pathology and anatomy of the female baboons have been characterized and shown to be similar to that of humans [D'Hooghe et al., 2008,]. Further, the phylogenetic similarities between baboons and the human than any other laboratory animal, justifies the use of baboons as suitable non-primate models for pre-clinical studies for human reproduction health [Mburi et al., 2007, D'Hooghe et al., 2008]. However, their vaginal and gland penis microbial ecosystem has not yet been extensively studied [Reet et al., 2012]. In addition, it is generally accepted that microbiota exists in the male lower genital tracts of human although their characterization has always lagged behind investigation in other body sites, including female genital tract.

1.1 LITERATURE REVIEW

1.1.1 General health

Microbiota of each biotope of human body is tightly integrated into host–microbiota ecosystem and complicated cross-talk takes place between its components. In addition to colonization resistance, our microbiota participates in metabolism, immune system stimulation and trophic function of epithelium [Reet *et al.*, 2012]. Male genital tract microbiota is not an exception in that respect although its microbial mass is undoubtedly lower than in intestinal tract where 2 kg of pure bacterial mass is considered to exist.

Microorganisms may translocate from each mucosal site, especially in case of immune deficiency, underlying diseases or dysbiosis, and consequently the infections outside of genital tract are possible [Ivanov *et al.*, 2009].

1.1.2 Impact of male genital tract microbiota on partner's health

The mucosal surface of the female genital tract is a complex bio system that provides a barrier against the outside world and participates in both innate and acquired immune defense systems. This mucosal compartment has adapted to a dynamic, nonsterile environment challenged by a variety of antigenic/inflammatory stimuli associated with sexual intercourse and endogenous vaginal microbiota [Nasu et al., 2010].

At the same time vaginal microbiota is an open ecosystem that can be significantly affected by sexual intercourse. Semen contains several factors like male reproductive proteins, markers of inflammation and microorganisms [Schwebke et al., 2004]. Alkalization of the vaginal niche during intercourse may enhance a shift from lactobacilli dominated microbiota to a BV-like type. Therefore the fluctuations in vaginal ecosystem are highly likely while significant dysbalance of this system may lead to several maladies including urinary tract infections and also infertility.

Several studies have shown that frequent sexual intercourse, multiple sex partners, frequent episodes of receptive oral sex, receptive anal sex before vaginal intercourse, and sex with an uncircumcised male partner may cause fluctuations of vaginal microbial communities and contribute BV episodes [Schwebke et al., 2004, Brotman et al., 2010]. It has also been shown that sexual partners harbor the same strains of BV-associated G. vaginalis [Eren et al., 2010].

[Leppäluoto et al., 2011] proposed a hypothesis that in addition to plymicrobial BV there could be a monobacterial form of BV, G. Vaginalis vaginitis, which may be a physiological post-coital condition for protection of ejaculated spermatozoa, characterized by ‘pure ’Gardnerella flora and elevated pH as an immediate result of an incidental unprotected coital act through neutralization of vaginal acid and replacement of Lactobacillus by Gardnerella flora. The author indicates that this hypothesis arose from their previous studies where in most of the women the dominant Lactobacillus morphotype flora seen in pre-coital smears was replaced by G. Vaginalis morphotypes dominant flora in post-coital smears [Leppäluoto et al., 2004, 2007].

At the same time [Verstraelen et al., 2010] suggested that BV may be considered a sexually enhanced disease (SED), with frequency of intercourse being a critical factor. This may relate to two distinct pathogenic mechanisms: (i) in case of unprotected intercourse alkalization of the vaginal niche enhances a shift from lactobacilli-dominated microflora to a BV-like type of microflora and (ii) in case of unprotected and protected intercourse mechanical transfer of perineal enteric bacteria is enhanced by coitus. A similar mechanism of mechanical transfer may explain the consistent link between non-coital sexual acts and BV.

Similar observations supporting the SED pathogenic model have been made for vaginal candidiasis and for urinary tract infection [Tchoudomirova et al., 1998, Reed et al., 2003]. Other studies have not confirmed the link between sexual activity and bacterial vaginosis. For example [Morison et al., 2005] examined the occurrence of bacterial vaginosis (BV) in a rural African setting using self-collected swabs on alternate days through four menstrual cycles. They had no association between BV and intercourse reported in the previous 4 days or intercourse frequency.

At the same time other studies like the ones below-described studies have revealed fluctuations in other vaginal bacteria and not so much BV-associated communities.

[Hooton et al., 1994] followed 40 women over a median period of 28 weeks to ascertain the effects on vaginal microflora of sexual intercourse alone compared with sexual intercourse associated with use of a diaphragm with a spermicide. Compared with no sex, the intercourse with use of a diaphragm/spermicide in the preceding 3 days was strongly associated with increases in rates of vaginal colonization with Candida sp. and uropathogenic flora, including E. coli, other gram-negatives, group B and D streptococci, while decrease in rates of lactobacillus colonization was noted. Except for E. coli colonization, no such increases in rates of vaginal colonization were seen after sexual intercourse without diaphragm/spermicide. This study indicates mostly the serious adverse effect of this contraception method on vaginal microbiota. In a very thorough study [Eschenbach et al., 2001] investigated 42 women before (1 month and 1–2 days) and after (8–12 h, 2–3 days, and 6–8 days) an index episode of sexual intercourse. The 22 subjects who used no condoms had significantly more E. coli in the vagina after intercourse. Also the 20 subjects who used condoms had a trend toward more vaginal E. coli and other enteric gram negative rods after intercourse.

A parallel increase in E. coli and enteric Gram negatives occurred in the urine of both groups. In this study intercourse with or without a condom had no effect on vaginal lactobacilli and also on pH as measured 8–12 h later. The results of this study are compatible with findings of other researchers [Foxman et al., 1997] that intercourse is associated with a transient increase of E. coli colonization in the vagina and urine. [Foxman et al., 2002] showed that uropathogenic E. coli were nine times more likely than other E. coli to be shared between sex partners.

[Newton et al., 2001] enrolled 617 women in a 1-year longitudinal study (baseline, 6 and 12 months) assessing the effect of sexual behaviors on the vaginal microflora. They found quite mild impact of sexual behaviors on the vaginal microbiota, yet Streptococcus agalactiae was associated with multiple partners and cunnilingus, while Candida spp. with fellatio and frequent sex.

[Santiago et al., 2011] performed longitudinal study of 17 women in which swabs and Gram stains were available for each day of two consecutive menstrual cycles. The swabs were cultured every 7th day and the bacteria were identified using tDNA-PCR and 16S rRNA gene sequencing. Due to low number of sexual intercourse events without condom the authors avoided conclusions about the influence of sex on vaginal microbiota.

Although male genital tract microbiota directly influences the partner's one, there are very few studies about the couples' genital tract microbiota because specimen collection from both partners in parallel is complicated. [Wittemer *et al.,* 2004] cultured endo-cervical, vaginal and seminal microbiota before in vitro fertilization in 951 couples.

The implantation rate was significantly diminished in case of endo-cervical bacterial growth. Positive cultures from both vagina and semen decreased clinical pregnancy rate and increased spontaneous miscarriage rate significantly more than vaginal infection alone.

[Kjaergaard *et al.,* 1997] investigated 11 couples with preterm, pre-labor, rupture of membranes (PPROM) and 18 couples with normal pregnancies. Urine and semen samples were collected from men while samples from vagina, cervix, urine and placenta from women. Pyospermia was found in three men of PPROM group (two of these couples were C. trachomatis positive) while in none of control group. The authors suggested that male genital tract microbiota is associated with preterm, pre-labor rupture of membranes in their spouses. However, C. trachomatis as sexually transmitted pathogen does not belong to microbiota.

Disturbed microbial communities that appear in male genital tract in case of prostatitis are very likely an important cause of changes in vaginal microbiota; however, there are no studies on this topic [Borovkova *et al.,* 2011]. This research group compared vaginal microbiota just before and 8–12 h after intercourse on the 6th–8th days of the menstrual cycle in 17 women who presented with infertility of the couple.

Semen samples from men were collected during menstruation of the partner, 3–5 days before the vaginal microbiota samples. In five men of this group inflammatory prostatitis was diagnosed according to leukocytospermia. In total, 67 different species or genera were isolated from aerobic, micro aerobic and anaerobic quantitative cultures; 36 microorganisms were isolated from men and 54 from women.

In women, the most frequently isolated bacteria were lactobacilli, coagulase-negative staphylococci, corynebacteria, anaerobic Gram-positives, and streptococci, while in men, corynebacteria, streptococci, coagulase-negative staphylococci, and anaerobic Gram-negative rods were most frequently found.

They increase in Nugent scores in 6 out of 17 women after intercourse although no cases of BV (score ≥7) emerged after intercourse. The Nugent score increase after intercourse was accompanied by shifts in cultured microbiota – some species disappeared while others emerged. These shifts were more prominent in partners of prostatitis patients, indicating the significant influence of prostatitis-associated microbial communities on vaginal microbiota. At the same time these shifts were less expressed in case of lower Nugent score indicating protective role of Lactobacillus dominant microbiota [Borovkova *et al.,* 2011].

1.1.3 Impact of male genital tract microbiota on male health

There is strong evidence that the composition of the reproductive tract microbiota is linked to reproductive health and resistance to STI in women [Riemersma *et al.,* 2003]. Lactobacillus spp. regulate the balance of proinflammatory cytokines in vaginal secretions, block colonization and invasion of some pathogens and produce lactic acid, hydrogen peroxide and bacteriocins that inhibit other vaginal microorganisms [Nelson *et al.,* 2012]

Reduction of vaginal Lactobacillus spp. is associated with the overgrowth of anaerobic bacteria that occurs in BV, and increased susceptibility to bacterial and viral STI [Nelson et al., 2010]. Much less is known about the effect of genital tract microbiota on male health yet it has been suggested that the bacterial colonization of male coronal sulcus and urethra might also impact the risk of STI [Riemersma et al., 2003, Ivanov et al., 2007].

Lactobacilli are not so prevalent in male genital tract than in vagina yet they have been identified in urine and urethral swabs [Dong et al., 2011, Nelson et al., 2012] and therefore they may have protective role against foreign microorganisms. [Nelson et al., 2010] found that urine microbiomes from STI positive men were dominated by fastidious, anaerobic and uncultivated bacteria while the same taxa were rare in STI negative individuals, and noted that similar BV-like communities in female genital tract are associated with increased risk for STI.

As concerns the CS microbiota then it is believed to mediate effects of circumcision on risk of HIV and other STI since the biotope for anaerobic and Gram negative communities disappear after circumcision [Nelson et al., 2012]. [Price et al., 2010] have proposed that the anoxic microenvironment of the sub preputial space may support pro-inflammatory anaerobes that can activate Langerhans cells to present HIV to CD4 cells in draining lymph nodes and that the reduction in putative anaerobic bacteria after circumcision may play a role in protection from HIV and other sexually transmitted diseases. However, the present data are clearly insufficient to conclude whether the STI-associated communities precede, are co-transmitted with or are established subsequent to STI.

1.1.4 Prostate pathologies and infertility

Prostate gland is the most commonly diseased internal organ of the human body. Human prostate pathologies (including prostatitis, benign prostate hyperplasia and prostate cancer) are one of the clinical problems with the greatest impact in the third millennium with important impacts in terms of social, health-related and individual costs, including impact on fertility and patient quality of life [Mazzoli et al., 2010, Turner et al., 2004]. Recent studies have shown that diseases of this organ are in more close association than supposed in the past.

Prostatitis that begins usually in younger age can be considered central that significantly interferes complaints of benign prostatic hyperplasia (BPH) and that is an important risk factor for prostate cancer [Dennis et al., 2002].

Also, the border between BPH and prostatitis is blurred [Nickel et al., 2005], and some believe that they may be the same disease [Kramer et al., 2006]. One of the factors stimulating the growth of prostate may be endotoxin of E. coli. Microorganisms have been found in prostate tissue in 21–44% of BPH patients while signs of inflammation in 90–100% of patients [Nickel et al., 1999].Prostatitis-associated inflammation may cause obstruction of male genital tract and impair spermatogenesis. High-grade oxidative stress in case of prostatitis is associated with alterations in metabolism, motility and DNA damage of spermatozoa [Dohle et al., 2003, Erenpreiss et al., 2006].

In a large WHO-conducted study, prostatitis has been found to comprise an important proportion (12%) and holding an outstanding 3rd place among principal causes of male infertility [World Health Organization, 1998]. In prostatitis cancer patients the bacteria have been found from prostate tissue in 81–89% of cases, among others E. coli, Bacteroides sp. [Keay et al., 1999], Propionibacterium acnes [Fassi et al., 2011].

Genital tract dysbiosis-related prostatitis is a condition that is characterized by oxidative stress (OxS) – an imbalance between the production and detoxification of reactive oxygen species that can cause tissue damage. This condition is present also in case of prostate cancer, reviewed by [Khandrika et al., 2009] and BPH [Pace et al., 2010]. OxS in the male genital tract is associated with infertility and deterioration of semen quality.

At the same time the prostatitis associated local OxS is accompanied by systemic one (that can be revealed in blood and urine) as shown by our studies [, Kullisaar et al., 2008, 2012].

Since the OxS is thought to be involved in the pathogenesis of many diseases including cancers, cardiovascular diseases and even mental diseases, the prostatitis-associated OxS may pose an increased risk for development of these diseases [Khandrika *et al.*, 2009]. It is worth mentioning that the patients with BPH have a considerably higher prevalence of cardiovascular diseases than the general population [Karatas *et al.*, 2010] that might be at least partially explained by systemic OxS in their organism.

1.2 Justification

Recognition that the human microbiome is an integral component of the human body, and, on the other hand, majority (up to 80%) of the bacterial species found in the human body being uncultured or even unculturable they play a very important role in health whether normal or pathological . Therefore characterization of the male genital tract microbiota which has always lagged behind investigations in other body sites, including female genital tract add weight to the need to study the microbiome of both the vaginal and glans penis of the baboon which are extensively used as models of reproductive health in humans. In addition, with the high emergency of the antibiotic resistance micro-organism and with the increased human animal interactions, the understanding of the human microbiome continues to grow rapidly.

The olive baboon has previously been used to provide detailed understanding of other aspects of reproduction applicable to women humans. However, there exists limited information on its vaginal and male glans penis microbial ecosystem. An understanding of the composition of its vaginal and glans penis microbial ecosystem is essential for understanding the impact of male genital tract microbiota to their health and to the females and on man's health, the etiology, prevention and treatment of reproductive tract infections.

This study utilized wild caught olive baboons (*Papio Anubis)* maintained at the institute of the primate research

CHAPTER TWO

2.0 Research Question

What are the vaginal, glans penis microbial niche and its impact in health of these Non-human primates (NHPs) olive baboons (*Papio Anubis*) wild caught in East Africa which has been useful and characterized over the years as models for studying human reproduction?

2.1 Objective

2.1.1 General objective

To determine the composition of the (*Papio Anubis)* baboon vaginal and glans penis microbial ecosystem

2.1.2 Specific objectives

1. Identify and isolate bacteria species found in the glans penis and vaginal flora

2. Determine the variation in the microbial flora colonization of the gland penis and vaginal in baboons

3. To determine baboons prostate specific antigen levels in prostatic diseases using the electrochemiluminescence immunoassay “ECLIA”

CHAPTER THREE

3.0 Materials and Methods

3.1 Study Site

The study was conducted at the Institute of Primate Research (IPR) , Nairobi, Kenya which is a World Health Organization (WHO) collaborating center that ethically utilizes non-human primates to improve human health and is guided by international and local standards.

This biomedical institution is mandated to carry out preclinical and biochemical research aimed at improving health care solutions for various diseases using scientific approaches and resources including animal models, also carry out studies that can inform policy with regard to management and conservation of the non-human primates and other biodiversity

3.2 Study Design

The study was an experimental case-control study to identify and isolate the bacterial species in the non-human primate's baboon (*Papio Anubis)* species which are useful models for studying human reproduction at the institute microbiology laboratory.

3.3 Study Population (Animals)

A total of 70 wild caught sexually mature baboons, 40 female cycling between the ages of 6-10 years and 30 males' ages 10-15 years' from East Africa in Kenya, were used for this study.

These baboons were wild caught and housed in group cages at the Institute of Primate Research (IPR), Nairobi, Kenya. The baboons were fed on commercial monkey cubes (Unga Feeds Ltd, Nairobi, Kenya) supplemented with fruits, vegetables and water *ad libitum*.

3.4 Inclusion Criteria

The baboons must have been a wild caught and held in the captive for not more than 3 months at the institute of primate research. Both the males and the females must have attained sexually mature, the females having undergone at least one menstrual cycle characterized by menstrual flow, while the males being considered as full adult size between the ages 10-15 years old. In addition the animals must have not a sign to any project or under any treatment especially with antibiotics and antifungal drugs.

3.5 Exclusion Criteria

1. All the olive baboons that was underage.
2. Animals that were under any treatment or having been treated within the last one month.
3. Olive baboons that had been a signed to any other project or held in captivate for more than 3 months.

3.5 Sample Size

To determine the sample size I applied the scientific rule "3Rs" of Russell and Burch (1959) that provide a framework for considering the humane use of animals in biomedical research:

Reduce- The number of animals should be reduced to the minimum consistent with achieving the scientific objectives of the study,

Replace- Animals should be replaced by less sentient alternatives such as invertebrates or in vitro methods whenever possible.

Refine- Experimental protocols should be refined to minimize any adverse effects for each individual animal.

In addition, the cost of acquiring, sampling and maintaining each animal per day was considered.

3.6 Data Sampling Techniques

The sampling frame included all the animals that passed the inclusion and exclusion criteria. The baboons were coded different identification numbers which were intern used to label the samples. The animals were under anesthesia during all experimental procedures. Anesthesia was induced by intramuscular injection of a mixture of ketamine hydrochloride and xylazine at a ratio of 5:3(100mg/kg body weight ketamine and 60mg/kg body weight xylazine). The vaginal and male urethra swabs were collected after every three weeks, total six different sampling time point for 6 months. Whole Blood for serum separation was collected at one time point.

3.7 Sample Collection and Protocols

3.7.1 Blood collection and serum preparation

Whole blood of 10ml from the non-human primates was collected in plain tubes for serum. The blood was allowed to stand for 1 hour at room temperature to clot, then kept overnight in a freezer at 4˚C for retraction. The Samples were spun at 1500rpm for 15 minutes and the serum separated aliquoted into equal volumes of 1.5mls in two vials.

3.7.2 Total specific antigen analysis

Electrochemiluminescence immunoassay "ECLIA it's a quantitative in vitro assay for diagnostic testing of total (free + complexed) prostate-specific antigen (tPSA) in human serum and plasma.

Reagents:

M-Streptavidin-coated micro particles: Streptavidin-coated micro particles preservative.

R1– Biotinylated monoclonal anti-PSA antibody (Anti-PSA-Ab~biotin); phosphate buffer100 mmol/L, pH 6.0; preservative

R2- Monoclonal anti-PSA antibody (mouse) labeled with ruthenium complex (Anti-PSA-Ab~Ru(bpy)); phosphate buffer 100 mmol/L, pH 6.0; preservative.

Test Sandwich Principle:

First incubation: 20 μL of sample, a biotinylated monoclonal PSA-specific antibody, and a monoclonal PSA-specific antibody labeled with aruthenium complexa reacted to form a sandwich complex. The 2nd incubation: After addition of streptavidin-coated micro particles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture was aspirated into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode.

Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The results were determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

3.8 Vaginal and Male Urethra Swabs

Sampling was carried out after animals were sedated by intramuscular injection with a mixture of 10% xylazine (Rompun®) and 10% ketamine (RotexMedica GMBH Tritau-Germany) mixed in the ratio of 10cc ketamine to 0.5cc of xylazine at 0.1 cc/Kg body weight. The external vulvar was disinfected using betadine to reduce fecal contamination of the genitalia.

The vaginal walls were spread with a speculum and vaginal swabs obtained aseptically by rolling moist sterile cotton swabs on the cervical and vaginal fonicles.

Two vaginal and two male thin urethra swabs were collected from each animal, placed into modified Stuart transport medium (Oxoid, Basingstoke, and UK) and delivered to the laboratory. Gram-stained slides were prepared from the vaginal and gland penis swabs from each animal.

3.8.1 Gram Stain and Culture

The slides were air dried, heat fixed, stained and evaluated by microscopy using oil immersion with × 100 magnifications to quantify and score the stained smears according to Nugent criteria [Pavlova et al., 2002]. The other swabs were used for bacterial culture. The swabs were removed from the transport medium and used to inoculate Rogosa agar for isolation of Lactobacilli and on Trypton Soy agar supplemented with 5% defibrinated sheep blood for isolation of all aerobic and anaerobic bacteria as described previously [Burton et al., 2002]. MacConkey agar was used for Enterobacteriaceae and Sabouraud dextrose agar for candida subcultures.

All culture media used were from Oxoid, Basingstole, UK. Anaerobic atmosphere was achieved by use of gas generating kit anaerobic system (Oxoid, Basingstoke, UK) in anaerobic jars. Colonies growing on the Rogosa agar were identified as Lactobacillus species on the basis of colonial morphology, Gram stain appearance and negative catalase test.

Initial identification for the rest of the isolates was based on colony morphology, Gram stain, oxygen availability and catalase test. The isolates were sub-cultured on fresh suitable culture media until pure colonies were obtained. For biochemical identification all tests were performed using reagents and methods provided by the manufacturer in the API® kits (Biomerieux® SA 69280 Marcy l'Etoile, France). The kits used included api 20A for biochemical identification of anaerobes, api Candida for yeast, api 20 Strept for most Streptococci and Enterococci and commonly related organisms, api 20E for Enterobacteriaceae and other non-fastidious gram-negative rods, api Coryne for Coryne form bacteria, api Staph for Staphylococci, Micrococci and related genera, api 50 CH for genus Lactobacillus and related genera, and api 50 CHB/E medium for Bacillus and related genera, Enterobacteriaceae and Vibrioaceae.

Analysis was done using apiwebTM stand-alone V 1.2.1 identification software (Biomerieux® SA 69280 Marcy l'Etoile, France).

3.9 Data Management and Analysis

The data collected from the gram stains and cultures were recorded on laboratory counter books and stored in lockable drawers. The data of biochemical tests of the pure cultures done using api® kits collected and subjected to analysis to identify the species of the microbes by apiwebTM standalone V 1.2.1 identification software were entered into Microsoft word and Ms excel spreadsheet in a password protected computer, backup copies were stored in an external hand drive and a flash disk, solely under my custody.

The data collected and stored in Ms. excel spreadsheet was analyzed using the same statistical software version 10. Descriptive statistics was used to summarize the collected data.

CHAPTER FOUR

4.0 Ethical considerations

The study is a nested study from the major project **"Developing olive baboons (Papio Anubis) as disease models for prostatic diseases particularly prostate cancer"** whose protocol was approved by the Institute of Primate Research-Institutional Review Committee (IPR-IRC) REF NO.IRC/04/14. The committee is guided by the institutional guidelines as well as international regulation, including those of the National Institutes of Health (NIH), Primate Vaccine Evaluation Network (PVEN) and Helsinki Convention. The baboons were procured, maintained and cared for according to IPR standard operating procedures (Barbra and Chai 1993) at the Institute of Primate Research (IPR).

CHAPTER FIVE

5.0 Results

Both males and females reach sexual maturity between four and six years of age. Males do not reach full adult size until they are about 10-15 years old in the wild and between seven and eight years in captivity. Sexual maturation in male olive baboons is characterized by enlargement of the testicles, growth of the canine teeth, which are much larger in males than females, a deepening of the voice, and an increase in body size and bulk. Females continue to grow and reach their adult size within three years after puberty and give birth one to two years after they begin cycling.

Mating among olive baboons is promiscuous; both males and females have multiple mates. One characteristic of baboon reproductive behavior is the formation of mating consort ships, defined as continuous close spatial association between a male and a sexually receptive female characterized by copulatory activity. Olive baboons form these exclusive relationships, lasting a few minutes or up to two weeks in length, in which they copulate frequently and where the male prevents other males from mating with his consort partner.

5.1 Total prostate specific antigens

The Total prostate specific antigens of all the 20 males' baboons tested were very low ranging between 0.01-0.06ng/ml compared to the references of the normal value ranging between 0.0-4.0ng/ml and the intermediate values of 2.5-10ng/ml seen in healthy men, benign prostatic hyperplasia BPH, prostatitis, gland manipulation and prostate cancer.

5.2 Gram stain assessment

Each Gram-stained smear of the swab was assessed and evaluated under oil immersion (×100 objective lens magnification) with standardized, quantitative bacterial cellular morphologies observed in samples using the Nugent criteria [Nugent *et al.*, 1991]. On microscopy differences were observed in the smears. Seven (17.5%) of the swabs had few large Gram positive rods of *Lactobacillus* morphotypes (Fig. 1), 19 (47.5%) had mixed microflora but devoid of lacto bacillary morphotypes (Fig. 2), 6 (15%) had Gram positive cocci only (Fig. 3), 8 (20%) had coco bacillary organisms that adhered to the cell borders (Fig. 4) and 11 (27.5%) had curved Gram variable rods and pus cells (Fig. 5). In addition, filamentous Gram positive rods (Fig. 6) were observed in more than 50% of the stained vaginal swab smears.

5.2.1 Gram stained vaginal swab smears from baboons

Figure 1: Few gram positive rods of lactobacillus morphotypes

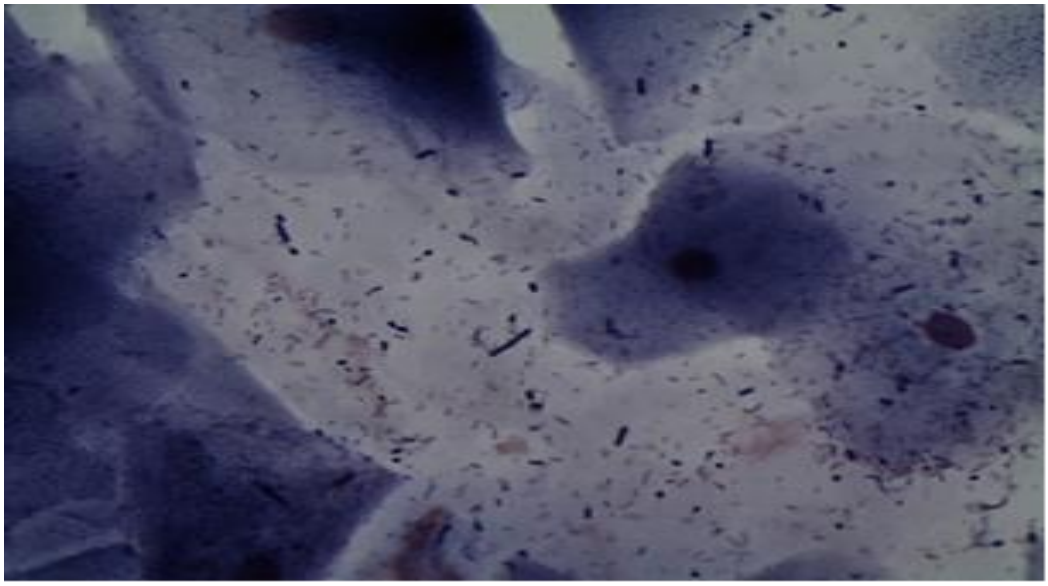

Figure 2 : Mixed microflora devoid of lactobacillay morphotypes

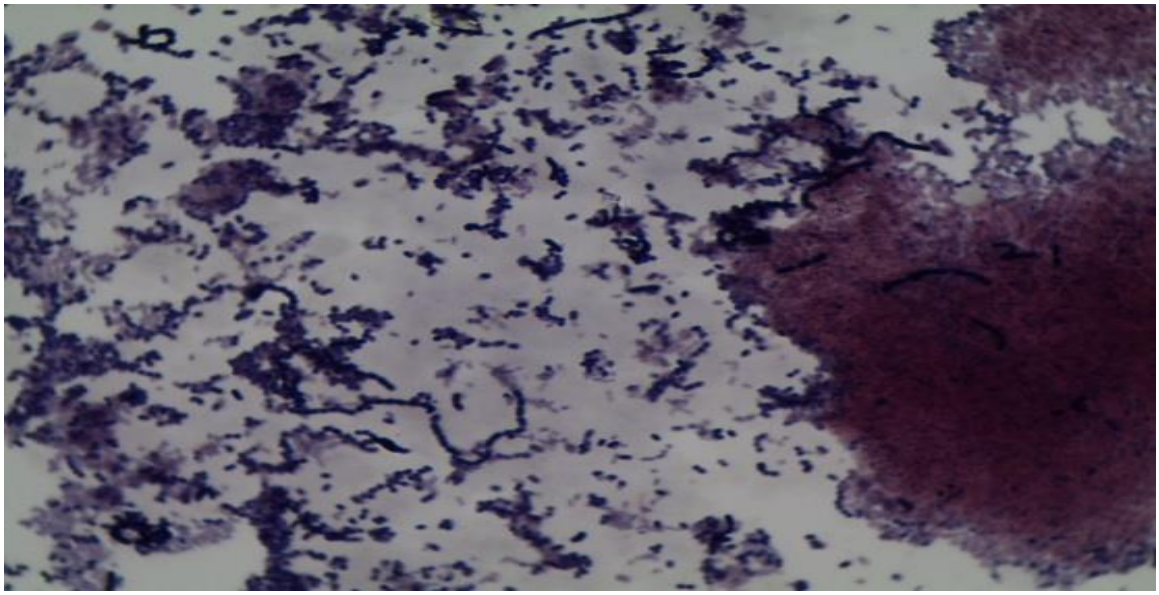

Figure 3: Gram positive cocci only

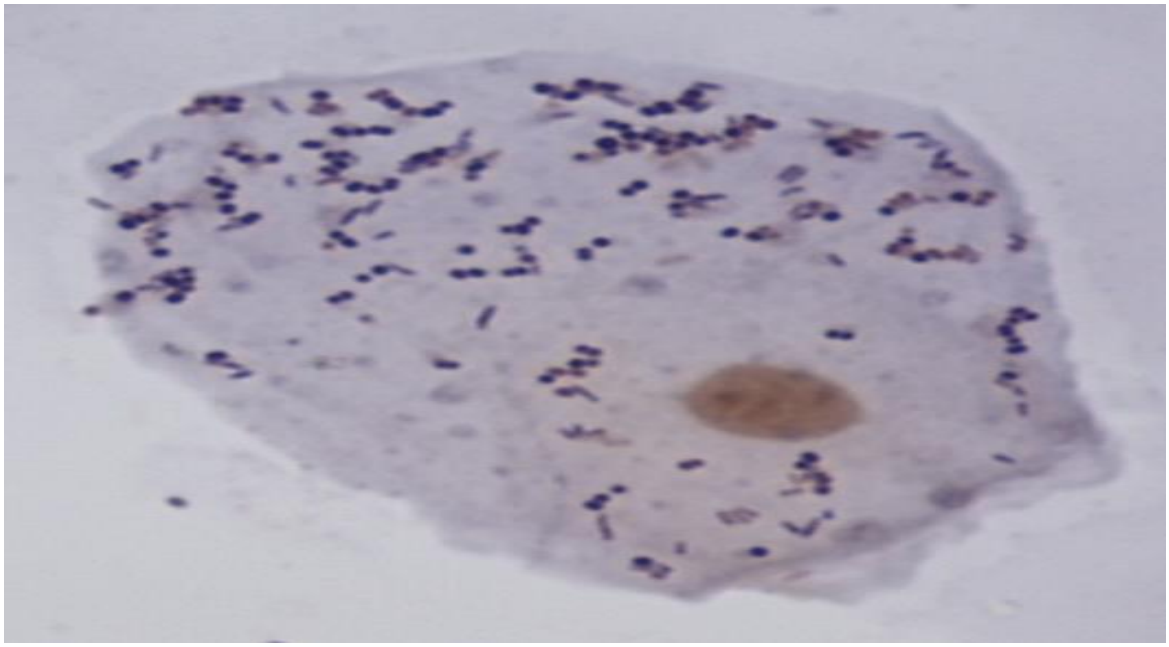

Figure 4: Clue cell showing epithelial cell coated with microflora and loss of cell borders caused by adherent coccoid gram negative to gram variable microbes

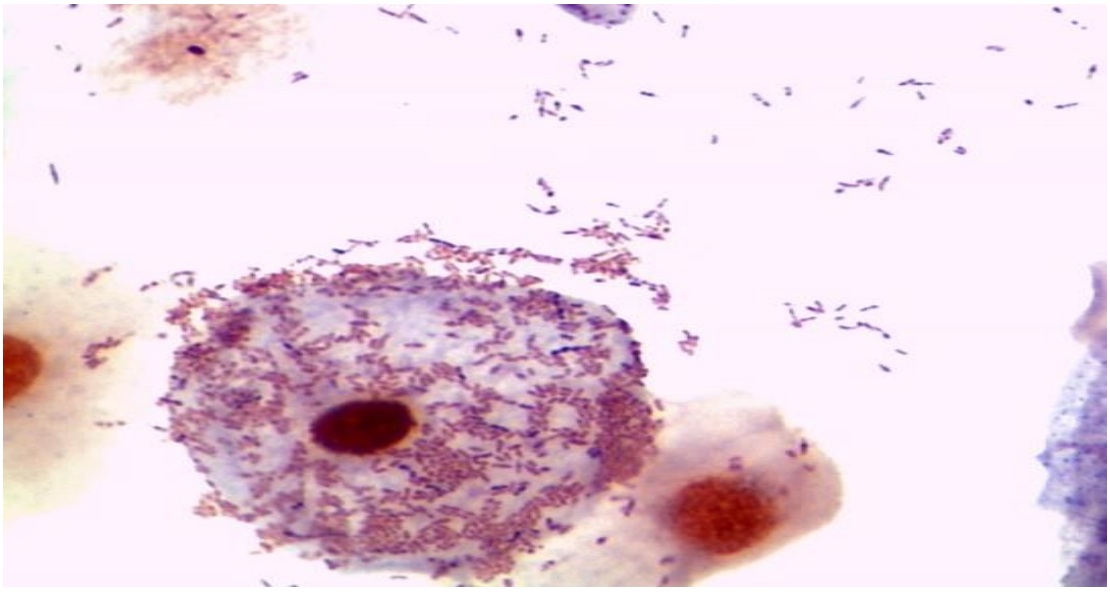

Figure 5: Curved Gram positive rods

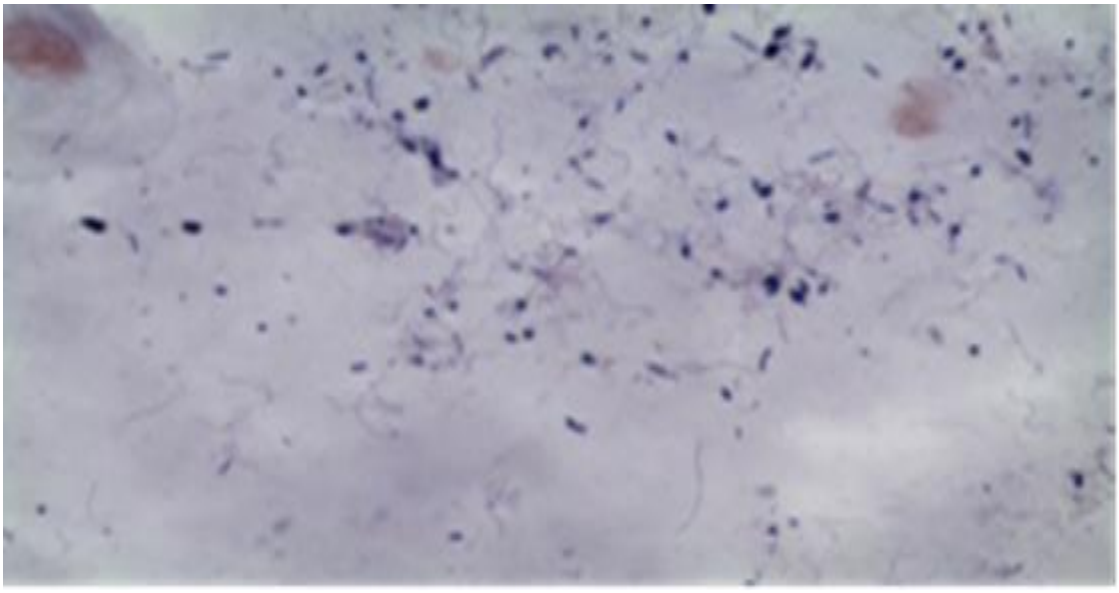

Figure 6: Gram negative rods and filamentous Gram positive rod

5.3 Microbial flora Composition and vaginal PH

Vaginal swabs of 40 female and urethra swabs from 30 males olive baboons were cultivated to assess the microbial composition of the vaginal microflora and the glans penis. Biochemical tests of pure cultures were done using api® kits and the data subjected to analysis to identify the species of the microbes by apiwebTM standalone V 1.2.1 identification software.

Cultivation-based analysis of the swabs resulted in identification of gram positive rods (Table 1), gram negative rods (Table 2), Gram positive cocci (Table 3) and yeast cells (Table 4)

Table 1: Gram positive rods isolates in the glans penis and vagina of the olive baboons.

Gram positive rods	**No. (%) of animals with indicated isolate**	
	Males	Females
***Lactobacillus* species**		
L. acidophilus	6(20%)	13(32.5%)
L. delbruechii	2(6.7%)	4(10%)
L. salivarius	1(3.3%)	6(15%)
L. paracasei	1(3.3%)	5(12.5%)
L. acqunticus	3(10%)	
L. rhamnosus		12(30%)
L. plantarum		8(15%)
L. crispactus		4(10%)
***Corynebacterium* species**		
C. glucuronolyticum	5(16.7%)	36(90%)
C. renale group	1(3.3%)	31(77.5%)
***Clostridium* species**		
C. perfringens		5(12.5%)
C. septicum		4(10%)
C. beijerinckii/butyricum		3(7.5%)
C. clostridioforme		2(5%)

***Bacillus* species**		
B. cereus	10(33.3%)	6(15%)
B. lentus	7(23.3%)	
B. firmus		6(15%)
B. coagulans		5(12.5%)
B. licherniformis		
B. megaterum	1(3.3%)	2(5%)
B. revibacterium	4(13.3%)	
B. subtilis	2(6.7%)	
Other Gram positives rods		
Leuconostoc lactis		14(35%)
Mobilluncus lenta		11(27.5%)
mesenterroides	5(16.7%)	5(12.5%)
Listeria grayi		2(5%)

Gram positive rods isolated included species of *Lactobacilli, Corynebacterium, Chlostridia,* and *Bacilli.* The proportion of swabs with *L. acidophilus* which was the most common species of *Lactobacillus* isolated was 32.5% (13/40) in female and 20% (6/30) in males.

Two Coryneforms namely *C. glucuronolyticum* and *C. renale* group which were among the predominant bacteria were isolated from more than 75% of the swabs and were found to be present in 36 (90%) and 31 (77.5%) of the females but very few in males 3 (10%) and 1 (3.3%) respectively. Each of the four species of *Clostridia* and five of *Bacilli* were isolated from less than 20% of the animals.

Six other Gram positive rod bacteria were isolated and *Leuconostoc lactis* was found to be the most common and was present in 14 (35%) of the animals.

Table 2: Gram negative rods isolates in the glans penis and vagina of the olive baboons.

Gram negative rods	**No.(%)of animals with indicated isolates**	
	Males urethra isolates	**Vaginal isolates**
Escherichia coli		21 (52.5%)
Klyuvera spp		5 (12.5%)
Psudomonas oryzihabitans		3 (7.5%)
Stentrophormonas maltophilia	1 (3.3%)	4 (10%)
Pseudomonas luteola		2 (5%)

Of the gram negative rods where only isolated in females baboons, *Escherichia coli* was the most common and was found to be present in 21(52.5%) of the female animals. *Stebotrophomonas multophila* was the only Gram negative bacteria isolated from males (Table 2).

Table 3: Gram positive cocci isolates in the glans penis and vagina of the olive baboons.

Gram positive cocci	**No.(%)of animals with indicated isolates**	
	Males urethra isolates	**Vaginal isolates**
Staphylococcus species		
S.aureus	17 (56.7%)	33 (82.5%)
S.epidermidis	12 (40%)	
S.intermedius	10 (33.3%)	
S.xylosus	12 (40%)	27 (67.5%)
S.chromogens	4 (13.3%)	
S.haemolyticus	8 (26.7%)	
S.hominis	7 (23.3%)	
S.warneri	4 (13.3%)	1 (2.5%)
S.lentus	9 (30%)	6 (15%)
S.hyicus		5 (12.6%)
S.simulans		12 (30%)
S.sciuri	6 (20%)	
S.capitis	4 (13.3%)	
Steptococcus species		
Aerococcus viridans	13 (43.3%)	37 (92.5%)
Streptococcus mitis		3 (7.5%)
Globicatella sanguinis		2 (5%)

Other Gram positive Cocci		
E.facium	11(36.7%)	
E. feacalis	9 (30%)	13 (32.5)
E. durans	8 (26.3%)	
Lactococcus raffinolactis		19 (47.5%)
K. varians	5 (16.7%)	4 (10%)
Micrococcus	4 (13.3%)	6 (15%)
Peptpstreptocuccus Spp		17 (42.5%)
Peptocuccus spp		7 (22.5%)

In the gram positive cocci group, 12 Staphylococci and three Streptococci were isolated. Both *S. aureus* and *S. xylosus* were isolated from more than 50% of the animals. However, S. aureus was the most common and was found to be present in 33 (82.5%) females and 17 (56.7%) in males of the animals. Other staphylococci species identified but not in female were *S. epidermidis*, *S.intermedius* at 12 (40%) and 10 (33.3%) of the males respectively. Of the Streptococci, *Aerococcus viridans* was the most common and was isolated from 37 (92.5%) and 13(43.3%) of the animals both the female and males respectively. In addition other streptococci with a higher no of isolate included *E.feacalis* at 13 (32.5%) females and 9 (30%) in males, *E.facium* bacteria isolates were detected at a higher level of 11 (36.7%) in males. Also isolated were seven other Gram positive cocci of which *Lactococcus raffinolactis* species was the most common and was found to be present in 19(47.5%) of the animals (Table 3). In addition to bacteria, the vaginal swabs were found to harbor yeast cells.

Biochemical identification of yeast cells revealed four species of Candida namely with *C. albicans, C. krusei, C. guilliermondii and C. tropicalis. C. candida* was the most common and was isolated from 16(40%) of the animals (Table 4).

Table 4: Yeast Cells isolated from the glans penis and vagina of the olive baboons.

Yeast cells	**No. (%) of animals with indicated isolate**	
	Males urethra isolates	**Females vaginal isolates**
Candida albicans		16 (40%)
C. Krusei		4 (10%)
C. guilliermondii		6 (16%)
C. tropicalis		5 (12.5%)
C. candida		8 (20%)

The vaginal PH

The olive baboon vaginal pH ranged between 4.5 and 7. The mean baboon vaginal pH I n this study was 5.2±1.1 ± SD).

CHAPTER SIX

6.0 Discussion

The baboon vagina is a dynamic and complicated environment composed of varying microbiological species in variable proportions. Many cultivation based studies of human vaginal flora have been conducted to date [Haggerty *et al.,* 2004]. Based on this methodology our study is the second three decades after Skrangalis's [*Skrangalis et al.,* 1979], to assess the baboon vaginal microbial flora and the first comparative study of the male tract and the vaginal microbial flora. In addition, it is the first to report the colonization of the baboon vagina with *Candida* species (Table 4). Despite the limitations of the conventional methods of cultivation including inability to readily cultivate some of the microbes [Estelle *et al.,* 2005, Burton *et al.,* 2002] its significance cannot be disregarded especially in low and middle income countries where resources are limited.

The present study of vaginal and male urethra tract swab samples from the 70 baboons provided valuable insight of what comprise the baboon vaginal microbial ecosystem and male urethra tract which have always lagged behind investigations in other body sites. It reflects an intermediate to high Nugent score of four to six in some animals (Fig. 1a) and seven to ten in others (Fig.1 b-f). Microscopic observation of Gram stained swab smears showed diminished *Lactobacillus* morphotypes and increased presence of Gram positive or variable rods and Gram positive cocci (Fig 1b-f). With the help of identification software we were able to extensively identify a more diverse array of other microbes present or absent in either the females or males including *Leuconostoc lactis*, *Eggerthela lenta*, *Bacillus firmus* and yeast cells that had not been previously reported. However, the current study was unable to identify some previously isolated microbes such as *Morganella Moxarella*, *Proteus* and *Propionibacterium*.

The presence of resilient species of fecal origin such as *Escherichia coli* in more than half the swabs could be explained by the fecal contamination of the external vagina. Due to the close proximity of the rectal and vaginal openings and the posture of the animals, the fecal material from the rectum gets deposited on the peritoneal area causing a great proportion of microbes that emerge from intestinal microbiota to ascend along the perineum to the vagina hence high chances of vaginal contamination by these microbes. In addition, baboons are sexually promiscuous. Males and females usually mate with several members of the opposite sex within a short period of time and this makes it possible to transfer vaginal microbes from one animal to another.

The baboon vagina and male genital tract harbors Gram-positive rods some of which have been considered pathogenic in addition to those considered beneficial to humans. As reported in previous studies [Rivera *et al.*, 2010], our results demonstrated that there was overwhelming diverse composition and appreciable differences in the composition of the microbiota from one individual to another. Though not dominated by *Lactobacilli*, which have been known as the common feature of the normal vaginal microbiota of humans but with gram positive coccus and other rods, the current study identified nine species of *Lactobacillus* some of which have previously been isolated from vaginas of normal asymptomatic women. The most common isolates of *Lactobacilli* included *L. acidophilus and L. rhamnosus* which were isolated from 13 (32.5%) and 12 (30%) of the female animals respectively, although they were not so prevalent in male genital tract than in vagina 6 (20%) (Table 1)

However, a proportion of the female baboons had other lactic acid producing bacteria [Rodriguez *et al.,* 1999, Zhou *et al.,* 2004] other than lactobacillus such as Leuconstoc, Pediococcus and Streptococcus that could play the protective role of *Lactobacilli* in the baboon vagina. Therefore, like in humans, distinct differences also occur in the composition of vaginal microbial flora within the *Papio* species.

In many previous studies in women, the occurrence of high numbers of *Lactobacilli* and pH < 4.5 have been associated with normal flora and being healthy [Boskey *et al.,* 2001, Martin *et al* ., 1999]. Previous studies have also reported that the protective role has largely been accomplished primarily by metabolic activities *Lactobacillus* species that lead to production of lactic acid to lower the vaginal pH, other by-products with antimicrobial properties [Boris *et al.,* 2000, Klebanoff *et al.,* 1991], and competition for nutrients or competitive exclusion [Kaewsrichan *et al.,* 2006]. In consequence, a depletion of vaginal *Lactobacilli* has been directly associated with an increase in the incidence of genital and urinary infections [Nelson *et al.,* 2010]. This may relate to two distinct pathogenic mechanisms with frequency of intercourse being a critical factor: (i) in case of unprotected intercourse alkalization of the vaginal niche enhances a shift from *Lactobacilli*-dominated microflora to a BV-like type of microflora (ii) in case of unprotected and protected intercourse mechanical transfer of perineal enteric bacteria is enhanced by coitus [Verstraelen *et al.,* 2010]. Despite the importance of this defense mechanism to women's health, differences in the species composition of vaginal bacterial communities among women have been found to occur. Cultivation based studies of human vagina samples have shown that a diverse range of other species such as *Staphylococcus, Ureaplasma, Corynebacterium, Streptococcus, Gardnerella, Bacteroides, Mycoplasma, Enterococcus, Escherichia, Veillonella, Bidifobacterim* and *Candida* can be present but often in low numbers

[Larsen *et al.,* 2001, Ley *et al.,* 2006]. Species of *Lactobacilli* that have been cultivated and identified based on phenetic characters include *L. jensenii, L. acidophilus, L. casei, L.crispatus, L. plantarum, L. fermentum, L. cellobiosus, L. brevis, L. minutes, and L. salivarius* [Giorgi *et al.,* 1987, Reid *et al.,*1998]. However, recent studies from asymptomatic and apparently healthy women based on culture-independent molecular profiles [Ravel *et al.,* 2011] have found that most vaginal communities are dominated by four species of *Lactobacillus* (*L. inners, L. crispatus, L. jensenii, and L .gasseri*). While numerous studies have shown that women with high numbers of *Lactobacillus* species have "healthy and normal" vaginal flora, and have equated unhealthy vagina with low numbers of or no *Lactobacilli,* other studies have found that this is not necessarily true [Forney *et al.,* 2006]. The vaginal bacterial communities of healthy women are not always dominated by *Lactobacillus* species. A proportion of women have vaginal communities dominated by lactic acid bacteria other than *Lactobacillus* such as *Atopobium, Strepotococcus* and a number of populations of the order Clostridiales [Zhou *et al.,* 2004]. A recent study [Ravel *et al.,* 2011] of vaginal microbiome of asymptomatic healthy reproductive-women from four ethnic groups namely Caucasian, Asian, Black and Hispanic has revealed that the proportions of each bacterial community varies significantly among the four ethnic groups. Bacterial communities dominated by species of *L. iners, L. crispatus, L. gasseri and L. jensenii* were present in more than 80% of Asian and Caucasian women, but in only about 60% in Hispanic and black women. The vaginas of women from these two ethnic communities are not dominated by *Lactobacillus,* but by a community of a large heterogenous group including *Prevotella, Dialister, Atopobium, Gardnerella, Megasphaera, Peptoniphilus, Sneathia, Eggerthella, Aerococcus, Finegoldia,* and *Mobiluncus*.

In addition, higher median pH values occur in Hispanic (pH5.0±0.59) and black (pH 4.7 ± 1.04) compared with Asian (pH 4.4+ 0.50) and Caucasian women (pH 4.2+ 0.3). This reflected the higher prevalence of communities not dominated by *Lactobacillus* in the two ethnic groups which formed a large heterogeneous group of organisms yet these women had no symptoms of BV.

Previous studies in humans have interpreted elevated pH values to indicate a BV disease state [Schwebke *et al.,* 2002, van de *et al.,* 2002]. These different pH values could reflect differences in the composition of vaginal secretions or in the activities of vaginal bacteria or both. Vaginal bacterial communities not dominated by species of *Lactobacillus* characterized by less production of lactic acid do not necessarily reflect a disease state, but are common and appear normal in black and Hispanic women [Ravel *et al.,* 2011].

Baboon vaginal pH in this study was 5.2±1.1 ($\bar{x}$ ± SD). Our previous studies [Obiero *et al.,* 2013] have found baboon vaginal pH to range between 4.5 and 7, hence comparable to Hispanic (pH5.0±0.59) and black (pH 4.7 ± 1.04) women. One of the ecological functions of vaginal bacterial communities, formation of lactic acid and maintenance of a low pH environment which is highly conserved among women of different racial groups despite the variations seen in community composition, is also evident in baboon vagina. Habits and practices such as personal hygiene, sexual behaviors, and methods of birth control in women may exert strong influence in humans [Schwebke *et al.,* 2009]. However, as in women, it is not clear whether the inter-individual species variation observed is governed by genetically determined factors between hosts which may include among others differences in innate and adaptive immune systems, composition and quantity of vaginal secretions and ligands on epithelial cell surfaces.

This current study has found that the composition of baboon vaginal and male genital tract microbial ecosystem is a reflection of human vaginal flora dominated by some bacterial communities in certain groups of women. However its diversity is comparable to heterogeneous bacterial vaginal communities of healthy women not dominated by species of *Lactobacillus*.

Results of our study suggest that baboons can be used as models to further understand the microbial physiology and immunology of the female and male reproductive tract and for pre-clinical trials of prevention and treatment of infections of the reproductive tract. However, it is not clear whether this diverse vaginal and male genital tract microbial ecosystem or inter-individual species variation observed may predispose the animals to prostatitis and urinary tract infections such as pelvic inflammatory disease, STIs, or risks of pregnancy complications including pre-term birth. An understanding of the ecological function of baboon vaginal and male genital tract microbiota and its relationship to infections of the reproductive tract is essential and needs to be explored to recognize any pathologic abnormalities.

CHAPTER SEVEN

7.0 Conclusion and Recommendation

Upper genital tract (including prostate tissue and vas deferens) is generally germ-free, except in case of infections (including prostatitis and also other prostate diseases). The studies of prostate-specific specimens have given contradictory results yet several investigations indicate that prostatitis patients have frequently abundant plymicrobial communities in their semen, expressed prostatic secretion and/or post-massage urine.

Again, whether this microbiota type is prerequisite, epiphenomenon or consequence of upper genital tract inflammation needs additional studies. Coryneforms bacteria (both culturable and unculturable) have been frequently found from male urogenital tract. They tend to be often overlooked as commensals but some authors have associated them with prostatitis and also urethritis.

Both the baboon (Papio Anubis) vaginal and lower gland penis microbiota is heterogeneous in terms of species composition and is typified by a scarcity of lactobacilli and majority of staphylococci aureus bacteria. Molecular techniques need to be applied to determine antibiotic resistance gene in these *staphylococci aureus*!

Understanding of the ecological function of baboon vaginal and male genital tract microbiota and its relationship to infections of the reproductive tract is essential and needs to be explored to recognize any pathologic abnormalities.

CHAPTER EIGHT

8.0 References

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CHAPTER NINE

9.0 Appendix

IPR/ Ethic and Institutional Review Committee letter