

Development of a vaginal microbicial contraceptive gel (UniPron) for the prevention of sexually transmitted infections and unwanted pregnancies

PhD Thesis

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DECLARATION

This thesis is my original work and has not been presented in any other institution for degree award or other qualifications.

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DEDICATION

This work is dedicated to my parents, the late Rev. Ainea Obiero and Mrs Christine Obiero, daughters Sheryl and Christine for their encouragement support on a course to follow the desires of my heart. May God bless them richly.

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Abbreviations

| | |
|-------------------------------|------------------------------------|
| AIDS | Acquired immunodeficiency syndrome |
| Lab | Albumin |
| ALP | Alkaline phosphatase |
| ALT | Alanine aminotransferase |
| ANOVA | Analysis of variance |
| ARV | Antiretroviral |
| AST | Aspartate aminotransferase |
| ATCC | American type culture collection |
| BV | Bacterial vaginitis |
| CRE | Creatinine |
| EDTA | Ethylene diamine tetraacetic acid |
| HDD | Historical demographic data |
| gm | Grams |
| HIV | Human immunodeficiency virus |
| HSV-2 | Herpes simplex virus-2 |
| H ₂ O ₂ | Hydrogen peroxide |

| | |
|------|--------------------------------|
| IPR | Institute of primate research |
| IRC | Institutional review committee |
| MDG | Millennium development goal |
| NaCl | Sodium chloride |
| NaOH | Sodium hydroxide |
| NHPs | Non-human primates |
| N-9 | Nonoxynol-9 |
| OCs | Oral contraceptives |
| OTC | Over the counter |
| PBS | Phosphate buffered saline |
| PCOS | Polycystic ovary syndrome |
| PCR | Polymerase chain reaction |
| PID | Pelvic inflammatory disease |
| TP | Total protein |
| Rpm | Revolutions per minute |

| | |
|--------|--|
| SIV | Simian immunodeficiency virus |
| STIs | Sexually transmitted infections |
| Spp | Species |
| STR | Systemic replacement therapy |
| UNFPA | United Nations Population funds |
| UNAIDS | Joint United Nations Programme on HIV and AIDS |
| Ure | Urea |
| UTIs | Urinary tract infections |
| WHO | World health organization |

ABSTRACT

Globally, unplanned pregnancies and sexually transmitted infections including HIV and AIDS are huge challenges to women's reproductive health. Development of a female-controlled vaginal preparation that has microbicidal and contraceptive properties has the potential to have widespread impact. Such a dual protection method could prevent unwanted pregnancies and reduce the risk of acquisition and transmission sexually transmitted infections. 'UniPron' is a candidate vaginal product that is undergoing testing and its potential applications include use as a personal lubricant, a vaginal contraceptive and/or a microbicidal product.

As a result of research carried out at the Institute of Primate Research, a premier biomedical World Health Organization collaborating centre in collaboration with a local Pharmaceutical Company-Universal Pharmaceutical Corporation Limited, UniPron was formulated, developed and patented (Patent No. KE 218) as vaginal microbicidal contraceptive gel. Uniron is an acidifying agent with highly buffered low pH that works by lowering the vaginal pH to enhance normal vaginal defence mechanism. It is formulated as a gel with concentrations of 0.4g, 0.8g and 1.2g of citric acid which is the active component. Also formulated and developed is a vaginal lubricating gel (Smuggle) the lubricant in Uniron. These products have since received three Awards namely, the National Council for Science and Technology, Kenya Public Service Innovation and African Union Innovation Awards. This study aimed at determining the safety of Smuggle and Uniron gels as vaginal products; effectiveness of Uniron as a microbicide against sexually transmitted infection pathogens *in vitro* and as a contraceptive using the baboon model (*Papio anubis*). The effect of Smugel was compared with KY Jelly, the commonly used commercial vaginal lubricant worldwide manufactured by the American company Johnson and Johnson. Vaginal pH, blood chemistry, vaginal flora and vaginal and cervical histology were

evaluated at baseline and after administration of 15 ml of the study gels intravaginally twice a week for eight weeks. Microbicidal effectiveness of UniPron was assessed against *Neisseria gonorrhoea*, *Gardnerella vaginalis*, *Haemophilus ducreyi*, *Candida albicans* and *Escherichia coli*. Contraceptive effectiveness of UniPron was assessed by determining the duration the low pH was maintained in the baboon vagina following its administration, its effect on baboon sperm parameters *in vitro* and by time mating sexually active female baboons with males of proven fertility after intravaginal administration of the product. Repeated application of Smugel or UniPron into the baboon vagina did not cause any detectable changes in the safety profiles investigated. In addition, UniPron was found to be effective as a microbicide against *N. gonorrhoea*, *G. vaginalis*, *H. ducreyi*, *C. albicans*, and *E. coli in vitro* and was effective as a non-hormonal vaginal contraceptive in the baboon model. Development of woman-controlled preventive methods that reduce the risk of sexually transmitted infections including HIV and unplanned pregnancies will not only improve human reproductive health which is a fundamental human right, but will also catalyse economic and social development for the realization of the Kenya government vision 2030 and the millennium development goals.

CHAPTER 1: INTRODUCTION

1.1 Background information

Over the past decade there has been increased interest in creating female-controlled vaginal preparation that could be microbicidal contraceptives. High rates of unintended pregnancies and sexually transmitted infections (STIs) seriously jeopardize the reproductive health mission of the United Nations, which seeks to ensure that every child is wanted, every birth is safe and every person is free of STI and human immunodeficiency virus (HIV) (UNFPA, 2013). An estimated 25% of all pregnancies worldwide end in abortions (Sibbald, 2004; Haddad, 2009) and an annual 499 million new cases of curable sexually transmitted infections (WHO, 2013) exponentially increase the risk of HIV and other viral STDs. Globally, unplanned pregnancies and STIs persist as significant threats to women's reproductive health (Randrianasolo *et al.*, 2008; United Nation Report, 2013). Overpopulation, particularly in low and middle income countries, is complicated by the pandemic of STIs including HIV. Preventable and curable STIs continue to severely compromise the health of people in sub-Saharan Africa; up to 85% of the estimated 340 million annual new cases of curable STIs worldwide occur in the continent and STIs account for 17% economic losses because of disease burden (Ezzati *et al.*, 2002). The importance of STIs has been more widely recognized since the advent of the HIV epidemic and there is evidence suggesting that the control of STIs can reduce HIV transmission (Mayaud & Mabey, 2004; Harwell *et al.*, 2003; Chun *et al.*, 2013). Young women aged 15-24 are 2.5 times more likely to be infected with STIs than young men (Minnis & Padian 2005, UNAIDS, 2012) and the high incidence of these infections is owing to heterosexual intercourse. In many cases, STIs go undiagnosed and eventually lead to long-term complications such as infertility and cervical cancer (Chan, 2005). These infections, in addition to unwanted pregnancies continue to cause

disease, disability and death among millions of women despite the prevention options implemented to date (Low *et al.*, 2006; UNAIDS, 2010). Worldwide, studies have demonstrated that condoms when correctly and consistently used are effective against prevention of both unwanted pregnancies and acquisition and transmission of STIs (Sarkar, 2008; Matson *et al.*, 2011), but the uptake of this method is still out of control for women in many societies as they often have little or no power to negotiate the use of condoms with their partners and are unable to protect themselves from non-consensual coercive sex (Centres for Disease Control and Prevention, 2010). As a result, consistent condom use has not reached a sufficiently high level, even after many years of widespread and often aggressive promotion (Potts *et al.*, 2008). In addition, condom use “at last sex” has fallen in all age groups, most dramatically, from 85.2% to 67.4% among men aged 15 to 24 and from 66.5% to 51% in women of the same age (Van der Linde, 2013).

Other physical barriers comprise diaphragms and cervical caps. The typical contraceptive use failure rates for these methods range from 15-30% (WHO, 2007). Despite the existence of a variety of effective contraceptives available, discontinuation or non-use remains high, primarily due to cost, side effects, inconvenient dosing schedules, poor access to prescription products, and/or poor acceptance of the method by the male partner, resulting in the unacceptably high rate of unintended or mistimed pregnancies (Stuart *et al.*, 2013). The use of hormonal contraceptives on the other hand requires absolute observance of contraindications which include actual suspected pregnancy, high risk of vascular disease, past thromboembolic disease, varicose veins of extremities, alular heart disease associated with pulmonary hypertension and thrombosis, arterial hypertension, ischaemic heart disease, active liver disease, porphyria, cholelithiasis, recent gestational trophoblastic disease, and breast and uterine cancer (Debski *et al.*, 2009;

Debski *et al.*, 2010). In addition many hormonal contraceptives have a direct and indirect influence on the susceptibility to STIs including HIV (Beaten *et al.*, 2007; Morrison *et al.*, 2004, Shust *et al.*, 2010) and other adverse effects including nausea, headache, mastalgia, bleeding, change in body weight, decrease in libido and mood swings tending toward depression (Medard & Ostrowska, 2010; Szlendak-Sauer *et al.*, 2009, Debski *et al.*, 2010).

This unacceptable situation demands the development of safe, effective and acceptable female-controlled methods (Mayaud & Mabey, 2004). This highlights that the need to develop and implement preventive strategies continues to be one of the highest public health research priorities and has led to an increased interest in female-controlled methods of STI prevention that also provide contraception (Ballagh *et al.*, 2008). In the midst of the global epidemics of both unwanted pregnancies and STIs, options that provide protection are ideal. Vaginal contraceptive products have been available for many years and usually contain the membrane surfactant nonoxynol-9 (N-9) as one of the main ingredients. However, the major drawback of using the surfactants is their detergent-type cytotoxic effect on vaginal cells which in turn increase the chances of STI/HIV transmission (Wilkinson *et al.*, 2002; van Damme *et al.*, 2002). Successful introduction of new family planning methods must be done in a systematic and strategic way. According to the World Health Organization (WHO), (WHO, 2010) the introduction of contraceptive methods should focus not on a technology-driven approach, but rather on how a new method responds to peoples' needs and rights, as well as on how it enhances overall quality of care and broadens the options available to clients. A strategy that encourages delayed sexual debut, monogamy and condom use is important to control the spread of STI/HIV, but this approach requires a level of female empowerment and control in sexual relationships often lacking for young women. This widespread disparity of gender power relationships makes the

development, evaluation and testing of safe and effective topical vaginal products that are effective against STIs and unwanted pregnancy urgent priority for young female populations. In the absence of a definitive cure or preventative vaccine for some STIs vaginal products with dual protection could offer an alternative or adjunct to condom for prevention pregnancy and guard against the acquisition and transmission of STIs. Current biomedical control techniques under research include vaccines, pre-exposure prophylaxis, treatment for infection prevention and microbicides.

1.2 Problem statement

Despite prevention options implemented to date, STIs, in addition to unwanted pregnancies continue to cause disease, disability and death among millions of women.

1.3 Justification for the study

Two of the major challenges to population health are the failure to reduce family size and STIs despite prevention options implemented to date. They are critical national and global health priorities that require rapid development of additional safe and effective control methods. Physical barriers such as condoms, cervical caps and diaphragm heavily depend on user adherence and partner cooperation and are associated with high failure rates. Highly effective contraceptives such as sterilization, intrauterine devices, and hormonal contraceptives typically provide little or no protection against STIs. In addition, hormonal contraceptive use is associated with several side effects and increased risk of STIs including HIV.

The latest additional tool for STI prevention is male circumcision (Siegfried *et al.*, 2009; Wiysonge *et al.*, 2011), this is also male controlled. The development of vaginal microbicial contraceptives lacking membrane toxicity may offer a significant clinical advantage over the

currently marketed detergent type spermicides. Further research in biomedical prevention is necessary in order to move development toward an easily accessible prevention method forward. Vaginal microbical contraceptives, self-administered agents designed for vaginal use to act at the mucosal surface may provide one such realistic method of intervention. Thus female-controlled protection methods against STIs and unwanted pregnancies are highly desirable and may have great potential for improving human health. The unmet need for women controlled dual protection contraceptive is quickly growing, particularly since women often find it difficult to negotiate condom-use with their partners. The prophylactic approach of vaginal microbicides offers a low-cost, user-friendly option for less-educated and low-income people, and by being virtually imperceptible and women-controlled, they may have an advantage over condoms. In appropriate formulation, these microbicides may also reduce friction-associated trauma duringcoitus (Dezzutti *et al.*, 2012a; and 2012b) and thus further inhibit STI/HIV transmission.

Development of vaginal microbical contraceptive products such as Acidform and Buffergel has been on-going for nearly two decades (Williams *et al.*, 2007; Keller *et al.*, 2012). Several other products are being investigated for their potential as vaginal microbicides and/or contraceptives, but there is none that is available for use by women so far (Obiero *et al.*, 2012a; 2013c). However, the recent success has renewed hopes in testing new vaginal products (Dezzutti *et al.*, 2012a; and 2012b; Cost *et al.*, 2012; Moss *et al.*, 2012), hence the need for further research on the development and testing of such new products.

Non-human primate model, the olive baboon (*Papio anubis*) has been used in this study. Reproductive physiology, fertility mechanism, immunology, pathology and anatomy of female baboons are well characterized and have been shown to be similar to that of humans. It is

therefore a suitable animal model for pre-clinical studies for human reproductive biology (D'Hooghe *et al.*, 2008).

As a result of research carried out at the Institute of Primate Research, in collaboration with a Pharmaceutical Company-Universal Pharmaceutical Corporation Limited, UniPron was formulated, developed and patented (**Patent No. KE 218**) as a vaginal microbicial contraceptive gel with the potential of preventing transmission of STIs (including HIV) and pregnancy, and acting as a vaginal lubricant. UniPron is an acidifying agent with a highly buffered low pH that works by lowering the vagina pH to enhance normal vaginal defence mechanism. It is formulated as a gel with concentrations of 0.4gm, 0.8gm and 1.2gm of citric acid which is the active component. The lubricating property of UniPron was further developed into two other products, Smugel and Smuscan, for improvement of sexual and reproductive health and maternal health respectively.

Development of simple preventive methods that are designed first and foremost for women especially the poor living in the developing world will be an effective strategy for empowering women in reproductive health. If developed as a microbicial contraceptive, UniPron will improve human reproductive health and therefore catalyse economic and social development. Stimulation of social and economic growth will no doubt accelerate the attainment of millennium development goals and the Kenya government Vision 2030.

1.4 Research questions

- i. What local and systemic side effects are associated with the use of Smugel and UniPron vaginal gels?
- ii. Do vaginal gels Smugel and UniPron reduce motility of the baboon spermatozoa?
- iii. Does UniPron vaginal gel have microbicidal effects on STI causing pathogens?
- iv. Is UniPron effective as a vaginal contraceptive gel in the baboon model?

1.5 Null Hypothesis

The use of vaginal products Smugel and UniPron is not associated with local and systemic side effects and UniPron is effective as reversible non-hormonal vaginal contraceptive gel.

1.6 Objectives

1.6.1 Overall Objective

To contribute towards improving human reproductive health by evaluating the safety, microbicidal and spermicidal effects of UniPron *in vitro* and its effectiveness as a reversible non-hormonal vaginal contraceptive gel in the baboon model.

1.6.2 Specific Objectives

- i. To evaluate the safety of Smugel and UniPron gels when applied vaginally.
- ii. To establish the effect of UniPron gel as a microbicide *in vitro*.
- iii. To determine the effect of UniPron gel on sperm function *in vitro*.
- iv. To establish the duration of efficacy and reversibility of UniPron gel in the baboon vagina.
- v. v) To determine the effectiveness of UniPron as a non-hormonal vaginal contraceptive gel.

CHAPTER 2: LITERATURE REVIEW

2.1 Physiology and ecology of the vagina

The vagina is lined by non-keratinised stratified squamous epithelium, which overlies a loose connective tissue, stroma, and has no glands. This specialized epithelial layer forms a natural barrier to pathogens (Eschenbach *et al.*, 2000; Farage & Maibach, 2006), has distinctive architectural features including basal cells serving as progenitors to the apical layers that are often anucleate, elongated and frequently are sloughed into the vaginal cavity (Hladik *et al.*, 2007; Farage & Maibach, 2011). The intermediate cell layers contain substantial amounts of glycogen and mucins that localize to vacuoles also present in basal cells (Ayehunie *et al.*, 2006; Farage & Maibach, 2006; Farage & Maibach, 2011). Furthermore, tight junction complexes are a hallmark of the vaginal mucosa (Siddique, 2003). Under specific conditions, the permeability of these complexes is altered to permit passage of innate immune effector molecules secreted by the vaginal epithelial cells. Antigen presenting cells which include macrophages and Langerhans cells are the most abundant cells in the vaginal epithelium (Hu *et al.*, 2000). Major components of the vaginal secretions that transudate through the vaginal walls include desquamated epithelial cells, cervical mucus from the upper genital tract and leucocytes. Oestrogens and sexual stimulation are examples of factors, which increase vaginal fluid. Major organic constituents of the vaginal fluid are proteins, carbohydrates, and fatty acids. The largest production of organic acids arises from metabolic by-products of vaginal bacterial flora, which cause vaginal odour, and show cyclic changes except in oral contraceptive users. The vaginal microbiota form a mutually beneficial relationship with their host and have major impact on health and disease

(Bing 2013 *et al.*, 2012). Thus, the presence of these metabolic by-products reflects the predominant microbial flora. All women produce acids, primarily acetic acid and lactic acids in the vagina. Vaginal bacteria are the primary source of lactic acid in the vagina (Boskey *et al.*, 2001). The acid production normally increases during the mid-menstrual cycle and decreases during luteal phase. In sexually active women, the levels of oestradiol and progesterone vary significantly under different natural and therapeutic conditions. The thickness of the ectocervical and vaginal epithelium change significantly throughout the course of the cycle. Thickness increases during the follicular phase, reaching a peak at around the time of ovulation, and then decreases. By the late luteal phase the epithelium is at its thinnest level (Ma *et al.*, 2001). The physiology and ecology of the vagina is highly susceptible to numerous endogenous and exogenous influences including microbial antibiosis, availability of receptors for adhesion to the vaginal epithelial, hormonal changes, disease states, drug therapy sexual activity and immunologic status of the person (Mastommarino *et al.*, 2002).

2.2 Vaginal lubrication and sexual intercourse

Insufficient lubrication during intercourse is a common complaint for women at all life stages. Although often associated with older patients, over 40% of women in the reproductive age also report intermittent episodes of vaginal dryness, occasional dyspareunia and post coital bleeding (Oberg *et al.*, 2004). Approximately 60% of post-menopausal women experience atrophic vaginitis, a common condition related to oestrogen deficiency, which results in cellular tissue changes characterized by dryness, itching, burning, irritation and dyspareunia. Secretions from Bartholin's secretory glands and vaginal wall decrease leading to reduced lubrication of vagina hence development of significant dyspareunia (Goldstein, 2010). The consequences of

dyspareunia can be both physical and psychological because of the associated difficulty of maintaining a sexual relationship.

The normal aging process of menopause is the most common aetiology of oestrogen deficiency. However, there are external causes for the interruption of ovarian oestrogen production such as radiation, chemotherapy, immunological disorders, surgical removal of ovaries, medications aimed at treating uterine fibroids, cigarette smoking (Lynch, 2009), and lactation (Palmer & Likis, 2003). Women use a wide range of products applied in a variety of ways inside the vagina to perform intravaginal practices such as cleansing and lubrication for a range of reasons including alleviation of vaginal symptoms and management of their sexual relationships (Martin *et al.*, 2010; Hoffman *et al.*, 2010). These practices could have adverse health consequences which include physical or chemical abrasions that could be exacerbated during sexual intercourse and directly increase HIV risk in the vaginal epithelium or cause mucosal inflammation that may lead to recruitment of HIV target cells. In addition, they may also indirectly increase the risk of HIV transmission by disrupting vaginal flora and increasing vaginal pH (van de Wijgert *et al.*, 2000; Atashili *et al.*, 2008) leading to bacterial vaginosis which is an established risk factor for HIV acquisition in many prospective studies. Vaginal stratified squamous epithelium is an important barrier to infection but, physical, chemical or biological factors associated with intravaginal practices could allow HIV to infect intraepithelial Langerhans cells or be taken up by migratory dendritic cells and disseminated to regional lymph nodes (Shattock & Moore, 2003). Frequent use of lubricants containing detergents, such as nonoxynol-9, a detergent-type spermicide with a long history as a contraceptive, has been found to increase the risk of HIV transmission possibly by increasing mucosal irritation, epithelial and

vaginal flora disruption, and increased risks of acquisition of other sexually transmitted infections (Wilkinson *et al.*, 2002a; and 2002b; van Damme *et al.*, 2002).

Current non-prescription treatment designed to lessen symptoms of vaginal dryness and dyspareunia include over-the-counter topical preparations. Personal lubricants such as petroleum jelly, saliva, oil and water are also commonly used to alleviate dryness and discomfort during intercourse (Gallo *et al.*, 2010). The use of oil-based lubricants which contain mineral oil as a common component may weaken the integrity of latex condoms (Voeller *et al.*, 1989). Most personal lubricants are currently regulated as medical devices with little published information on product-specific vaginal tolerance or irritation potency, despite their frequent use by millions of women. In contrast, newly developed drug formulations are thoroughly evaluated for irritation potential, because inflammation can increase disease transmission (van Damme *et al.*, 2002; van De Wijgert *et al.*, 2001). To date, majority of candidate microbicides, including Tenofovir gel which has been found to be effective in reduction of HIV by an estimated 39% overall, and by 54% (Abdool Karim *et al.*, 2010) in women with high gel adherence are formulated as gels to act as lubricants when used during sex. Exogenous lubrication does not only alleviate vaginal symptoms, but also improves sexual health and intercourse by increasing sexual pleasure in both partners. A better understanding of vaginal lubricant use is thus important because of their possible contribution to the acquisition of STIs including HIV.

2.3 Vaginal flora in humans

The microbiota normally associated with the human body have an important influence on human development, physiology and immunity. The vast majority of these indigenous microbiota exist in a symbiotic relationship with their human host, although few are opportunistic pathogens that

can cause both chronic infectious and life-threatening diseases. These microbial communities are believed to constitute the first line of defence against infections by completely excluding invasive nonindigenous organisms that cause diseases (Dethlefsen *et al.*, 2007). The human vagina and the bacterial communities that reside therein are an example of this finely balanced mutualistic association. In this relationship, the host provides benefits to the microbial communities in the form of the nutrients needed to support bacterial growth. This is of importance as bacteria are continually shed from the body in vaginal secretions, and bacterial growth must occur to replenish their numbers. Some of the required nutrients are derived from sloughed cells, while others are from glandular secretions. On the other hand, the indigenous bacterial communities play a protective role in preventing colonization of the host by potentially pathogenic organisms, including those responsible for symptomatic bacterial vaginosis, yeast infections, STIs and urinary tract infections (van De Wijger *et al.*, 2000; Wiesenfeld *et al.*, 2003).

Recent studies have shown that several distinct kinds of vaginal communities with markedly different species composition occur and the frequency of these types of microbiota varies in different ethnic groups (Zhou *et al.*, 2007; Zhou *et al.*, 2010; Ravel *et al.*, 2011). It is hypothesized that differences in species composition may correlate with how vaginal communities respond to disturbances (Sundquist *et al.*, 2007; Zhou *et al.*, 2007). Conceptually this is important because vaginal communities continually experience various kinds of chronic and acute disturbances caused by human behaviours such as use of antibiotics, hormonal contraceptives and other methods of birth control, sexual activity, vaginal lubricants, douching and other intravaginal practices, in addition to many other intrinsic factors such as the innate and adaptive immune systems of the hosts (Turnbaugh *et al.*, 2007). Further, a disturbed state itself

may constitute the clinical syndrome known as bacterial vaginosis (BV), which as a disruption of ecological equilibrium is believed to increase the risk of invasion by infectious agents.

2.3.1 Bacterial vaginosis

Abnormal vaginal flora, probably due to bacterial vaginosis (BV), is a highly prevalent vaginal disorder in reproductive-age women, but its diagnosis and treatment are disappointing and often ineffective. BV is often vaguely characterized as the disruption of the equilibrium of the normal vaginal ecosystem. It is one of the most common gynecological disorders 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* spp; and anaerobic *Peptostreptococcus* species, with a consequent increase in vaginal pH. This is believed to contribute to the development of pelvic inflammatory diseases (PID), (Ness *et al.*, 2005), reproductive tract infections, obstetric sequelae (Goldenberg *et al.*, 1997; Hillier *et al.*, 1995; Meis *et al.*, 1995) adverse pregnancy outcomes including amniotic fluid infection, premature birth, low infant birth weight, histologic chorioamnionitis, postcesarean and post-abortal endometritis (Hillier *et al.*, 1995; Haggerty *et al.*, 2004). In addition, BV is an established independent risk factor for the acquisition of STIs (Martin *et al.*, 1999; Chernes *et al.*, 2003; Wiesenfeld *et al.*, 2003) and the acquisition and transmission of HIV (Martin *et al.*, 1999; Coleman *et al.*, 2007; Spear *et al.*, 2007).

2.3.2 *Lactobacillus* species

Colonization of the vaginal mucosa by certain bacterial species has been found to be beneficial, as they constitute the primary microbiological barrier against infections by vaginal pathogens (Hillier, 1993). Members of the genus *Lactobacillus* are commonly identified as the hallmark of a normal or healthy vagina (Donders *et al.*, 2000; Gupta *et al.*, 1998; Martin *et al.*, 1999) and are the dominant vaginal bacterial species in a majority of women. Since they were first identified by cultivation in vaginal secretions in the late 19th century, *Lactobacillus* species have been thought to play a major role in protecting the vaginal environment from non-indigenous and potentially harmful microorganisms.

In many previous studies, the occurrence of high numbers of lactobacilli and pH of 3.5-4.5 has been associated with normal flora and being healthy (Boskey *et al.*, 2001; Pavlova *et al.*, 2002). Previous studies have also shown that the protective role has largely been accomplished primarily by metabolic activities of *Lactobacillus* species that lead to production of lactic acid resulting in a low and protective vaginal pH (3.5-4.5), other by-products with antimicrobial properties such as broad-spectrum H₂O₂, target specific bacteriocins (Klebanoff *et al.*, 1991; Boris & Barbes, 2000), competition for nutrients or competitive exclusion, (Kaewsrichan *et al.*, 2006; Voravuthikunchai *et al.*, 2006) and by countering inflammation (Fichorova *et al.*, 2011; Rose *et al.*, 2012). It is believed that commensal organism metabolism of the abundant glycogen stores present in vaginal epithelial cells *in vivo* facilitates the symbiotic relationship helping to provide a uniform physical barrier against pathogen adhesion and establishment of infection (Farage & Maibach, 2011; Spurbeck *et al.*, 2011; Boskey *et al.*, 2001). Consequently, the depletion of vaginal lactobacilli due antibiotic therapy, douching, sexual activity, pathologies or

other factors have been directly associated with increased susceptibility to subsequent genital and urinary infections (Martin *et al.*, 1999; Anton *et al.*, 2008 Baeten *et al.*, 2009).

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* (Levison *et al.*, 1977; Reid *et al.*, 1996; Giorgi *et al.*, 1987). Culture-independent methods have also demonstrated that, when surveyed cross-sectionally, several kinds of vaginal communities exist in normal and otherwise healthy women, each with a markedly different bacterial species composition. These communities either are dominated by one of four common *Lactobacilli* species ; *L. crispatus*, *L. inners*, *L. gasseri* and *L. jensenii*, or do not contain significant numbers of lactobacilli, but instead have a diverse array of strict and facultative anaerobes (Zhou *et al.*, 2007; Zhou *et al.*, 2010; Ravel *et al.*, 2011). Vaginal microbiome of asymptomatic healthy reproductive-women from four ethnic groups namely white, Asian, black and Hispanic has revealed that the proportions of each bacterial community varies among the four ethnic groups significantly, with bacterial communities dominated by species of *L. iners*, *L. crispatus*, *L. gasseri* and *L. jensenii* being present in more than 80% of Asian and white women, but in only about 60% in Hispanic and black women (Ravel *et al.*, 2011)

2.3.3 Other types of vaginal microbiota

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*, *Bidifobacterium* and *Candida* can be present but often in low numbers (Larsen & Monif, 2001; Marrazzo *et al.*, 2002;

Redondo-Lopez *et al.*, 1990). 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). Recent studies have found that 20-30% of asymptomatic, otherwise healthy women harbour vaginal communities that lack appreciable numbers of *Lactobacillus*, but include a diverse array of or strictly anaerobic bacteria that are associated with somewhat higher vaginal pH (5.3-5.5) (Zhou *et al.*, 2007; Zhou *et al.*, 2010; Ravel *et al.*, 2011).

The vaginas of 40% of women from two ethnic groups namely Black and Hispanic women are not dominated by *Lactobacillus*, but by a community of a large heterogenous group of bacteria including *Prevotella*, *Dialister*, *Atopobium*, *Corynebacterium*, *Gardnerella*, *Megasphaera*, *Peptoniphilus*, *Sneathia*, *Eggerthella*, *Aerococcus*, *Fingoldia*, and *Mobiluncus* (Zhou *et al.*, 2007; Zhou *et al.*, 2010; Ravel *et al.*, 2011). Higher median vaginal pH values have been reported in Hispanic (pH 5.0±0.59) and black (pH 4.7 ±1.04) compared with Asian (pH 4.4+ 0.50) and white (pH 4.2+ 0.3) women (Ravel *et al.*, 2011). High vaginal pH reflected the higher prevalence of communities not dominated by *Lactobacillus* spp. in the two ethnic groups which formed a large heterogeneous group of organisms yet these women had no symptoms of BV. Many previous studies have interpreted elevated vaginal pH values to indicate a disease state of BV (Schwebke, 2000; van De Wijgert *et al.*, 2000; Zhou *et al.*, 2004).

These different pH values could reflect differences in the composition of vaginal secretions or in the activities of vaginal bacteria or both. The incidence of vaginal bacterial communities with high proportions of phylotypes related to strictly anaerobic bacteria and not dominated by

species of *Lactobacillus*, hence less production of lactic acid, are thus common and appear normal in black and Hispanic women (Ravel *et al.*, 2011). Therefore, it is apparent that there is a highly significant effect of ethnicity on the frequency of bacterial community types among women in different racial groups. Elevated vaginal pH ≥ 4.5 and vaginal communities not dominated by lactobacillus but anaerobic Gram positive bacteria does not necessarily reflect a disease state as interpreted in many previous studies, but is a characteristic of vaginal microbial communities normal to women in some ethnic groups. An appreciable proportion of asymptomatic, otherwise healthy women have vaginal microbiota lacking significant numbers of *Lactobacillus* spp and harboring a diverse array of facultative and strictly anaerobic microorganisms. These studies in humans have shown significant differences in the vaginal bacterial community composition within racial groups in women thus allowing the grouping of women in various categories depending on the dominant community. The findings challenge the common wisdom that the occurrence of high numbers of lactobacilli and a vaginal pH of <4.5 are synonymous with “normal” and “healthy.” Previous studies have hypothesized non-*Lactobacillus*-dominant vaginal microbiota may be nonetheless able to maintain functional vaginal ecosystems by preserving lactic acid production and possibly other important functions (Zhou *et al.*, 2004; Ravel *et al.*, 2011; Gajer *et al.*, 2012,). Vaginal communities dominated by many underappreciated lactic acid bacteria other than lactobacillus such as members of *Atopobium*, *Streptococcus*, *Staphylococcus*, *Megasphaera* and *Leptotrichia* and a number of populations of the order *Clostridiales* are capable of homolactic or heterolactic acid fermentation (Rodriguez *et al.*, 1999; Zhou *et al.*, 2004). The highly diversified microbial community may have accommodated duplications of functions allowing for the stability of the ecosystem in the face of perturbations (Warlde, 2000).

In the absence of symptomology, these types of vaginal bacterial communities might be considered normal or even healthy, although the composition of these communities closely resembles those associated with symptomatic BV. An understanding of the composition of the vaginal microbial ecosystem in relation to vaginal health is essential for comprehensively understanding the aetiology, prevention and treatment of reproductive tract infections.

2.4 Sexually transmitted infections

STIs, defined as infections that spread primarily from person to person through sexual contact are a major public health problem in all regions of the world (WHO, 2010). However, estimates available from the Joint United Nations Programme on HIV and AIDS (UNAIDS, 2010) and the WHO (WHO, 2001) indicate that Africa remains the continent worst affected (Figure 2.1). STIs impose an enormous burden of morbidity and mortality, both directly through their impact on reproductive and child health (Wasserheit, 1989), and indirectly through their role in facilitating the sexual transmission of HIV infection (Lega *et al.*, 1994; Mayaud and McCormick, 2001). In many cases, STIs go undiagnosed and the greatest impact can be seen among women in whom severe complications include pelvic inflammatory disease, chronic pain and adverse pregnancy outcomes (ectopic pregnancies, endometritis, spontaneous abortions, stillbirths, low birth weight) and infertility in both men and women. A growing number of malignancies are attributed to STIs, notably cervical, anal and penile cancers as well as hepatocellular carcinoma. Some STIs lead to congenital infections in the new-born including congenital syphilis, neonatorum ophthalmia and pneumonia (Mayaud & McCormick 2001; Chan 2005; Centers for Disease Control and Prevention, 2010). These infections cause disease, disability and death among millions of women despite the prevention options implemented to date (Low *et al.*, 2006; UNAIDS, 2010). STIs disproportionately affect the health and social and social well being of

women by producing significant impact on their reproductive potential, and adolescent women are at increased risk of STIs due to ignorance of appropriate preventative measures, unplanned or coercive sexual intercourse, where it may be difficult or impractical to negotiate safer sex (Mayaud & McCormick, 2001; Centers for Disease Control and Prevention 2010). The rising impacts of the infections suggest limited impact of prevention approaches to date. Rates of STIs differ significantly between different neighbourhoods and communities (Fichtenberg *et al.*, 2010). The variations are strongly associated with various features of socioeconomic environment such as poverty, income, employment, gender inequalities, food insecurity, violence, educational attainment and access to labour markets, decision-making autonomy, migration, family structure,

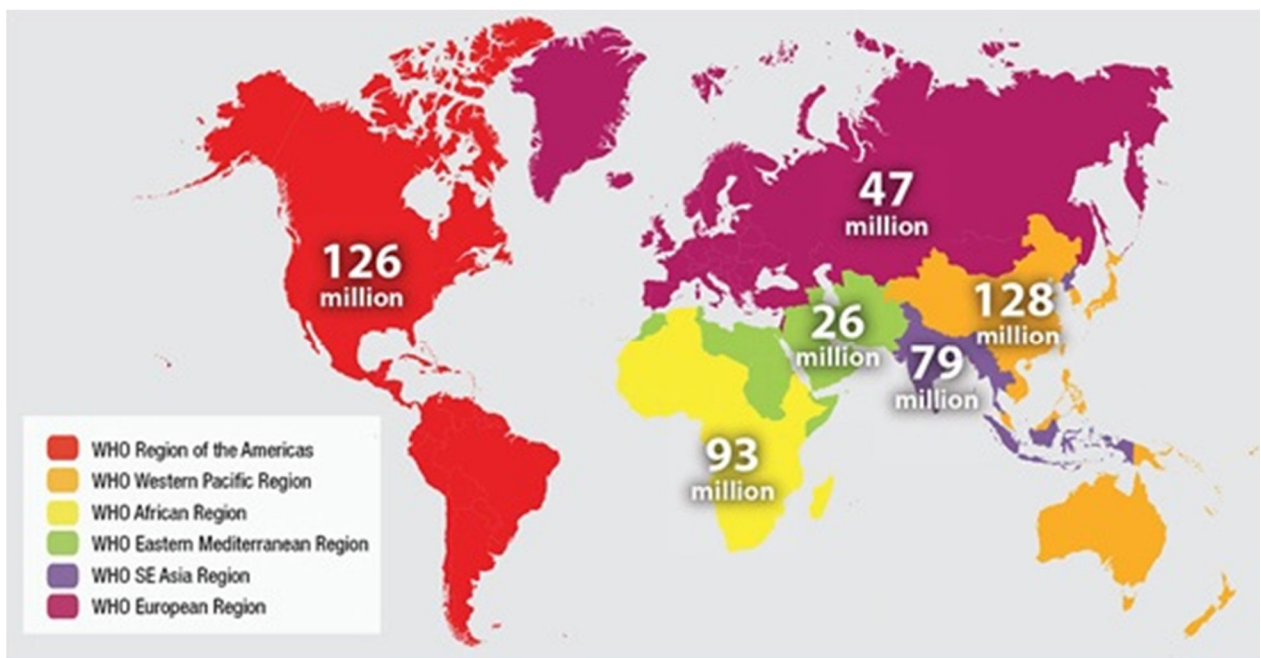


Figure 2.1. Estimated new cases of curable STIs (gonorrhoea, chlamydia, syphilis and trichomoniasis) among adults by WHO region, 2008. Global total 499 million.

community physical disorder, racial/ethnic composition, social capital, and racial/ethnic social marginalization. In particular, poor neighbourhoods suffer disproportionately with high STI rates (Sérgio *et al.*, 2010; Tsai *et al.*, 2012, Vogenthaler *et al.*, 2013).

Studies have demonstrated that condoms when correctly and consistently used are effective against acquisition and transmission of STIs (Sarkar, 2008). Hence condom use remains the key method for the prevention of STIs worldwide (Matson *et al.*, 2010). Existing inequalities in gender based norms and values, reinforce women's lower social and economic status and makes them unable to negotiate safe sex practices. However, in many countries, only a small proportion of the sexually active population use condoms and those who do may do so irregularly and only with selected partners. Barriers to consistent use of condoms include high price in some settings, low availability and inadequate social marketing, but above all, lack of appeal to potential users. Women may also be forced into unprotected intercourse as a result of unequal power relations between men and women (Mayaud & McCormick, 2001). As a result, consistent condom use has not reached a sufficiently high level, even after many years of widespread and often aggressive promotion (Potts *et al.*, 2008). Vaginal infections including vaginal candidiasis and bacterial vaginosis are very common among women in Sub-Saharan Africa and are the leading cause of vaginitis among these women (Hilber *et al.*, 2010).

Despite decades of promotion on prevention methods, there is a rising incidence of several STIs. Preventable and curable STIs continue to severely compromise the health of people in sub-Saharan Africa; up to 85% of the estimated 340 million annual new cases of curable STIs worldwide occur in the continent and STIs account for 17% economic losses because of disease

burden (Ezzatiet *al.*, 2002). The burden of STIs falls primarily on low- and middle income countries with 110 million in Africa, 71 million in South and South-East Asia and 109 million in the Western Pacific regions. In addition many people are infected with non-curable STIs, mainly viral diseases such as HIV and AIDS, hepatitis B or genital herpes. (Looker *et al.*, 2008; UNAIDS, 2010). The importance of STIs has been more widely recognized since the advent of the HIV epidemic and there is evidence suggesting that the control of STIs can reduce HIV transmission (Mayaud & Mabey, 2004; Harwell *et al.*, 2003; van De Wijgert *et al.*, 2001). Despite the availability of several simple, cheap and cost-effective interventions to combat STIs, little progress has been made. An estimated 499 million new cases of curable STIs occurred globally in 2008, suggesting no improvement over the 2005 estimate of 448 million cases. STIs have been overshadowed in recent years by the heightened public-health focus on HIV treatment, despite the strong association between STIs and HIV acquisition. Effective treatment is currently available for several STIs; however, the emergence of drug-resistant organisms (WHO, 2013) and conditions such as BV (Mayaud & McCormick, 2001) may undermine STI control in some settings. There are problems in the effective implementation of control programmes because STIs are not just biological and medical problems, but also behavioural, social, political and economic problems that have not been adequately addressed in the past (Mayau & McCormick, 2001). Prevention of STIs is a fundamental component of global sexual and reproductive health (WHO, 2013).

2.5 Population statistics

A United Nations (UN) report (UN, 2013), projected that the world population will reach 7.2 billion in July 2013, 8.1 billion in 2015, and 9.6 billion in 2050. Much of the increase in the world population between 2013 and 2050 is projected to take place in Africa, where the

population now stands at approximately 1.1 billion, but is expected to increase four-fold to 4.2 billion by 2100. Population growth in the world's developing regions is projected to increase from 5.9 billion in 2013 to 8.2 billion in 2050. In the same period, the population of developed countries is expected to remain largely unchanged at 1.3 billion. In Kenya, for example, the historical demographic data (HDD) indicate that the population was 2.5 million in 1897, 8.2 in 1962, 21.4 in 1989, 28.7 in 1999, 38.6 in 2009 and is currently more than 40 million (HDD, 2013). The huge surge in population is expected to cause mega-city populations to swell, which could worsen environmental woes and overcrowding. However, the projections could change based on three major components namely; fertility, mortality and migration.

2.6 Contraception

Provision of access for voluntary family planning, especially effective contraceptive methods, for women and men is not only crucial to directly improve reproductive health outcomes. It is also positively associated with improvements in health, schooling and economic outcomes (Ahmed *et al.*, 2012; Cleland *et al.*, 2012; Caniing & Schultz, 2012). Family planning programmes in sub-Saharan Africa show varying success in reaching all social segments, but inequities persist in all countries (Creanga *et al.*, 2011). Contraceptive use supports maternal and reproductive health by averting unintended and closely spaced pregnancies and reducing unsafe abortions. Unwanted pregnancy is the major precursor of abortion which is a major contributor to maternal morbidity and mortality not only in adolescents, but also in married women (Asekun-Olarinmoye *et al.*, 2013). Not surprisingly, maternal mortality in Africa tends to be lower in countries where levels of contraceptive use and skilled attendance at birth are relatively high. These countries are mostly found in Northern Africa and Southern Africa (Figure 2.2). The Millennium Development Goal (MDG) report shows that Sub-Saharan Africa as a whole has the

world's highest maternal mortality ratio, contraceptive prevalence of only 25 per cent and low levels of skilled attendance at birth (UN-MDGs report, 2013). The high population growth has contributed to environmental degradation, increased poverty and a deteriorating quality of life for the majority of the people in the region (Palamuleni, 2013). Some observational studies (Morrison *et al.*, 2004; Mohllajee *et al.*, 2006) have suggested that hormonal contraception may increase the risk of acquisition of STIs and that progestins increase susceptibility to a variety of STI pathogens in animal models (Achilles *et al.*, 2002; Kaushic *et al.*, 2003). The causal mechanisms of this putative effect are not well known. One hypothesis is that contraceptive

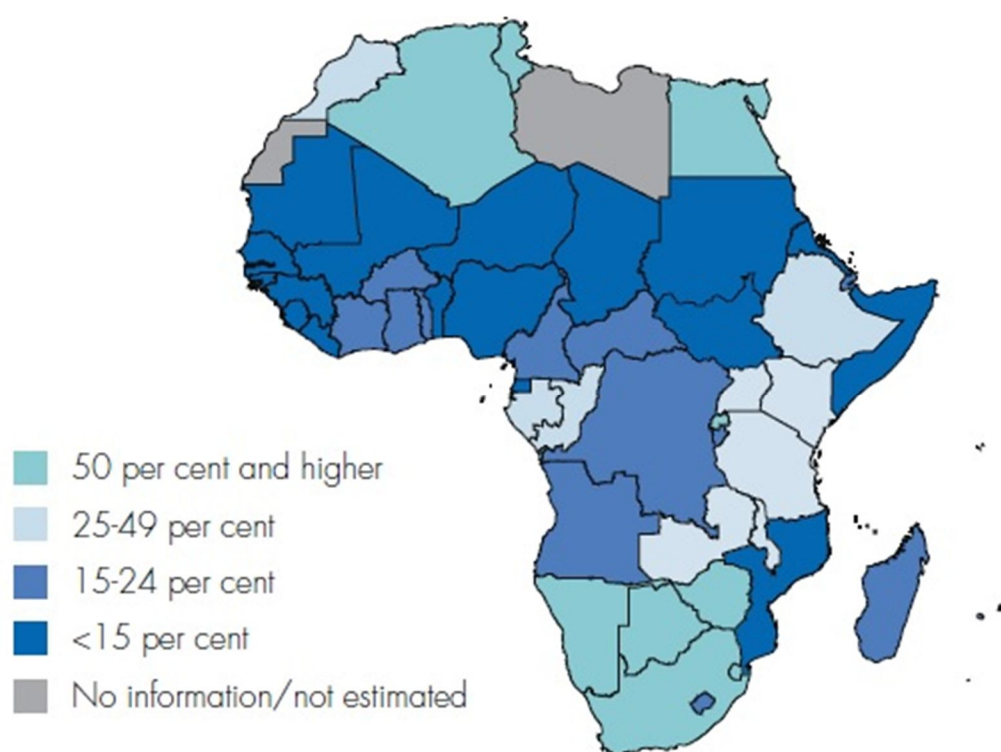


Figure 2.2.Proportion (%) of women aged 15-49, married or in union, who are using some method of contraception, most recent survey (2000-2012)

hormones alter genital tract epithelial structure, that is, epithelial type, and/or pathogen receptors, thereby making the tissue more susceptible to infection (Jacobson *et al.*, 2000; Khanna *et al.*, 2002). Another hypothesis suggests that progestin based hormonal contraception alters the immune system (Truvona *et al.*, 2006).

Progesterone thins the vaginal epithelium, increases the vaginal pH, reduces the amount of cervical mucus and increases its viscosity. In the rhesus macaque model, it has been shown that progesterone treatment results in atrophy of the vaginal epithelium and increases incidence of systemic simian immunodeficiency virus (SIV) infection after intravaginal exposure (Marx *et al.*, 1996). A thin vaginal epithelium may allow more virions to move through it, or susceptible target cells such as Langerhans dendritic cells may be more exposed in a thinner epithelium, potentiating their infection and spread of virus into the lamina propria to T cells (Miller *et al.*, 1992; Frankel *et al.*, 1996).

Oral contraceptives (OCs) remain the most frequently used therapy for women seeking prevention of pregnancy. Research efforts have been directed primarily towards maximizing the benefits of contraceptives while minimizing their potential risks specifically venous thromboembolic disorders. The effects of OCs on metabolic function have not been comprehensively or systematically studied. Studies evaluating the use of OCs have been performed in populations that differ in anthropometric parameters, age and genetic makeup (Vrbilova & Cibula, 2005). Despite the heterogeneity, there are numerous reports suggesting that OCs could worsen glucose tolerance and augment insulin resistance in both healthy women and those with polycystic ovary syndrome (PCOS) (Mastorakos *et al.*, 2006).

Women who use diaphragm along with a spermicide are at increased risk of acquiring urinary tract infections (UTIs). Studies from several countries employing different methodologies have shown the risk to be approximately 2 to 3.3-fold greater than that for sexually active women not using diaphragm (Fihn *et al.*, 1986). Initially, the mechanism of this effect was attributed to ill-fitting diaphragms that impinged upon the urethra, causing obstruction and residual volume. Subsequent studies have disapproved this notion and have suggested that the effects of the spermicide on the vaginal flora are the dominant factor (Fihn *et al.*, 1986). Women using diaphragm/spermicide, even those with no history of UTI, typically have higher levels of introital and periurethral colonization with uropathogenic organisms than those using other methods of contraception. The levels of colonization approximate those seen in women with recurrent UTI. Among women using spermicidal foam of diaphragm/spermicides, bacteriuria with *E. coli* after intercourse is more frequent (Stephen *et al.*, 1996).

Accumulating evidence suggests that spermicidal agents promote colonization of the vaginal introitus, an important step in the pathogenesis of UTI, by altering the normal vaginal flora and enhancing adherence of pathogens to the vaginal mucosa (Hooton *et al.*, 1991). *In vitro* pharmacologic concentrations of nonoxynol-9, the most commonly used spermicidal agent in the United States, inhibit the growth of the normal constituents of the vaginal flora, while failing to exert a similar effect on uropathogenic *E. coli*. Moreover, certain strains of *E. coli* demonstrate greater adherence to vaginal cells treated with N-9 than to control cells (Hooton *et al.*, 1991). Because diaphragm/spermicide users are more prone to UTI, bacteria that infect women who use other types of contraceptives may also easily infect them (Stapleton *et al.*, 1991). The presence of genital lesions increases women's susceptibility to HIV by disrupting the epithelial barrier. The ulcerative infections, for example due to herpes simplex virus-2 (HSV-2), have been associated

with greater risk of HIV acquisition than are inflammatory infections like gonorrhoea, chlamydia and trichomoniasis (Gray *et al.*, 2004). However, pro-inflammatory cytokines associated with chronic genital inflammation may dysregulate immune mechanisms that typically limit HIV replication (Decrion *et al.*, 2005). Spermicides currently make a negligible contribution to the contraception options and the commonly used Nonoxynol-9 (N-9) has been found to increase the risk of transmission of HIV and other STIs (Wilkinson *et al.*, 2002), hence the need to test all new spermicides on HIV infection.

2.7 The role of non-human primates in biomedical research

The use of experimental models to investigate aspects of human disease involves highly specific considerations in order to use appropriate model framework. Non-human primates (NHPs) remain the premier animal models for studying the transmission, immunology and pathogenesis of infections. It is often necessary to use NHPs in development and evaluation of new compounds prior to clinical trial with humans (Veazey *et al.*, 2012). The purpose of biomedical research involving non-human primates (NHPs) is to improve diagnostic, therapeutic, prophylactic procedures, understanding aetiology and pathogenesis and to assess the likely risks and benefits to human health. They remain essential for the pre-clinical safety and efficacy testing of candidate products. Research with NHPs plays a role in advancement of human and animal health. NHPs share 96% of human genes, reflect the biological, reproductive, anatomical, physiological, pathological and behavioural make up of humans, hence provide irreplaceable bridge between laboratory studies and clinical use (www.ca-biomed.org). They have continued to play a critical role in medical progress on a wide variety of diseases (Dancet *et al.*, 2013; Kyama *et al.*, 2014). Animal models therefore are uniquely valuable because they allow for controlled experimental studies when comparable data are not available in humans.

2.8 Reproductive status and cyclicity in female baboons

Reproductive status can be assessed by a variety of methods. Sex skin evaluation is the least expensive, quickest, and easiest because it requires no invasive measures at all (Bambra & Chai, 1993). In the baboon, but not in the rhesus or cynomolgus monkeys, it is possible to perform non-invasive perineal skin monitoring to determine the phase of the menstrual cycle. Assessment of the perineal skin has shown that dramatic changes in colour and size occur throughout the cycle. The degree of inflation or tumescence indicates the stage of the cycle in the animal. The baboons are sexually promiscuous and males and females usually mate with several members of the opposite sex within a short period of time. There is no seasonality in births and inter-birth intervals are affected by both environmental and social factors. An adult female weighs 11-15 kg and a new-born 870-945 g. Sexual maturity is attained at about 5 years in both males and females. Females show pronounced sexual swelling around the external genitalia characterized by a bright red-pink colouration which indicates sexual receptiveness during follicular phase of ovulatory cycle (Stevens, 1997). At maximum inflation the skin is deep red-pink and shiny and there is an absolute absence of wrinkles. The sex skin is large and gives the impression of an over inflated balloon. If the animal has been cycling frequently the tautness of the skin is so extreme that cuts appear which bleed. Ovulation occurs in the last two or three days of the maximum inflation of an animal (Bambra & Chai, 1993). The menstrual phase is characterized by flow of the menstrual blood while the luteal phase is characterized by flat, pale and wrinkled sex skin. Menstrual flow variation in the baboon is usually overt. There is a high degree of individualism in duration (3 to 6 days) and extent of discharge (Bambra & Chai, 1993). These cyclic changes in the appearance of the sexual skin usually reflect cyclic fluctuations in the secretion of ovarian hormones during the female cycle. Females give birth to one infant at a time

and the average gestation period ranges from 160–180 days. Females do not mate when they are pregnant or lactating, a period lasting about 18 months. Perineal inflation and deflation correspond with follicular and luteal phase respectively, while ovulation occurs about two days before perineal deflation (Stevens, 1997). Timing of puberty is highly dependent upon the environmental conditions in which the animal has been raised. Nutritional status is the key issue. Animals, which are in the wild, can be as much as one year behind their captive compatriots in age at puberty. Puberty is determined by the onset of cyclicity, which can be expected at about five years of age for female baboons in captivity.

The female baboon is comparable to women with respect to cycle length (33 ± 2 days); duration of menstruation (3 to 6 days); maximum serum estradiol level attained per cycle (245 ± 30 pg/ml) and maximum progesterone level attained per cycle ($11.5\pm$ ng/ml) (Stevens, 1997). The hormonal profile of *Papio* species is characterized by increasing concentrations of circulating oestradiol, which peaks at over 300pg/ml prior to the luteinizing hormone (LH) surge and subsequent ovulation. LH concentration rises from $2\mu\text{g/ml}$ or below to above $10\mu\text{g/ml}$ at the pre-ovulatory spike. Progesterone rises in the first few days of the postovulatory period from less than 1ng/ml to plateau throughout the remainder of the luteal period at around 5-7ng/ml (Bambra & Chai 1993; Stephens, 1997).

2.9 Immunobiology of the reproductive tract in a female baboon

Baboons (*P. anubis* and *P. cynocephalus*) are important NHP models for human disease that are increasingly used for biomedical research in human health studies, including transplantation, cardiovascular physiology, infectious disease, stomatology and reproduction (D'Hooghe *et al.*, 1996). Humans are more closely related to old world monkeys, such as baboons than the new

world monkeys. The phylogenetic proximity (how species have evolved together) is related to antigenic proximity (how close species are immunologically) (Wykrzykowska *et al.*, 1996). In a study to assess the suitability of various anti-human antibodies directed against immunocompetent cells to identify components involved in cellular and humoral immune responses in the immune organs of a female baboon, most anti-human antibodies were found to cross react with the baboon antigens (D'Hooghe *et al.*, 2000). The same reagents were used to evaluate the immunobiology of its reproductive tract. The distribution of immune cells in the reproductive tract of the female baboon has also been found to be comparable to that in the human (D'Hooghe *et al.*, 2000). Owing to closely shared genetic similarities between baboons and humans, cross-reactive human steroid assays, antibodies or polymerase chain reaction (PCR) primers can be used in the baboons in the context of research in other reproductive disorders (D'Hooghe *et al.*, 2006).

2.10 The baboon semen

Electroejaculation is the most commonly used method to obtain sperm in baboons (Bornman *et al.* 1988; Amboka & Mwethera 2003). As part of the development of baboon model for reproduction, baboon spermatology has been documented based on semen volume, pH, sperm concentration, motility, morphology and size (Amboka & Mwethera 2003; Nyachieo *et al.*, 2012). Baboon semen parameters of breeder males of proven fertility (with at least one infant in captivity) have been described; volume (0.33-0.5 ml), pH (7.2-8.2), motility (41.18-77.7%), sperm concentration 51.5-54.2 million/ml. Fertile breeder baboons have >85% normal sperm morphology and sperm length is ~73.1 μm with inter-individual variability (Nyachieo *et al.*, 2012). Except for larger sperm size and volume, baboon spermatology data are comparable to

those obtained in fertile human donors; pH (7.2–8.0) (Mortimer *et al.*, 1999; WHO, 2010), motility (mean, 53.4%) (Wildt, 1986) and concentration (53.1 million/ ml) (Wildt, 1986),

2.11 Microbicides

Microbicides are products that can be applied vaginally or rectally to kill or disable disease-causing organisms such as viruses, bacteria, fungi or protozoa (D’Cruz and Uckun, 2004; Isaacs *et al.*, 2006). The ideal microbicide would be active against a range of STI-causing organisms, available in both spermicidal and non-spermicidal formulations, effective over relatively long periods, acceptable to potential users, easy to use and affordable, bio-diffusible, bio-adhesive, be effective immediately, be stable at high temperatures, maintain or enhance normal vaginal ecology, preserve or enhance mucosal immunity and neither be irritating to mucosal surfaces nor be absorbed systemically. Because most women at risk for HIV infection are of reproductive age, effective use of dual-function microbicide is important to prevent unintended pregnancies and acquisition and transmission of STIs. Both contraceptive and non-contraceptive microbicides have been under investigation (D’Cruz & Uckun, 2004).

Microbicides that have been developed include detergents that disrupt viral, bacterial and cell membranes; acid-buffering agents that maintain the natural vaginal acidity; sulphated or sulphonated polysaccharides that bind to viruses or bacteria to prevent them from attaching to and infecting healthy cells and antiretroviral (ARV) agents that prevent the replication of the pathogen after it has entered the cell. Some of the microbicide trials have been prematurely terminated due to safety concerns (Obiero *et al.*, 2012a; and 2012b). Further research is ongoing to develop not only vaginal microbicides, but also rectal microbicides for prevention of transmission and acquisition of STIs through rectal intercourse which is currently on the rise in

both men and women (McGowan, 2012). Currently, there is no microbicide in the market but a number are at different stages of experimentation.

CHAPTER 3: INTRAVAGINAL ADMINISTRATION AND SAFETY OF SMUGEL GEL IN BABOONS

3.1 Introduction

Vaginal lubricants are commonly used at personal level to treat pain or dryness during intercourse not only in postmenopausal women, but also in women of reproductive age (Dunn *et al.*, 2002; Oberg *et al.*, 2004). The use of lubricants, such as oils, creams, petroleum jelly, saliva or tested commercial products such as KY Jelly to minimize pain during sex or enhance sexual pleasure is a common vaginal practice (Galo *et al.*, 2010; Hilber *et al.*, 2010). Many over-the-counter (OTC) lubricants have been available for decades and are used frequently during sexual intercourse, but they have not been extensively tested for biological effects that might influence STI and HIV transmission. Substances not specifically marketed as sexual lubricants are also commonly used, especially in low-income households and developing parts of the world where people do not have access to and cannot afford to purchase sexual lubricants. However, it is not clear whether their use as lubricants poses a risk to sexual health (Begay *et al.*, 2011). To date, majority of candidate microbicides, including Tenofovir gel which has been found to have potential in reduction of HIV by an estimated 39% overall and by 54% (Abdool Karim *et al.*, 2010) in women with high gel adherence are formulated as gels to act as lubricants when used during sex. A better understanding of vaginal lubricant use is thus important because of their possible contribution to the acquisition of STIs including HIV. The safety of Smugel, the lubricant in UniPron was tested in the baboon model and was compared with KY Jelly, a commercial vaginal lubricant commonly used worldwide.

3.2 Materials and Methods

3.2.1 Animal selection

Ten healthy sexually mature cycling female olive baboons (*P. anubis*) used in this study were housed at the IPR, Nairobi, Kenya. IPR is a WHO Collaborating Centre that ethically utilizes non-human primates to improve human health. IPR research is guided by international and local standards including Primate Vaccine Evaluation Network, the Council for the International Organizations of Medical Sciences and the National Institutes of Public Health Service policies. Animals were handled humanly and all animal procedures and care were conducted in accordance with internationally accepted standard operating procedures. Prior approval for use of baboons was obtained and sample collection performed following approved Institutional Review Committee (IRC) protocols. All experimental animals were fed on commercial monkey cubes with fruits, vegetables and water. 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.

3.2.2 Smugel and KY-Jelly vaginal gels

Smugel

Smugel is water based vaginal lubricating gel and is composed of Carbomer, Sodium Benzoate, Glycerine, EDTA, NaOH, and purified water (aqua).

KY-Jelly

KY Jelly (Johnson & Johnson, 51120 Sezanne, France) is commercially available vaginal lubricant that is commonly used worldwide.

3.2.3 Sample collection and processing

Samples were collected from all the animals to obtain baseline data for vaginal pH, vaginal flora, and blood chemistry, vaginal and cervical mucosal integrity. Thereafter, the animals were randomized into two groups of equal sizes and allocated to treatment with either Smugel or KY Jelly lubricating gels. KY Jelly is commercially available vaginal lubricant worldwide and has been used for a very long time. It was used as a positive control in this study. The packaged 15ml of the study gels in prefilled single use applicators were applied into the vagina of each animal twice a week for five weeks, followed by sample collection during the entire treatment period and after the treatment. During sample collection, the animals were at various stages of the menstrual cycle namely menstrual, follicular and luteal. Vaginal and cervical biopsies were collected at baseline and at the end of vaginal gel application. Before any vaginal insertions for pH assessment, sample collection or gel application, the vulva was disinfected using betadine.

3.2.3.1 Vaginal pH

Vaginal pH was assessed for five weeks before treatment to obtain baseline data. Thereafter the assessment was done during the entire period of application of Smugel or KY jelly and one week post application. The pH was assessed twice per week by placing pH indicator paper (E. Merck, D-6100 Darmstadt, F. R. Germany) on the vaginal wall (Obiero *et al.*, 2008).

3.2.3.2 Clinical Chemistry

Ten (10) ml of blood was collected from all the animals before treatment to obtain baseline data. During treatment, blood was collected from each group at week two, four, six and eight and centrifuged at 1610 rpm for 10 minute (Jouan C422 Labcare USA) to separate plasma samples for subsequent analysis of serum chemistry to evaluate systemic toxicity. The aliquoted sera were used for analysis of total protein (TP-g/dl), albumin (ALB-g/dl), urea (Ure-mg/dl), creatinine (CRE-mg/dl), aspartate aminotransferase (AST-U/L), alanine aminotranferase (ALT-U/L), alkaline phosphatase (ALP-U/L) and total bilirubin (TBIL-mg/dl). All the tests were performed using reagents and methods provided by the manufacturer in a kit (Max-Planck-ring 21-D 65205 Wiesbaden-Germany) and biochemical analysis was performed using Humalyzer 2000 (Human GmbH, D65205, Wiesbaden, Germany).

3.2.3.3 Vaginal microbiology

Vaginal swabs were collected once a week for five weeks from all the animals to establish baseline data. Thereafter, the collection was done weekly from the two groups during the entire period of gel application and one week post application. A moist sterile cotton swab (0.9% NaCl) was aseptically inserted to obtain high vaginal swabs. Three swabs were used to obtain samplings from the anterior vaginal wall. One swab was rolled onto a slide for a vaginal smear which was Gram stained, allowed to air dry and examined. The other two were placed into modified Stuart transport medium and delivered to the laboratory. Vaginal swabs were later removed from the transport medium and used to inoculate Rogosa agar for isolation of lactobacilli and on Trypton Soya agar supplemented with 5% defibrinated sheep blood for isolation of all aerobic and anaerobic bacteria. MacConkey agar was used for Enterobacteriaceae

and Sabouraud dextrose agar for candida subcultures. All culture media used were obtained from Oxoid, Basingstoke, Hampshire, England.

3.2.3.4 Collection of sheep blood

Fifty millilitres of blood was collected per sheep per bleed (Veterinary Laboratories, Kabete, Nairobi). The blood was collected from the jugular vein using a 21 gauge needle after sterilizing the neck region with 70% ethanol. It was then emptied into 100 ml bottle containing glass beads, which had been sterilized by autoclaving. The bottle was continuously shaken and on reaching the laboratory it was placed on a shaker to ensure no clotting of blood occurred before being used in the preparation of blood agar medium.

3.2.3.5 Culture media preparation

The culture media were prepared as per the manufacturer's instructions and dispensed into sterile tubes and plates (petridishes). On gelling and cooling they were labelled, stack and sealed in plastic bags to prevent loss of moisture and to reduce risk of contamination before being stored in a dark room at 2-8 °C.

Preparation of Stuart Transport Medium

Typical formula (g/l): Sodium glycerophosphate 10.0, Sodium thioglycollate 0.5, Cystein hydrochloride 0.5, Calcium chloride 0.1, Methylene blue 0.001, Agar 5.0, pH 7.4 ± 0.2 at 25°C.

Sixteen grams of the medium was suspended in one litre of distilled water, boiled to dissolve completely and dispensed in screw-cap 7 ml bottles. Sterilization was done by autoclaving at 121°C for 15 minutes.

Preparation of Rogosa agar

Typical formula (g/l): Tryptone 10.0, Yeast extract 5.0, glucose 20.0, sorbitan mono-oleate 1.0, Potassium dihydrogen phosphate 6.0, Ammonium citrate 2.0, Sodium acetate (anhydrous) 17.0,

Magnesium sulphate 0.575, Manganese sulphate 0.12, Ferrous sulphate 0.034, Agar 20.0, pH 5.4 \pm 0.2 at 25° C.

Eighty-two grams of the medium was suspended in 1000 ml distilled water and boiled to dissolve completely. 1.32 ml glacial acetic acid was added, mixed thoroughly and heated to 90-100° C in a water bath for two to three minutes with frequent agitation.

Preparation of Tryptson Soya agar supplemented with 5% defibrinated sheep blood

Typical formula (g/l): Pancreatic digest of casein 15.0, Enzymatic digest of soya bean 5.0, Sodium chloride 5.0, Agar 15, pH7.3 \pm 0.2 at 25° C.

Forty grams of the medium was suspended in 1000ml distilled water and boiled to dissolve completely. Sterilization was done by autoclaving at 121° C for 15 minutes. 5-10% sheep blood was added to the sterile agar which had been cooled to 45-50° C.

Preparation of Sabouraud dextrose agar

Typical formula g/l: Mycological peptone 10.0, Glucose (dextrose) 40.0, Agar 15.0; pH 5.6 \pm 0.2 at 25° C.

Sixty five grams of the medium was added to 1000 ml of distilled water and boiled to dissolve completely. Sterilization was done by autoclaving at 121°C for 15 minutes.

Preparation of MacConkey agar

Typical formula g/l: Peptone 20.0, Lactose 10.0 Bile salts 5.0, Sodium chloride 5.0, Neutral red 0.075, Agar 12.0, pH 7.4 \pm 0.2 at 25° C.

Fifty-two grams of the medium was suspended in 1000 ml distilled water and boiled to dissolve completely. Sterilization was done by autoclaving at 121°C for 15 minutes.

3.2.3.6 Inoculation and incubation of culture media

The culture media were allowed to warm to room temperature before being used. Visual contamination or any change in appearance which may indicate deterioration of the media was checked. The swab was gently applied to a small area of a well-dried plate near the peripheral area (the 'well'). A nichrome wire loop of 2 mm diameter, 6 cm long and with thickness of 26 standard wire gauge (swg) was flame sterilized. It was used to thinly spread the inoculum in parallel lines in different segments of the media on the plate to show whether a culture was pure or mixed, and also to provide single colonies for identification. The loop was flame sterilized and cooled between different streaks. All inoculations were carried out in a safety hood cabinet, which was sterilized with 70% ethanol before and after inoculation. Inoculated media were immediately kept at 37°C for at least 12 hours or as long as the microorganisms took to develop cultural characteristics which recognized it. In the plates that had more than one colony, individual colonies were sub cultured to obtain pure colonies. Anaerobic cultures were obtained by placing inoculated media in a ten plate anaerobic jar suitable for use with oxygen removing sachets (Becton Dickinson and Company, Cockeysville, USA). Colonies growing on the Rogosa agar were identified as *Lactobacillus* species on the basis of colonial morphology and Gram stain appearance and negative catalase test. Initial identification for the rest of the isolates was based on colony morphology (cultural characteristics), Gram stain and oxygen availability and catalase test. The isolates were sub cultured on suitable culture media until pure colonies were obtained.

3.2.3.7 Gram stain

Preparation of smears for staining

Every slide was clearly labelled with the animal's number and a drop of normal saline was placed on the slide. Using a flame sterilized loop, a pure colony was picked and emulsified in

normal saline to make a thin preparation on a slide. The smear was spread evenly covering an area of about 15-20 mm diameter. The smear was allowed to air-dry completely and heat fixed by passing the slide, with smear uppermost, rapidly three times through the flame and laid on the back of the hand to ensure too much heat had not been used. The smear was allowed to cool before staining.

Preparation of Gram stain reagents

Acetone-Alcohol decolorize (1 litre):

Composition; Acetone 500 ml, Ethanol absolute 475 ml, distilled water 25 ml.

Distilled water was mixed with absolute ethanol and the solution transferred to a screw-cap bottle. Acetone was added immediately to the solution, mixed, labelled and stored at room temperature.

Crystal violet

Composition: Crystal violet 20 gm, ammonium oxalate 9 gm, Ethanol absolute 95 ml, distilled water.

Crystal violet was weighed and transferred to a brown bottle marked to hold one litre. Absolute ethanol was added and mixed until the dye was completely dissolved. Ammonium oxalate was weighed, dissolved in about 200 ml distilled water and added to the stain to make one litre.

Lugol's Iodine Solution

Composition: Potassium iodide 20 gm, Iodine 10 gm, Distilled water.

Potassium iodide was weighed and transferred to a brown bottle. About a quarter of the volume of water was added, mixed until the iodide was completely dissolved. Iodine was weighed, added to the mixture, made up to 1 litre and stored in a dark place at room temperature.

Neutral red

Composition: Neutral red 1.0, Distilled water 1 litre

Neutral red was weighed and transferred to a bottle of 1 litre capacity. About a quarter of the volume of water was added, mixed until the dye was completely dissolved and the remainder of the water added and mixed.

Gram stain procedures

The fixed smears were covered with crystal violet stain for 30-60 seconds and rapidly washed off with water. The smear was then covered with Lugol's iodine for 30-60 second, washed off with clean water before being decolorized (few seconds) with acetone-alcohol and washed immediately with clean water. Lastly, the smear was covered with neutral-red stain for about 60 seconds, washed off with clean water and air dried.

3.2.3.8 Microscopic observation

The smears were examined under a light microscope (002-10247, N.V-S.A, Axioskop; CarlZeiss, Gottingen, West Germany), first with the x40 objective lens to check the staining and the distribution of bacteria, and then with the x100 oil immersion objective to observe the cellular morphology of bacteria.

3.2.3.9 Biochemical tests and identification of microorganisms

The isolates were subcultured on 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 (Biomérieux[®] 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 coryneform

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

3.2.3.10 Vaginal and cervical biopsies

Every time application of both Smugel and KY Jelly was done and before collection of biopsies, there was physical examination of pelvic (with the naked eye) to assess any signs of mucosal irritation. Vaginal biopsies were collected once from each animal before treatment to obtain baseline data and at the end of the product application, processed and stained using haematoxylin and eosin (H & E). Cervical biopsies were collected at the same time by using an endoscopic cup (Karl Storz, GmbH & Co. KG, Germany) to pinch the epithelium at the external os of the cervix and processed in a similar way.

3.2.3.11 Tissue processing and staining

The biopsy tissues were processed by the paraffin wax infiltration method and stained using H & E.

Tissue processing materials: Fixative (10% neutral buffered formalin), Dehydration solutions (80%, 95%, 100% ethanol), Clearing solutions (Toulene/Xylene), Embedding medium (Paraplast), Embedding rings, Gelatin powder, Wax dispenser (Tissue embedding centre), vacuum incubator, Microtome, Microtome knife or blades, Frosted end microscope slides, Graphite pencil, Vertical staining racks, Coplin jars.

Preparation of 10% Buffered formalin

Composition; Formalin (37-40%), 100 ml, Distilled water 900ml, Sodium phosphate monobasic 4.0 gm, Sodium phosphate dibasic (anhydrous) 6.5 gm

The salts were suspended in distilled water and mixed to dissolve. 100 ml formalin was added, mixed and distilled water added to make 1 litre.

Tissue staining solutions

Materials: Xylene, varying concentrations of alcohol (80%, 95%,100% ethanol), Harris haematoxylin stain, Eosin, Ammonia water or 2-3% lithium carbonate, 0.5% acid alcohol, Staining racks, Tissue sections, Timer.

Preparation of Harris haematoxylin stain

Compostion g/l; Haematoxylin crystals 5.0, alcohol (absolute) 50 ml, ammonium or potassium alum 100 gm, distilled water 1000 ml, mercuric oxide (red) 2.5 gm, Glacial acetic acid 30 ml.

The haematoxylin was dissolved in alcohol and the alum in water using the aid of a low heat on the hot plate and magnetic stirrers. The two solutions were removed from heat and boiled rapidly while agitating for 1 minute. The solution was removed from the heat and mercuric oxide added slowly before reheating to a simmer. Immediately it turned dark purple, it was removed from the heat and placed in a basin of cold water. On cooling, 25-30 ml of glacial acetic acid was added mixed and stored.

Preparation of Eosin solution

Stock solution: Composition g/100 ml: Eosin 1 gm, Distilled water 20 ml, Ethanol 95% 80 ml.

Working solution (1 litre); Eosin stock solution 250 ml, 80% ethanol 750 ml, Glacial acetic acid 5 ml.

Eosin stock solution was added to alcohol and mixed. Glacial acetic acid was slowly added while stirring constantly and the stain filtered before use.

Preparation of acid alcohol, 0.5%

Composition; 70% ethanol 1 litre, Concentrated hydrochloric acid 5 ml.

The concentrated acid was carefully added to 70% ethanol in a strong glass bottle.

Ammonia water

Composition; ammonia 3 ml, Tap water 1 litre.

Twenty five percent (25%) ammonia solution was slowly and carefully added into tap water using a glass pipette.

3.2.3.12 Tissue processing

The tissues were collected and fixed in 10% buffered formalin for two weeks. The tissues were removed from the fixative, cut into 1-2 mm and dehydrated by passing through alcohol as described below;

i) 80% ethanol 1 hr

ii) 95% ethanol 4 hrs

iii) 95% ethanol 2 hrs

iv) 100% ethanol 2 hrs,

v) 100% ethanol 1 hr

vi) 100% ethanol 1 hr

After dehydration, the tissues were cleared by passing them through three changes of Toluene at 1 hour intervals. Thereafter, the tissues were transferred to fresh paraffin bath and placed in a vacuum oven at 58-60° C for 30 minutes to 3 hours to ensure complete paraffin infiltration. This procedure was repeated after one hour. Immediately following infiltration the tissues were transferred to a pre-warmed mold containing warm paraffin (58-60°C) for embedment. The mold was transferred to a cold surface to allow wax block to solidify and blocks stored at 4°C before trimming and sectioning.

3.2.3.13 Tissue staining

The tissues sections (6 μ) were placed on clean labelled microscope slides. The slides, arranged in vertical staining racks were transferred into a dust free oven preset at 58° C for 30-60 minutes. Thereafter the slides were removed from the drying oven, allowed to cool to and stained as described below;

i) Deparaffinization with Xylene 5 minute

ii) Rehydration of the section; 100, 95, 85% ethanol for 5, 5 and 3 minute
respectively

iii) Washing with running tap water 1 minute

- | | | |
|---|--------------|---------------|
| iv) Staining with Harris haematoxylin | | 10-15 minutes |
| v) Washing with running tap water | | 1 minute |
| vi) Decolorizing with 0.5% acid alcohol | | 2 dips |
| vii) Wash with running tap water | | 1 minute |
| viii) Ammonia water | | 45 seconds |
| ix) Wash with running tap water | | 1 minute |
| x) Dehydration | 96% ethanol | 14 dips x2 |
| | 100% ethanol | 14 dips x2 |
| xi) Clearing with Xylene | | 14 dips x 2 |
| xii) Mounting with DPX | | |

3.2.3.14 Microscopic observation

The tissues were examined under a light microscope (002-10247, N.V-S.A, Axioskop; Carl Zeiss, Gottingen, West Germany) using x100 oil immersion objective.

3.2.3.15 Data analysis

The results were analyzed by one-way analysis of variance (ANOVA) using the GraphPad Prism software (Version 3.0). P-values of, 0.05 were considered statistically significant.

3.3 Results

To allow for vaginal pH evaluation, vaginal swab and biopsy collection, the baboon vaginal was opened using a speculum (Figure 3.1).

3.3.1 Evaluation of vaginal pH

Vaginal pH at baseline was determined to have a mean±SD of 5.8±0.8 (data not shown). There was no statistically significant difference in vaginal pH of Smugel treated animals compared with KY Jelly ($p>0.05$) or baseline pH. Similarly, no significant difference was observed one week post treatment (Table 3.1).

3.3.2 Blood Chemistry Parameters

The parameters evaluated included TP, ALB, URE, TBIL, CRE, ALP, ALT and AST. Variations were observed in the levels of parameters evaluate at baseline, during treatment and two weeks post treatment. However, the differences observed were considered not significant ($p>0.05$) (Table 3.2).



Figure 3.1 Opening of a baboon vagina using a speculum to allow for vaginal sampling (pH evaluation, vaginal swab and biopsy collection).

| | Sample points (Weeks) | | | | | |
|-----------------|-----------------------|---------|---------|---------|---------|---------|
| Lubricating Gel | 1 | 2 | 3 | 4 | 5 | 6 |
| Smugel | 5.8±0.6 | 5.7±1 | 5.8±0.1 | 5.8±0.8 | 6.1±0.9 | 5.7±0.8 |
| KY-Jelly | 5.7±0.7 | 5.8±0.8 | 6.1±0.8 | 6.2±1 | 5.8±0.8 | 5.8±1 |

Table 3.1 Values of vaginal pH evaluated during weekly application of 15 ml Smugel or KY-Jelly for five weeks and thereafter one week post application ($\bar{x} \pm SD$). The values are presented as mean±SD and compared with mean vaginal baseline pH of 5.8±0.8 (data not shown on the table).

| Test Component | Baseline | Smugel | KY-Jelly | Post treatment | |
|----------------------------|-------------|-------------|-------------|----------------|-----------|
| | | | | Smugel | KY Jelly |
| Total protein | 6.6±1.5 | 6.2±1.2 | 6.5±0.9 | 6.4±1.5 | 6.5±1.7 |
| Albumin | 4.8±0.6 | 4.8±0.8 | 4.7±0.7 | 4.8±0.3 | 4.7±0.9 |
| Urea | 49.4±6.9 | 44.5±6.9 | 47.6±11 | 46±8.1 | 44.8±9.2 |
| Total Bilirubin | 0.26±0.17 | 0.26±0.13 | 0.25±0.13 | 0.24±0.16 | 0.26±0.18 |
| Creatinine | 1.2±0.4 | 0.9±0.1 | 0.9±0.2 | 1.0±0.3 | 0.9±0.3 |
| Alkaline phosphatase | 263.5±114.9 | 323.6±183.7 | 268.3±109.2 | 289.3±105 | 266.9±116 |
| Alanine aminotransferase | 30.3±10.4 | 23±10 | 24.5±10.5 | 26.6±8.9 | 29.7±9.4 |
| Aspartate aminotransferase | 39.7±12.5 | 33.8±6.6 | 33.9±6.5 | 35.3±8.7 | 36.3±7.1 |

Table 3.2 Blood chemistry of parameters evaluated at baseline, during treatment with Smugel or KY-Jelly lubricating gels and two weeks post treatment ($\bar{x} \pm SD$). The differences observed were not significantly different ($p>0.05$).

3.3.3 Vaginal microbiology

Despite the uniform environmental conditions and diet significant inter individual variations were observed in the type of microflora isolated. The variations were observed both at baseline and during treatment with Smugel or KY Jelly, hence not attributable to application of any of the vaginal lubricating gels. The variations on vaginal flora isolated were also observed one week post treatment (data not shown). Gram positive rods isolated from the baboon vagina included species of *Lactobacilli*, *Corynebacteria*, *Clostridia* and others related to genera *Lactobacillus*. The proportion swabs with *L. acidophilus*, which was the most common species of *Lactobacillus* isolated was 18% (9/50) at baseline and 20% (5/25) in both Smugel and KY-Jelly treatment groups (Table 3.3a). Two Coryneforms namely *C. renale* group and *C. glucuronolyticum* were isolated and *C. glucuronolyticum* was found to be most common. It was isolated from more than 50% of the swabs both at baseline and across the two treatment gel arms; baseline (54%; 27/50), Smugel (84%; 21/25) and KY Jelly (80%; 20/25) (Table 3.3a). Figure 3.2a shows the Gram stained smear of *C. renale* group. Figure 3.2b shows the results of biochemical tests of api strip inoculated with the microorganism which were reported as positive (+) or negative (-) and separated into groups of three and a value indicated for each on the result sheet for api[®] Coryne (Figure 3.2c). Analysis of the biochemical test results (Figure 3.2d) was done using apiweb[™] stand alone V 1.2.1 identification software (Biomérieux[®] SA 69280 Marcy l'Etoile, France).

Several Gram positive cocci were isolated and included species of *Staphylococci* and *Streptococci*. *S. aureus* was the most common *Staphylococcus* species and was isolated from more than 50% of all the swabs both at baseline and across the two treatment gel arms; baseline (52%; 26/50); Smugel (56%;14/27); KY Jelly (68%; 17/25). This was followed by *S. xylosus* baseline (38%;

19/50); Smugel (64%; 16/25); KY-Jelly 60%; 15/25). *Aerococcus viridans* (*Streptococcus viridans*) was found to be the most common *Streptococcus* species and was isolated from more than 50% of the swabs collected from each group of animals; baseline (54%; 27/50); Smugel (64%; 16/25); KY Jelly (68%; 17/25) (Table 3.3b).

Of the Gram negative rods, *E. coli* was isolated from the swabs in all the groups; baseline (18%; 9/50); Smugel (16%; 4/25); KY Jelly (12%; 3/25). Other gram negative rods including *Gardnerella vaginalis*, which are normally considered pathogenic in women, were isolated from the vagina of healthy baboons though in low numbers; baseline (6%; 3/50); Smugel (8%; 2/25); KY Jelly(8%; 2/25). Yeast cell were also isolated from the swabs collected from the baboon vagina. Biochemical identification of yeast cells revealed four species of *Candida* namely with *C. albicans*, *C. krusei*, *C. guilliermondii* and *C. tropicalis* (Table 3.3c). Except for *C. guilliermondii* that was only isolated at baseline (4%; 1/50), the rest of the *Candida* species were isolated from the both at baseline and across the two treatment arms. However, the percentage frequency isolation was very low.

| Gram positive rods | % isolation | | |
|--|-------------|--------|--------------|
| | Baseline | Smugel | KY- Jelly |
| <i>Lactobacillus</i> species | N=10 | N=5 | N=5 |
| <i>L. acidophilus</i> | 18 | 20 | 20 |
| <i>L. brevis</i> | 12 | 0 | 4 |
| <i>L. crispatus</i> | 2 | 8 | 0 |
| <i>L. debrueckii ssp debrueckii</i> | 0 | 0 | 4 |
| <i>L. fermentum</i> | 8 | 20 | 12 |
| <i>L. paracasei ssp paracasei</i> | 0 | 0 | 4 |
| <i>L. pentosus</i> | 2 | 28 | 12 |
| <i>L. plantarum</i> | 4 | 8 | 4 |
| <i>L. salivarius</i> | 12 | 36 | 16 |
| Coryneform | | | |
| <i>Corynebacterium glucuronolyticum</i> | 54 | 84 | 84 |
| <i>Corynebacterium renale</i> group | 25 | 32 | 32 |
| Others | | | |
| <i>Lactococcus raffinolactis</i> | 25 | 12 | 16 |
| <i>Leuconostoc lactis</i> | 20 | 36 | 40 |
| <i>L. lactis ssp lactis</i> | 4 | 8 | 8 |
| <i>Leuconostoc mesenteroides ssp mesenteroides</i> | 4 | 4 | 0 |
| <i>L. mesenteroides ssp cremoris</i> | 2 | 0 | 4 |
| <i>Leisteria gray</i> | 2 | 0 | 0 |
| <i>Bacillus</i> species | 14 | 20 | 16 |
| <i>Clostridium</i> species | 8 | 4 | 2 |

Table 3.3 a. Percentage frequency isolation of Gram positive rods

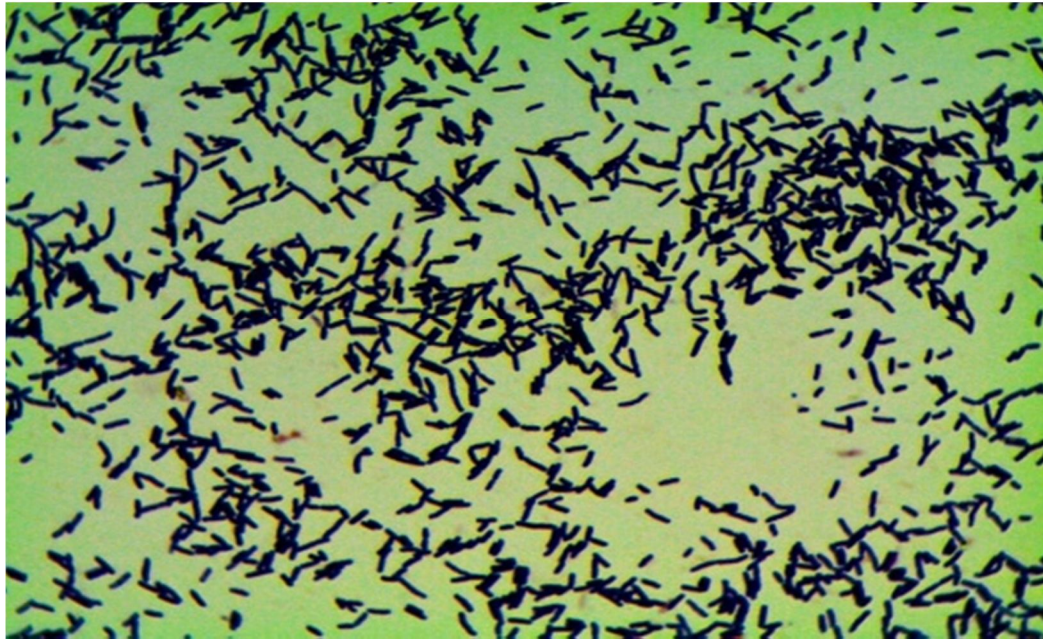


Figure 3.2a Gram stained smear of *C. renale* group showing pleomorphic Gram positive rods. Magnification $\times 100$ oil immersion.

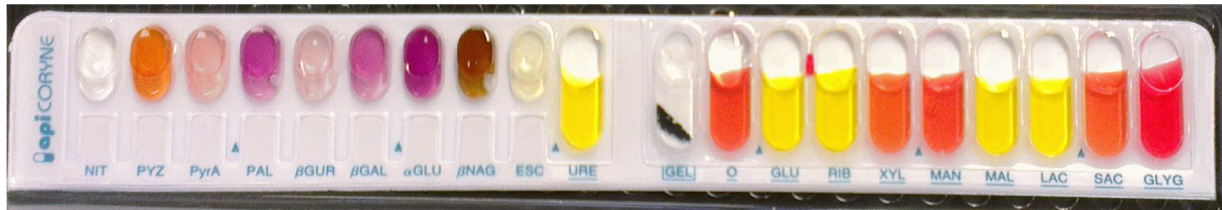


Figure 3.2b. Biochemical test results of inoculated api Coryne strip. The colour change represents either a positive or negative result.

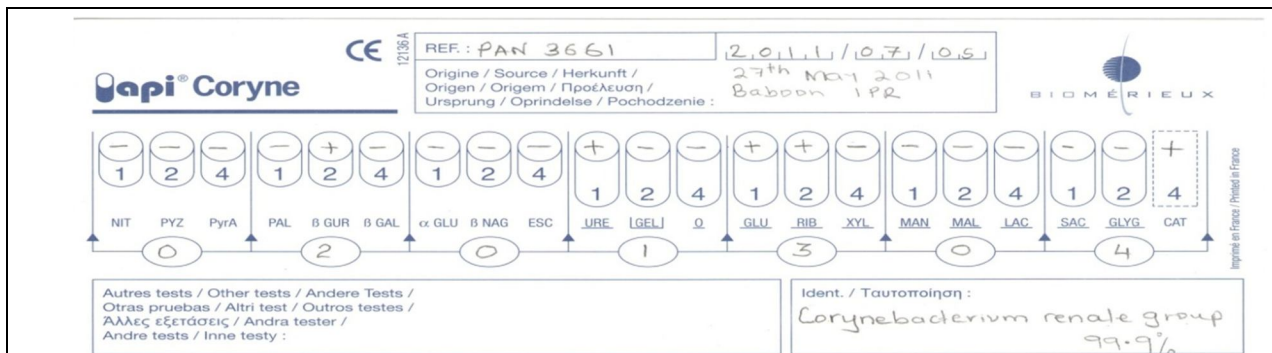
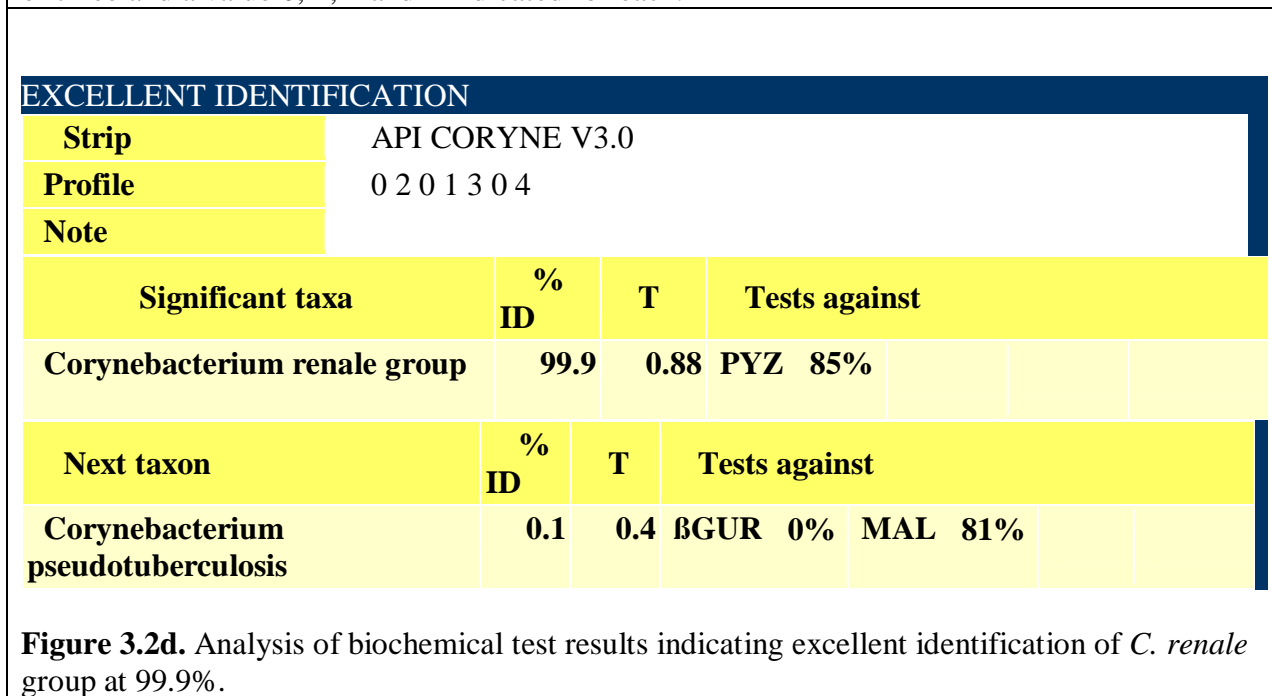


Figure 3.2c. Result sheet of biochemical tests, positive (+) or negative (-) separated into groups of three and a value 0, 1, 2 and 4 indicated for each.



| Gram positive coccus | % isolation | | |
|--|-------------|--------|----------|
| | Baseline | Smugel | KY-Jelly |
| <i>Staphylococcus</i> species | N=10 | N=5 | N=5 |
| <i>S. aureus</i> | 52 | 56 | 68 |
| <i>S. xylosus</i> | 38 | 64 | 60 |
| <i>S. hyicus</i> | 12 | 24 | 28 |
| <i>S. chromogenes</i> | 4 | 8 | 4 |
| <i>S. sciuri</i> | 4 | 12 | 12 |
| <i>S. hominis</i> | 2 | 16 | 20 |
| <i>S. lentus</i> | 6 | 4 | 4 |
| <i>S. simulans</i> | 18 | 12 | 4 |
| <i>S. saprophyticus</i> | 4 | 0 | 4 |
| <i>S. capitis</i> | 2 | 0 | 0 |
| <i>S. haemophylus</i> | 2 | 0 | 0 |
| <i>S. epirdemidis</i> | 0 | 4 | 0 |
| <i>S. warneri</i> | 0 | 8 | 0 |
| <i>S. pneumonia</i> | 4 | 0 | 0 |
| Others | | | |
| <i>Pediococcus pentosaceus</i> | 2 | 4 | 0 |
| <i>Kocuria varians/rosea</i> | 4 | 0 | 0 |
| <i>Micrococcus spp/Kocuria varians/rosea</i> | 10 | 0 | 4 |
| <i>Streptococcus</i> species | | | |
| <i>Aerococcus viridans</i> | 54 | 64 | 68 |
| <i>S. mitis</i> | 0 | 0 | 4 |
| <i>Globicatella sanguinis</i> | 0 | 0 | 8 |

Table 3.3b. Percentage frequency isolation of Gram positive cocci

| Gram negative rods and yeast cells | % Isolation | | |
|------------------------------------|-------------|---------|-----|
| | Baseline | Smulgel | KY |
| Gram negative rods | N=10 | N=5 | N=5 |
| <i>Enterococcus faecalis</i> | 0 | 8 | 24 |
| <i>Escherichia coli</i> | 18 | 16 | 12 |
| <i>Gardnerella vaginalis</i> | 6 | 8 | 8 |
| <i>Klyuveraspp</i> | 0 | 4 | 4 |
| <i>Pseudomonas oryzihabitans</i> | 8 | 4 | 0 |
| <i>P. luteola</i> | 2 | 4 | 0 |
| <i>Stentrophomonas maltophilia</i> | 8 | 0 | 0 |
| <i>Serratia rubidaea</i> | 2 | 0 | 0 |
| <i>Mobiluncuss spp</i> | 5 | 0 | 2 |
| <i>Candida species</i> | | | |
| <i>C. albicans</i> | 12 | 16 | 12 |
| <i>C. krusei</i> | 8 | 4 | 4 |
| <i>C. guilliermondii</i> | 4 | 0 | 0 |
| <i>C. tropicalis</i> | 8 | 4 | 8 |

Table 3.3 c. Percentage frequency isolation of Gram negative rods and yeast cells

3.3.4 Biopsies

Evaluation of histological sections by light microscopy indicated that, in comparison to baseline biopsies, Smugel like KY-Jelly did not induce any detectable vaginal or cervical irritation or histological changes (Fig. 3.3 & 3.4). Both biopsies were collected when at the same time when the animals were in follicular phase of the menstrual cycle. Biopsies in Fig. 3.3 were characterized with increased thickness of the vaginal mucosa of the follicular phase of the menstrual cycle.

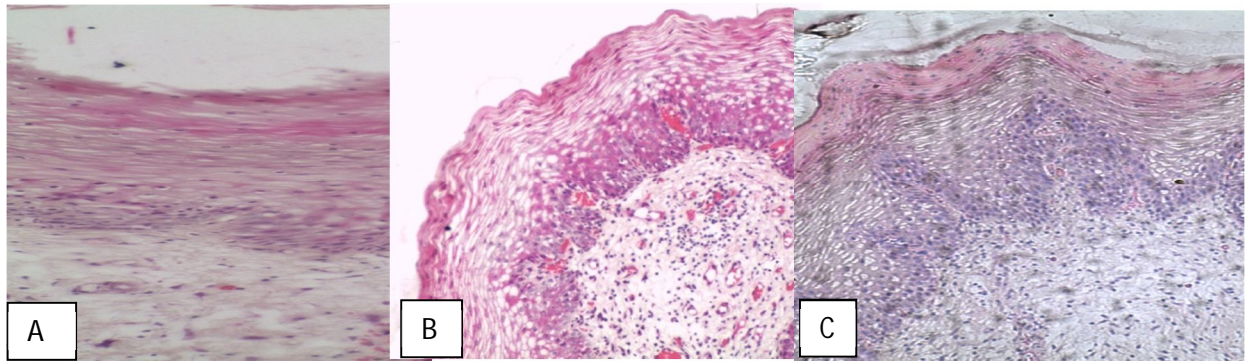


Figure 3.3 Vaginal biopsy tissues at baseline and after application of Smugel and KY Jelly during follicular phase of the menstrual cycle (H & E \times 100). A: follicular biopsy at baseline, B: follicular biopsy after application of Smugel, C: follicular biopsy after application of KY Jelly. No detectable histological changes were observed after application of Smugel lubricating gel.

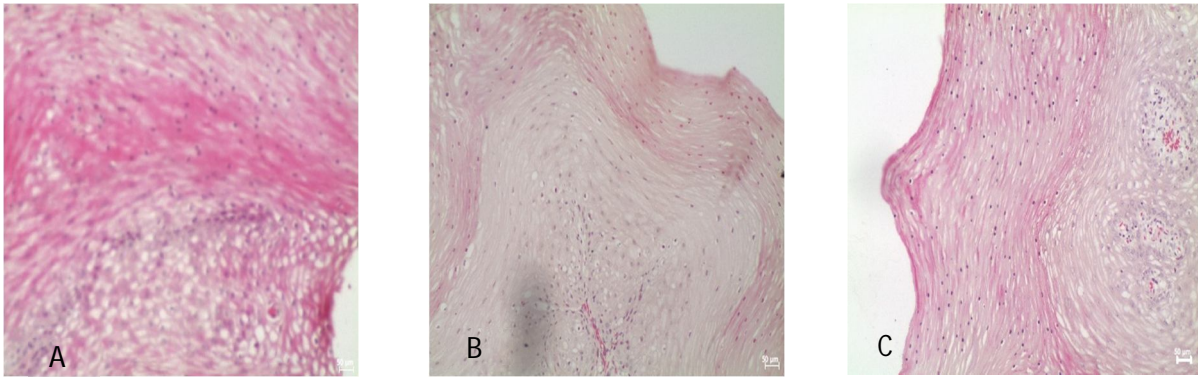


Figure 3.4 Cervical biopsy tissues at follicular phase of the menstrual cycle: A; baseline biopsy, B and C, biopsies after Smugel and KY Jelly application respectively. No cervical histological changes were observed after Smugel application. (H & E \times 100).

3.4 Discussion

Norms and practices regarding lubrication during sex exist in many different countries. Despite significant variation, common themes about vaginal lubrication emerge. In the majority of countries, women's genital hygiene is highly valued and women are expected to achieve a moderate amount of vaginal lubrication during sex that is neither excessive nor inadequate. Women try to achieve this by engaging in a wide variety of vaginal practices (Braunstein & van de Wijgert, 2005; Martin *et al.*, 2010; Hoffman *et al.*, 2010). Coital lubricants are generally used for symptomatic relief of dyspareunia, one of the most common gynaecological conditions. The symptoms do not frequently present as primary complaints to the medical practitioner due to frequent self-medications with OTC lubricants. Dyspareunia is not, however, limited to the peri- or postmenopausal groups with atrophic vaginitis which is an important contributor to postmenopausal sexual dysfunction, but it also affects the reproductive population (Goldstein & Alexander, 2005). It has been shown to affect up to 46% women of reproductive age. Frequent use of some vaginal formulations over long periods may induce mucosal irritation and damage vaginal epithelium which is one of the natural barriers to infection (Wilkinson *et al.*, 2002). Vaginal inflammation and ulceration increase the susceptibility to sexually transmitted pathogens during sexual intercourse (Van Damme *et al.*, 2002).

Symptom relief to date has been based on either low doses of oestrogen applied directly to the vagina (Suckling *et al.*, 2006), or systemic oestrogen replacement therapy (SRT) which may also be given through the vagina to provide better relief than other dosing form (Ballagh, 2005). However, despite the fact that the benefits of SRT in preventing vaginal atrophy and reducing the incidence of related symptoms are well established, estrogen has a mutagenic effect on breast cells and such therapy is thought to be contraindicated in women successfully treated for breast

cancer to avoid stimulating disease progression, and it is not an acceptable option for some populations (Pandit & Ouslander, 1997; Lynch, 2009). While the efficacy of all local therapy has been demonstrated, the safety of systemic hormone therapy remains of concern because of a potential increased risk of breast cancer and heart disease within a subgroup of women (Goldstein, 2010; Palmer & Likis, 2003; Bachmann & Nevadunsky, 2007) and may not cure vaginal symptoms in up to 45% of users (Barlow *et al.*, 1997). As a result, the decision to use topical vaginal oestrogen therapy comes with tremendous stigma and breast cancer survivors presenting with symptoms of vaginal atrophy are often denied estrogen and offered moisturizers and lubricants (Dew *et al.*, 2003). Women who breastfeed have high levels of prolactin that exert an antagonistic action on oestrogen production and may lead to hypoestrogenemia throughout lactation period (Palmer & Likis 2003; Pandit & Ouslander, 1997). A lubricating gel may be used to provide relief from vaginal dryness and dyspareunia. Non-medicated vaginal lubricants or moisturizers offer moderate relief of vaginal dryness, dyspareunia and itching (Nachtigall, 1994). Water based lubricants such as Smugel may provide temporary relief from vaginal dryness and dyspareunia. Because sexual health is a fundamental human right and an integral part of human right, the safety of any lubricating gel is an important parameter to consider when developing such products due to their use on regular basis.

Non-human primate models have been used in pre-clinical research to test the safety, mechanisms of action and efficacy of new diagnostic or therapeutic approaches before they can be used in human (Dancet *et al.*, 2013). In this study, the baboon was used to evaluate the effects of Smugel, a new vaginal lubricant and compared with KY Jelly. Smugel, as with KY Jelly was not associated with any changes in vaginal pH, flora, and clinical chemistry profile, vaginal and cervical integrity of the baboon (Obiero *et al.*, 2012c; 2013b). The inter and intra species

variation observed in the vaginal microbial composition, at baseline, during treatment with both lubricating gels and one week post treatment could not be attributed to the respective gel use. These could have been due to a number of factors including host-specific relationships, composition of vaginal secretions and receptors on vaginal epithelial cell surfaces or vaginal tract tolerance to a variety of different strains of bacteria (Rivera *et al.*, 2010), a feature that might contribute to survival of primate species.

3.5 Conclusion

Compared with KY Jelly, Smugel was found to be safe as a vaginal lubricant and was not associated with local or systemic toxicity when the gel was administered intravaginally in the baboon vagina. Nevertheless, expanded safety studies should be carried out to establish whether its application may lead to inflammation of the vaginal mucosae which may facilitate transmission of STI pathogens including HIV. Its attribute of being water based gives it advantage over oil based lubricants. These results also suggest that Smugel has advantage over presently marketed over the counter vaginal lubricating gels that have no information on their safety profiles. As a locally manufactured product, its accessibility and affordability to the common person in low and middle-countries will be achieved.

CHAPTER 4: INTRAVAGINAL ADMINISTRATION AND SAFETY OF UNIPRON GEL IN BABOONS

4.1 Introduction

The role that the vaginal flora plays in protecting the host from STIs, including HIV infection is becoming increasingly appreciated. Due to increased incidence of STIs, the development of vaginal products with microbicidal activities is a high priority in contraception research (Potts, 1994). Research and development of topical microbicidal contraceptives is now being widely considered, as they would ideally provide a convenient, readily available method of self-protection against STIs in addition to preventing pregnancy. This female-controlled method of protection would also empower women because it offers a system that does not require consent from men. However, a major challenge has been to design mechanism-based products that are highly effective against pregnancy and STIs/HIV infections while lacking detergent-type effects on epithelial cells and normal vaginal flora (Elias & Heise, 1994). Over the past two decades, both contraceptive and non-contraceptive microbicidal vaginal products have been undergoing development and investigation (Ashis *et al.*, 2013; Sokal *et al.*, 2013; Grammen *et al.*, 2014). There is concern over the vaginal and cervical irritation caused by some of the products under investigation because these epithelial changes are thought to increase the risk of STI/ HIV acquisition for women at high-risk for infection, especially with frequent application (Wilkinson *et al.*, 2002; van Damme *et al.*, 2002; Obiero *et al.*, 2012a; and 2012b). It is essential that the evaluation of the potentially harmful effects of these compounds on the vaginal epithelium and/or normal vaginal flora be made before subjecting women to their use. This study aimed to assess the safety of vaginal dosing of 0.4 gm, 0.8 gm and 1.2 gm of UniPron in terms of local

and systemic effects in the baboon model. Smugel gel whose safety profile on the parameters to be assessed has been established (Obiero *et al.*, 2012d; 2013a; and 2013b) was used as a placebo.

4.2 Materials and Methods

4.2.1 Animal selection

Twenty healthy sexually mature cycling female olive baboons (*P. anubis*) used in this study were housed at the Institute of Primate Research (IPR), Nairobi, Kenya. The animal handling was performed as described in section 3.2.1.

4.2.2 UniPron and Smugel vaginal gels

UniPron is an antimicrobial and spermicidal agent that contains citric acid as the active as the active compound. UniPron is a clear fluffy, acidic, water based and non-detergent lubricating gel. The product is heavily buffered with 0.4 gm, 0.8 gm and 1.2 gm concentrations of citric acid having a pH of 3.47, 3.02 and 2.7 respectively. It contains the following raw materials and compounds: Carbomer, sodium benzoate, sodium carboxymethyl cellulose, EDTA, disodium hydrogen phosphate, purified water and citric acid. The buffering agent in UniPron is carbomer (carboxyvinyl polymers of high molecular weights). UniPron therapeutic classification is antifertility and/or microbicide agent. The product is very stable at both room (approximately 22°C) and body temperature and has a shelf life of at least 24 months. It is patented, Patent Number KE 218. UniPron's mechanism of action is by lowering vaginal pH immediately after administration and maintaining the acidity for about 3 hours after which the pH returns to the normal range without causing any detectable irritation on vaginal epithelium. UniPron has the ability to preserve an acidic vaginal microenvironment due to its highly buffered low pH. As a result of research carried out at the Institute of Primate Research, in collaboration with a Pharmaceutical Company-Universal Pharmaceutical Corporation Limited, UniPron was

formulated, developed and patented (**Patent No. KE 218**) as a vaginal microbicide contraceptive gel with the potential of preventing STIs and pregnancy and acting as a vaginal lubricant. Smugel gel (section 3.2.2) was used as the placebo.

4.2.3 Sample collection and processing

Samples were collected from all the animals to obtain baseline data of vaginal pH, vaginal flora, and blood chemistry, vaginal and cervical mucosal integrity. Thereafter, the animals were randomized 1:1:1:1 to receive placebo (Smugel), UniPron 0.4 gm, 0.8 gm or 1.2 gm. The packaged 15 ml of the study gels in prefilled single use applicators were applied into the vagina of each animal twice a week for eight weeks, followed by sample collection during the entire treatment period and after the treatment. During sample collection, the animals were at various stages of the menstrual cycle, that is, menstrual, follicular and luteal phases. Vaginal and cervical biopsies were collected at baseline and at the end of vaginal gel application. Before any vaginal insertions for pH assessment, sample collection or gel application, the vulva was disinfected using betadine.

4.2.3.1 Vaginal pH

Vaginal pH was assessed for five weeks before treatment to obtain baseline data. Thereafter the assessment was done every week during the eight weeks treatment period with Smugel or UniPron and one week post treatment (section 3.2.3.1).

4.2.3.2 Clinical Chemistry

Ten mls of blood was collected from all the animals before treatment to obtain baseline data. During treatment, blood was collected from each group at week two, four, six, eight, ten (two weeks post treatment) and processed and analysed (section 3.2.3.2).

4.2.3.3 Vaginal microbiology

Vaginal swabs were collected once a week for five weeks from all the animals to establish baseline data. Thereafter, the swabs were collected from each of the four treatment groups during the entire period of gel treatment and one week post treatment. The vaginal swabs were processed and vaginal flora determined from Gram-vaginal smears, vaginal culture and biochemical tests (section 3.2.3.3 to 3.2.3.9).

4.2.3.4 Vaginal and cervical biopsies

Every time application of both Smugel and UniPron was done and before collection of biopsies, there was physical examination of the vagina and the cervix to assess any signs of mucosal irritation. Vaginal biopsies were collected once from each animal before treatment to obtain baseline data and at the end of the product application, processed and stained using haematoxylin and eosin (H & E). Cervical biopsies were collected at the same time by using an endoscopic cup (Karl Storz, GmbH & Co. KG, Germany) to pinch the epithelium at the external os of the cervix and processed in a similar version (section 3.2.3.10 to 3.2.3.14).

4.2.3.5 Data analysis

The data were analysed as described in section 3.2.3.15

4.3 Results

4.3.1 Evaluation of baboon vaginal pH

Vaginal pH at baseline was determined to have a mean \pm SD of 5.2 \pm 0.8 (data not shown). The variation observed in vaginal pH during the eight weeks treatment was not statistically significant ($P>0.05$) across all gel arms (Table 4.1). Similarly, no significant difference was observed in vagina pH one week post application.

4.3.2 Blood Chemistry Parameters

The parameters evaluated included TP, ALB, URE, TBIL, CRE, ALP, ALT and AST. The differences observed across all gel arms were considered not significant ($P>0.05$) (Table 4.2). Similarly, no significant differences were observed two weeks post gel application.

| Vaginal gel | Sample points (weeks) | | | | | | | | |
|------------------|-----------------------|----------|---------|---------|---------|---------|---------|---------|---------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Smugel (Placebo) | 5.6±0.8 | 5.5±0.3 | 6.0±0.6 | 5.6±0.5 | 5.9±0.5 | 6.2±0.7 | 5.6±0.5 | 5.6±0.8 | 5.7±0.9 |
| UniPron 0.4 gm | 5.3±0.6 | 5.2± 0.5 | 5.3±0.7 | 6.1±0.8 | 5.6±1.0 | 4.6±0.7 | 5.6±1.0 | 5.6±0.8 | 6.0±0.7 |
| UniPron 0.8 gm | 5.6±1.0 | 6.5±1.0 | 6.0±0.9 | 4.9±0.9 | 5.6±0.5 | 5.5±0.9 | 6.0±1.0 | 5.6±0.9 | 4.7±0.9 |
| UniPron 1.2 gm | 5.4±0.6 | 5.2±0.5 | 5.7±0.6 | 5.7±0.7 | 6.2±0.7 | 5.7±0.8 | 6.1±0.8 | 6.3±0.8 | 5.6±1 |

Table 4.1. Vaginal pH of animals treated with Smugel (Placebo) or UniPron($\bar{x} \pm SD$). The pH was evaluated during weekly treatment with Smugel or UniPron and one week post treatment

| Test component | Baseline | Placebo (Smugel) | UniPron | | | Post treatment |
|----------------|-------------|------------------|-------------|------------|-------------|----------------|
| | | | 0.4 g | 0.8 g | 1.2 g | |
| TP (g/dl) | 6.7±1.5 | 6.1±1.4 | 6.3±0.9 | 5.7±0.7 | 5.7±1.2 | 6.8±1.2 |
| ALB (g/dl) | 4.8±0.7 | 4.7±0.7 | 4.9±0.6 | 4.7±0.9 | 4.7±0.8 | 4.6±0.8 |
| URE (mg/dl) | 50.7±1.0 | 49.4±6.4 | 45.0±6.6 | 50.0±8.1 | 49.0±8.5 | 51±4.5 |
| TBIL (mg/dl) | 0.25±0.18 | 0.27±0.17 | 0.24±0.15 | 0.25±0.19 | 0.26±0.2 | 0.24±0.17 |
| CRE (mg/dl) | 1.1±0.4 | 0.9±0.2 | 1.1±0.5 | 1.0±0.2 | 1.1±0.1 | 0.9±0.4 |
| ALP (U/L) | 254.0±106.8 | 204.4±53.9 | 263.3±111.4 | 239.6±90.1 | 254.9±105.6 | 235.1±119.1 |
| ALT (U/L) | 31.2±10.7 | 27.2±10.0 | 30.7±8.1 | 33.1±7.6 | 32.1±8.9 | 32.8±4.1 |
| AST (U/L) | 43.3±23.6 | 34.8±7.5 | 36.9±7.6 | 34.5±7.5 | 33.5±6.5 | 36.3±8.4 |

Table 4.2. Blood chemistry profiles at baseline, during treatment period with Smugel or UniPron and at two weeks post treatment ($\bar{x} \pm SD$). The chemistry profiles evaluated included TP, ALB, URE, TBIL, CRE, ALP, ALT, and AST.

4.3.3 Vaginal microbiology

A total of 20 sexually mature cycling female baboons were used in this study. Diverse species of both Gram positive, Gram negative bacteria and yeast cells/*Candida* were isolated from the vaginal swabs collected from the animals. Of the Gram positive rods, eight species of *Lactobacilli*, two Coryneform and five other species were isolated. Four species of *Lactobacilli*, namely *L. acidophilus*, *L. brevis*, *L. fermentum* and *L. rhamnosus* were found to be frequent. The proportion of swab results with *L. acidophilus*, for example, were reported as follows; baseline (27%; 17/100); Smugel (23%; 9/40); UniPron 0.4 gm (20%; 8/40); UniPron 0.8 gm 25%; 10/40); UniPron (1.2%; 23/40). Of the coryneform bacteria, both *C. glucuronolyticum* and *C. renale* group were found to be common in the baboon vagina with baseline data having more than 50% of the swabs with both species of bacteria. The proportion of swab with these bacteria was found to be high across the four gel groups (Table 4.3a).

In the Gram positive cocci group, six species of *Staphylococci* and one *Streptococcus* were isolated. The *Staphylococci* included *S. aureus*, *S. xylosus*, *S. hyicus*, *S. chromogenes*, *S. hominis* and *S. lentus*. *S. aureus* was found to be the most common staphylococcus species; baseline (46%; 46/100); Smugel (38%; 15/40), UniPron 0.4 gm (45%; 18/40), UniPron 0.8 gm (33%; 13/40). UniPron 1.2 gm (38%; 15/40). *A. viridans*, the only *Streptococcus* species isolated, was the most predominant bacteria and was found in more than 50% of the swabs both at baseline and across the four gel groups (Table 4.3b). Also isolated were four species of Gram negative rods namely *E. faecalis*, *E. coli*, *G. vaginalis* and *Klyuvera* spp. *E. faecalis* and *E. coli* were more common than the other rods in this group (Table 4.3c). Figure 4.1a shows the Gram stained smear of *E.coli*. Figure 4.1b shows uninoculated strip of api[®] 20E and Figure 4.1c shows inoculated strip with biochemical test results which were reported on the result sheet (Figure

4.1d) as either positive (+) or negative (-). Analysis of the biochemical test results (Figure 4.1e) was done using apiwebTMstand alone V 1.2.1 identification software (Biomerieux[®] SA 69280 Marcy l'Etoile, France).

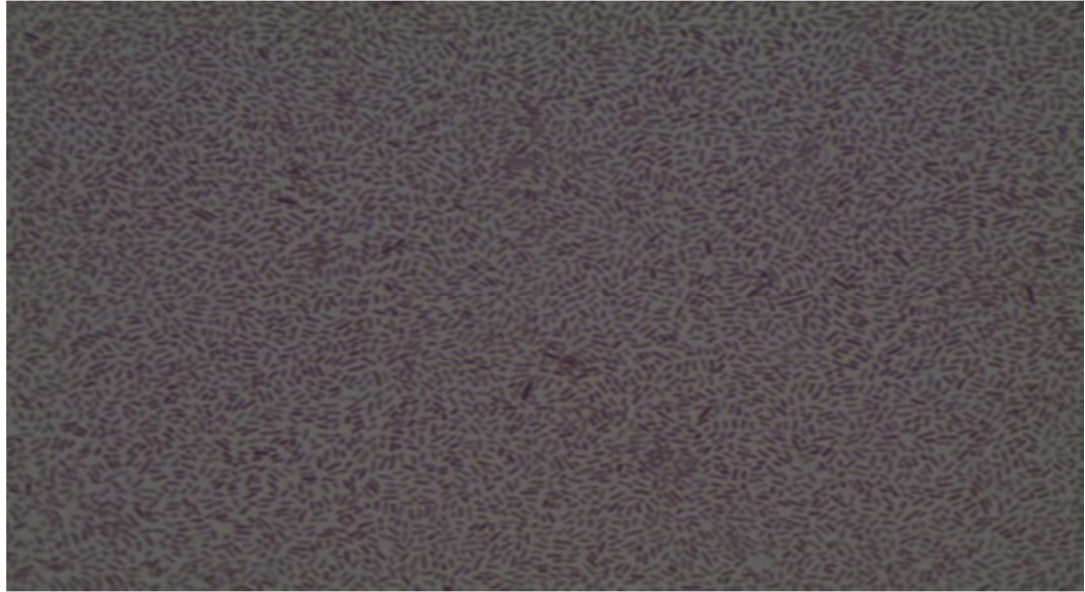


Figure 4.1a. Gram stained smear of *E. coli* showing Gram negative rods. Magnification $\times 100$ oil immersion



Figure 4.1b. Uninoculated strip of api 20E consisting of 20 microtubes containing dehydrated substrates for the demonstration of enzymatic activity or the fermentation of carbohydrates.



Figure 4.1c. Inoculated strip of api 20E indicating biochemical test results of *E. coli*. The colour change represents either a positive or negative result.

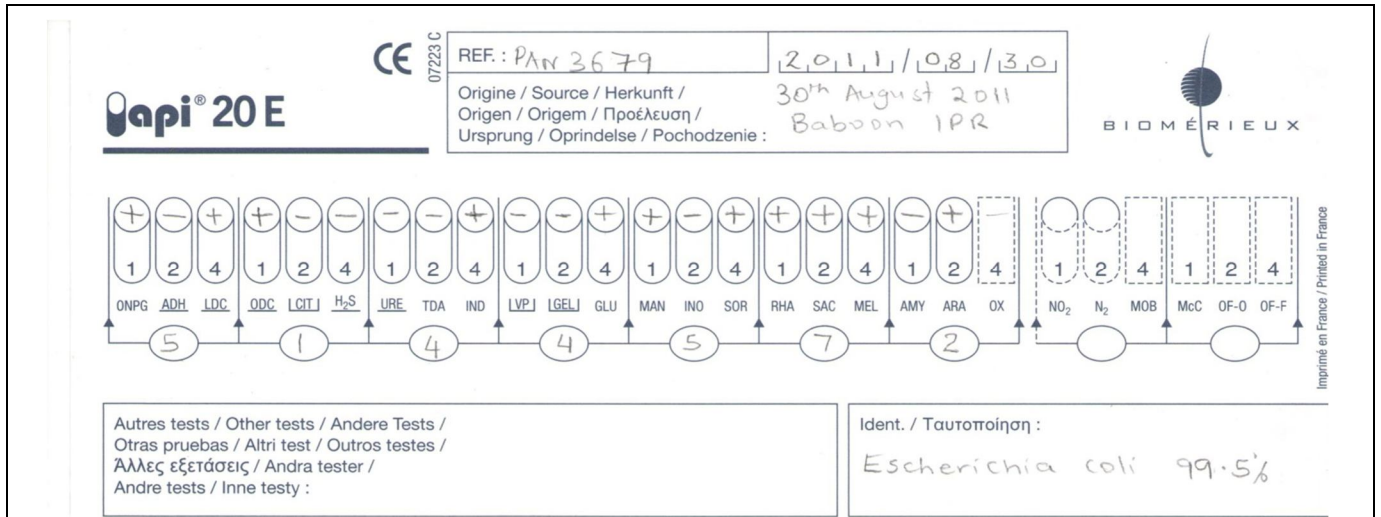


Figure 4.1d. Result sheet of biochemical tests, positive (+) or negative (-) separated into groups of three and a value 1, 2, 4, 5 and 7 indicated for each.

| EXCELLENT IDENTIFICATION | | | | | |
|---------------------------------|----------------------|------------|----------------------|--|--|
| Strip | API 20 E V4.1 | | | | |
| Profile | 5 1 4 4 5 5 2 | | | | |
| Note | | | | | |
| Significant taxa | % ID | T | Tests against | | |
| Escherichia coli | 99.9 | 1.0 | | | |

Figure 4.1e. Analysis of biochemical test results indicating excellent identification of *E. coli* at 99.9%.

In addition to bacteria, the swabs were also found to harbour four species of *Candida*; *C. albicans*, *C. crusei*, *C. guilliermondii* and *C. tropicalis*. *C. albicans* was found to be the most common candida in the baboon vaginal swabs collected (Table 4.3c). A few microbes such as *S. lentus*, *Kluyvera* spp and *C. tropicalis* were not reported at baseline, but later appeared from a few swabs collected during treatment with the study gels. Similarly, a few other microbes including *L. rafterolactis*, *S. chromogenes*, *G. vaginalis* and *C. krusei* were reported at baseline, but did not appear during treatment at certain gel arms. Other than the few missing isolates, the proportion of vaginal swabs with microbes was approximately equally distributed both at baseline and across the four treatment groups (Tables 4.3a, 4.3b, and 4.3c).

| Gram positive rods | % isolation | | | | |
|---|-------------|--------|-------------|------|------|
| | Baseline | Smugel | UniPron gel | | |
| | | | 0.4g | 0.8g | 1.2g |
| <i>Lactobacilli</i> species | N=20 | N=5 | N=5 | N=5 | N=5 |
| <i>L. acidophilus</i> | 27 | 23 | 20 | 15 | 23 |
| <i>L. brevis</i> | 12 | 18 | 10 | 10 | 15 |
| <i>L. crispatus</i> | 4 | 0 | 5 | 5 | 8 |
| <i>L. delbrueckii ssp delbrueckii</i> | 4 | 3 | 8 | 0 | 5 |
| <i>L. fermentum</i> | 21 | 15 | 13 | 18 | 20 |
| <i>L. pentosus</i> | 16 | 28 | 30 | 23 | 30 |
| <i>L. plantarum</i> | 8 | 0 | 0 | 8 | 10 |
| <i>L. rhanmosus</i> | 13 | 18 | 15 | 20 | 13 |
| <u>Coryneform</u> | | | | | |
| <i>Corynebacterium glucuronolyticum</i> | 69 | 40 | 43 | 45 | 33 |
| <i>Corynebacterium renale group</i> | 54 | 35 | 33 | 40 | 25 |
| <u>Other gram positive rods</u> | | | | | |
| <i>Lactococcus raffinolactis</i> | 21 | 0 | 10 | 5 | 5 |
| <i>Leuconosto lactis</i> | 19 | 15 | 13 | 8 | 15 |
| <i>L. mesenteroide sssp mesenteroides</i> | 3 | 3 | 3 | 3 | 3 |
| <i>Bacillus</i> species | 16 | 23 | 13 | 18 | 15 |
| <i>Clostridium</i> species | 11 | 8 | 3 | 4 | 33 |

Table 4.3a. Percentage frequency isolation of Gram positive rods at baseline and during treatment with Smugel (placebo) or UniPron.

| Gram positive cocci | % isolation | | | | |
|-------------------------------------|-------------|--------|-------------|--------|--------|
| | Baseline | Smugel | UniPron gel | | |
| | | | 0.4 gm | 0.8 gm | 1.2 gm |
| <i>Staphylococci species</i> | N=20 | N=5 | N=5 | N=5 | N=5 |
| <i>S. aureus</i> | 46 | 38 | 45 | 33 | 38 |
| <i>S. xylosus</i> | 14 | 10 | 18 | 15 | 18 |
| <i>S. hyicus</i> | 2 | 3 | 8 | 13 | 5 |
| <i>S. chromogenes</i> | 3 | 0 | 0 | 0 | 3 |
| <i>S. hominis</i> | 3 | 0 | 3 | 0 | 3 |
| <i>S. lentus</i> | 0 | 0 | 0 | 3 | 3 |
| <u><i>Streptococcus species</i></u> | | | | | |
| <i>Aerococcusviridans</i> | 71 | 58 | 53 | 55 | 63 |

Table 4.3b. Percentage frequency isolation of gram positive cocci at baseline and during treatment with Smugel (placebo) or UniPron.

| Gram negative rods & Candida species | % isolation | | | | |
|--------------------------------------|-------------|--------|-------------|--------|--------|
| | Baseline | Smugel | UniPron gel | | |
| | | | 0.4 gm | 0.8 gm | 1.2 gm |
| Gram negative rods | N=20 | N=5 | N=5 | N=5 | N=5 |
| <i>Enterococcus faecalis</i> | 13 | 10 | 18 | 18 | 15 |
| <i>Escherichia coli</i> | 15 | 20 | 10 | 5 | 18 |
| <i>Gardnerella vaginalis</i> | 2 | 3 | 3 | 3 | 0 |
| <i>Klyuvera spp</i> | 0 | 3 | 3 | 3 | 0 |
| <i>Candida species</i> | | | | | |
| <i>C. albicans</i> | 16 | 8 | 20 | 15 | 15 |
| <i>C. crusei</i> | 2 | 0 | 0 | 8 | 5 |
| <i>C. guilliermondii</i> | 4 | 5 | 3 | 0 | 0 |
| <i>C. tropicalis</i> | 0 | 5 | 3 | 3 | 0 |

Table 4.3c. Percentage frequency isolation of Gram negative rods and yeast cells at baseline and during treatment with Smugel (placebo) or UniPron.

4.3.4 Biopsies

There was no detectable incidence of abnormal pelvic examination finding (seen with the naked eye) after repeated exposure to UniPron and placebo gels. Evaluation of histological sections by light microscopy indicated that, application of UniPron 0.4 gm, 0.8 gm and 1.2 gm twice weekly did not cause any detectable alterations in the cervicovaginal lining of the baboon vagina as no incidence of abnormal pelvic examination finding was observed. The epithelial architecture of vagina of the baboons treated with UniPron across gel arms was largely comparable to baseline and placebo treated biopsies with no detectable alterations such as erythema, edema, excoriations or erosion of the cervicovaginal epithelial lining and leucocyte infiltration being observed (Figures 4.2 and 4.3).

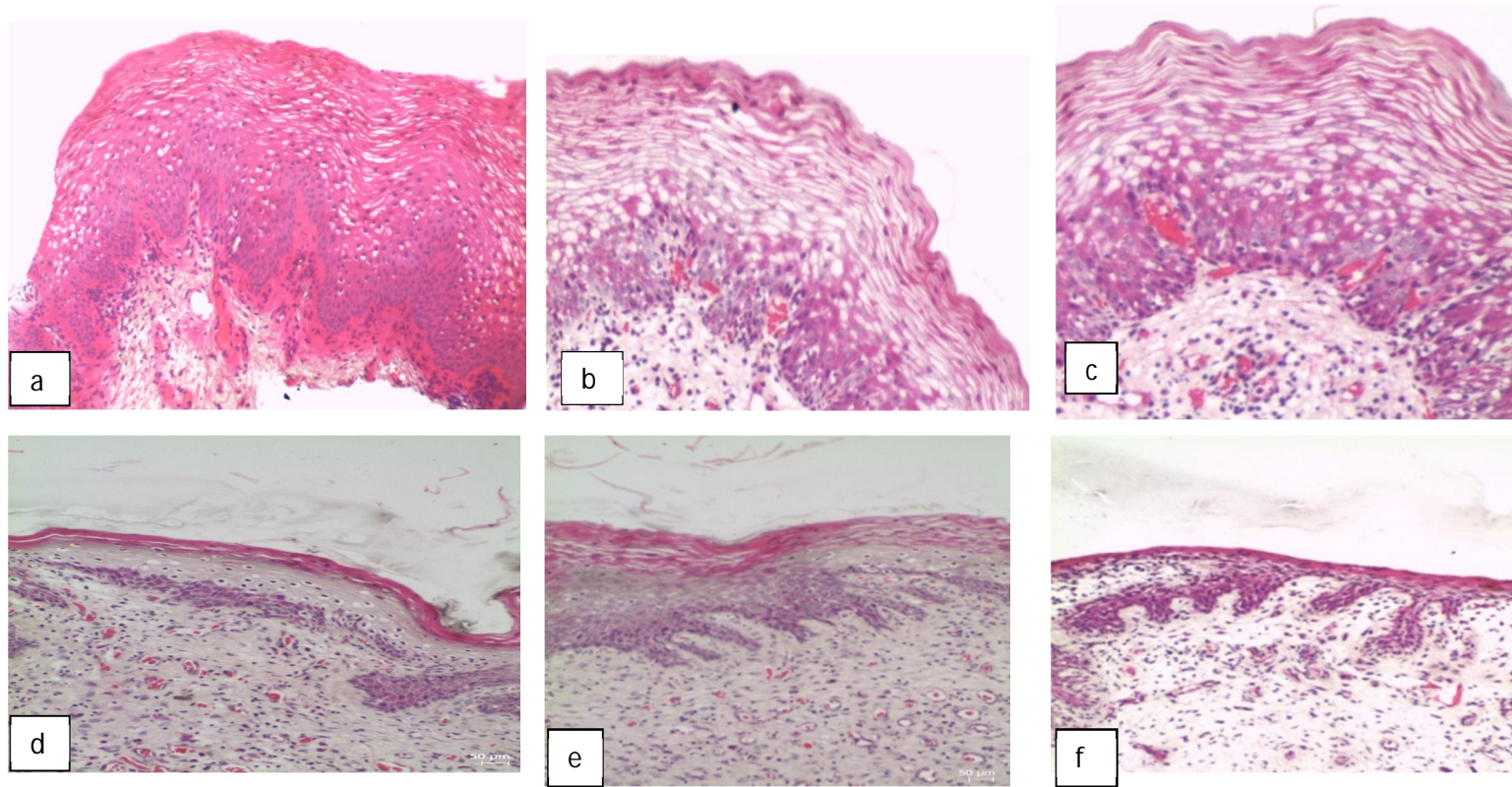


Figure 4.2a-f. Histology of the vaginal mucosa of baboons at baseline and after treatment with Smugel and UniPron. Vaginal biopsies were obtained from the animals in the follicular and luteal phases of the menstrual cycle. (a) Baseline follicular; (b) Smugel treated follicular; (c) UniPron 0.4gm treated follicular; (d) Baseline luteal; (e) UniPron 0.8gm treated luteal; (f) UniPron 1.2gm treated luteal (H&E $\times 400$).

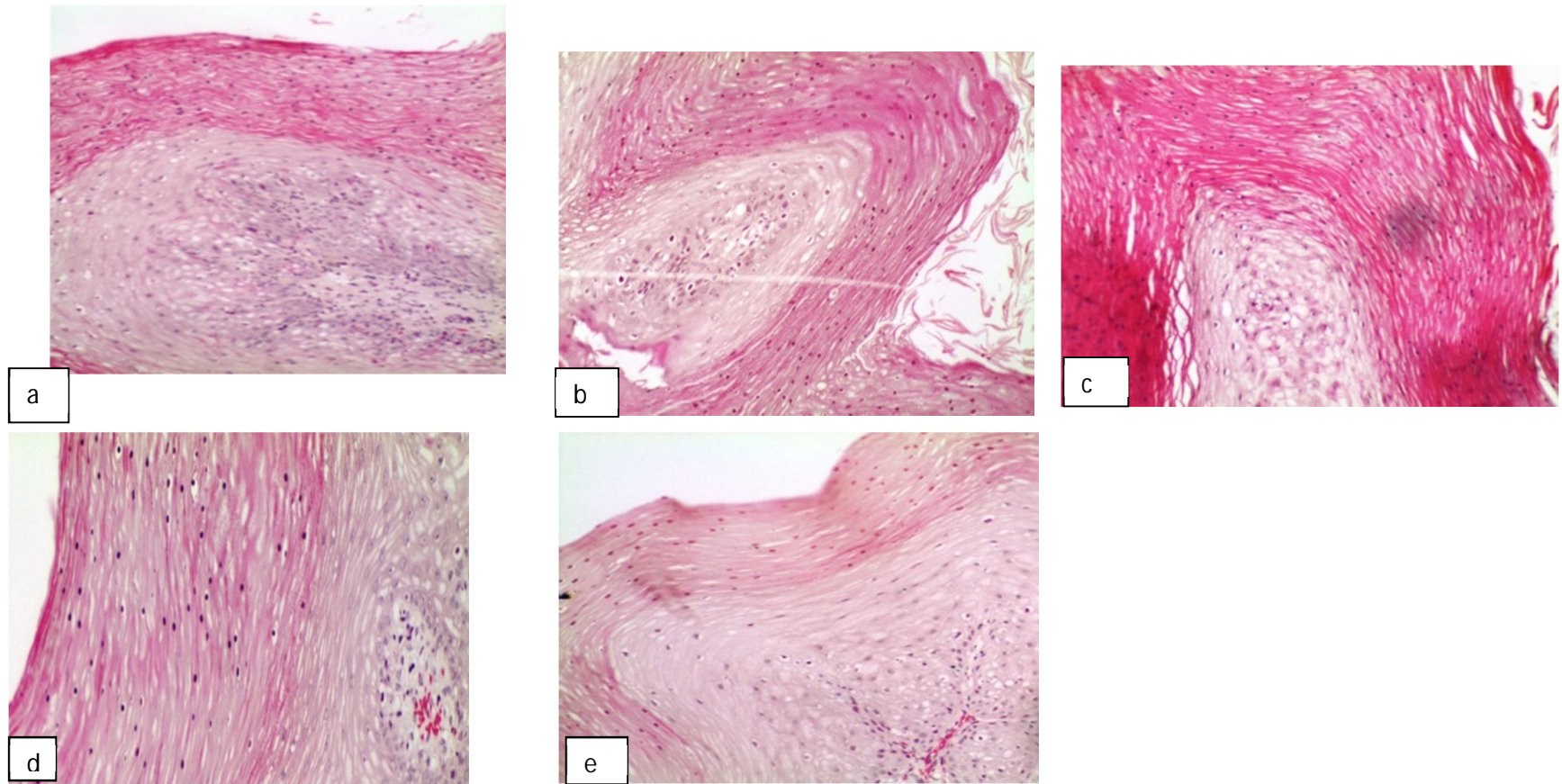


Figure 4.3a-e. Histology of the cervical mucosa of baboons during luteal phase of the menstrual cycle. (a) Baseline; (b) Smugel treated; (c) UniPron 0.4 gm; (d) UniPron 0.8 gm treated; (e) UniPron 1.2 gm treated (H&E $\times 400$)

4.4 Discussion

This study evaluated the safety of UniPron 0.4 gm, 0.8 gm and 1.2 gm compared to placebo administered twice weekly for eight weeks in the baboon model. The baseline data were collected before administration of any of any intravaginal product to provide for comparisons in the subsequent topical product testing. Exposure of the baboon vagina to the three concentrations of UniPron did not induce detectable toxicity (Obiero *et al.*, 2010; Obiero *et al.*, 2013). Assessment of product safety included vaginal pH, clinical chemistry profile, vaginal microflora and cervicovaginal mucosal integrity. The baseline vaginal pH observed was 5.2 ± 0.8 and no significant differences in the pH ($P>0.05$) was found to have occurred during treatment across the four gel arms and one week post treatment (Table 4.1). Similarly, analysis of blood chemistry parameters revealed no significant differences between baseline and the data obtained during treatment and two weeks post treatment with respect to TP, ALB, CRE, AST, ALT, ALP and TBIL (Table 4.2b) that relate to liver and kidney functions. These findings indicate that the gel neither interfered with baboon vaginal pH, nor clinical chemistry profiles of the parameters tested.

Like the human vagina, the baboon vagina is a dynamic and complicated environment composed of varying microbiological species in variable quantities and proportions. Elucidating how a vaginal product interacts with the vaginal microenvironment constitutes a critical step in evaluating their safety, as the disturbance of this microenvironment has been linked with several disease states (Sobel, 2000, Ursell *et al.*, 2013) and associated with increased susceptibility to STIs possibly due to related changes in innate defense responses from the epithelial cells (Rose *et al.*, 2012). UniPron gel usage did not alter the baboon vaginal microbial flora within the confines of the current study design. However, there were slight variations in the percentage

frequency isolation of all the microbes isolated (Tables 4.3a, 4.3b, 4.3c). The differences were not significant and could not be conclusively linked to the use of any of the study gels. For instance, the percentage frequency isolation was high for some microbes such as *C. glucuronolyticum*, *C. renale group* and *A. virudance* (Table 4.3a, 4.3b) both at baseline and across the four gel arms, with *A. viridans* being isolated from more than 50% of the vaginal swabs from each group. For other microbes such as *S. chromogenes*, *S. lentus*, *Klyuvera spp*, *G. vaginalis* and all species of *Candida* except *C. albicans*, (Tables 4.3b, 4.3c) the percentage frequency isolation was less than 10% both at baseline and across the four gel arms. The diverse composition of the baboon vaginal microbiota and the variation in percentage frequency isolation could not be attributed to treatment with any of the study gels, but could be as a result multiple factors such as host-specific relationships, composition of vaginal secretions and receptors on vaginal epithelial cell surfaces or vaginal tract tolerance to a variety of different strains of bacteria (Rivera *et al.*, 2010), a feature that might contribute to survival of primate species. In addition the vaginal flora characteristic is also of transient nature.

The healthy cervicovaginal mucosa represents an efficient barrier against STIs and dissemination of pathogens (Fichorova *et al.*, 2011). Histopathology results of biopsies obtained from UniPron treated animals showed that UniPron concentrations of 0.4 gm, 0.8 gm and 1.2 gm were non-toxic and did alter the integrity of the cervicovaginal mucosa (Figures 4.1 & 4.2). Abnormal pelvic examination finding (seen with the naked eye) such as edema, erythema excoriations or abrasions were not identified. Several vaginal products, microbicide or microbicide contraceptive trials have been terminated due to safety concerns (Obiero *et al.*, 2012). Vaginal contraceptive products have been available for many years and usually contain the membrane surfactant N-9 as one of the main ingredient. This has been associated with cytotoxic effect on

the vaginal cells and increased genital tract inflammation thereby potentiating the transmission of infectiousness of STIs including HIV by recruitment of white blood cells to the genital tract and possibly, by up regulation of genital cytokines (Stafford *et al.*, 1998). In addition, N-9 is also known to inactivate lactobacilli, which form the normal flora in vaginal tissues (Richardson *et al.* 1998). It is essential that the evaluation of the potentially harmful effects of these compounds on the vaginal epithelium and/or normal vaginal flora should be made before these products are used in human clinical trials. Partly due to the recent failures in human microbicide trials (Veazey, 2008) there is an emerging consensus that pre-clinical safety and efficacy testing of vaginal microbicial contraceptive candidates in nonhuman primates should precede to human trials.

4.5 Conclusion

There is renewed emphasis on the development of multifunctional prevention technologies, that is, products designed to address multiple STIs. Dual-protection contraceptive microbicial products are being designed to prevent STIs and pregnancy. Since consistent and correct use of these products will be critical to their effectiveness, the active pharmaceutical ingredients must be delivered in acceptable vaginal dosage forms such as gels. The development of different dosage forms will help ensure that women can find a method to protect themselves from pregnancy, and potential STIs. Evaluation of the vaginal pH, microbiologic and histology of the baboon model I has characterized the baboon vaginal environment's response to repeated topical product application in the absence of the exogenous factors of intercourse and potential infectious ejaculate. The three concentrations of UniPron appeared to be safe and well tolerated based on the results of the study in the baboon model. However, further studies are needed to assess whether UniPron may cause genital tract inflammation over a period of time. These data

support the need for future studies of this product and demonstrate that it is feasible to use the baboon model for assessment of potential vaginal microbicides.

CHAPTER 5:

ASSESSMENT OF MICROBICIDAL ACTIVITY OF UNIPRON ON STI PATHOGENS AND SPERMICIDAL ACTIVITY ON BABOON SEMEN *IN VITRO*

5.1 Introduction

STIs and unintended or mistimed pregnancies are prevalent and morbid problems worldwide (Thurman *et al.*, 2011). Rates of STIs are continuing to rise in many countries particularly among young people. Published estimates, available from the UNAIDS and WHO, indicate that Africa remains the continent worst affected (UNAIDS, 2010; WHO, 2011). In addition, almost half of all pregnancies worldwide, estimated to be over 100 million annually, are unintended or mistimed (WHO, 2007; Singh *et al.*, 2009). In 2008, this resulted in 43 million abortions, half of which were performed under unsafe conditions, leading to almost 100,000 maternal deaths and 5 million women left with temporary or permanent disabilities (Singh *et al.*, 2009). Although distinct different issues with varying causes, the same behaviour, unprotected intercourse, puts a woman at risk for both problems. Therefore, prevention of two or more of these conditions could potentially be targeted by one multipurpose product.

Vaginal acidifying agents may work as microbicides and spermicides by enhancing the normal vaginal defence mechanism. Healthy human vagina in women in the reproductive age is acidic, with a pH ranging from 3.5 to 4.5, primarily because of lactic acid. This acidic environment is an important component of the natural vaginal defence against infections in addition to inactivating sperm. The natural defence mechanism can be overcome either by the seminal fluid alkaline pH or the depletion of normal vaginal bacterial flora (Garg *et al.*, 2001). *In vitro* studies have shown

that an acidic pH prevents infection due to a variety of disease-causing organisms including HIV, herpes simplex, chlamydia, gonococci, Gardnerella and BV-associated organisms, *Treponema pallidum* and *Haemophilus ducreyi* (Mahmoud *et al.*, 1995; O'Connor *et al.*, 1995; Pettit *et al.*, 1999). To protect spermatozoa from the vaginal environment, semen contains a variety of buffering proteins. The buffering quality of the ejaculate also effectively neutralizes the vaginal defences for pathogens.

In response to a global epidemic of STIs and unintended pregnancies, vaginal products are being developed to address these challenges (Ballagh *et al.*, 2008; Shepherd *et al.*, 2012). The development of compounds that protect the acidic vagina, either by buffering the seminal pH or by maintaining the vaginal bacterial flora is one of the strategies that could enhance the vaginal defense system. The rationale for using an acidifying agent is to maintain an acidic pH in the vagina that will resist the buffering effect of semen, work as a spermicide and provide protection against acid-sensitive microbes. In this study, the antimicrobial and spermicidal potential of UniPron on STI pathogens and baboon semen function *in vitro* were assessed.

5.2 Materials and Methods

5.2.1 Microbiological samples

5.2.1.1 Clinical and American Type Culture Collection microbes

Clinical isolates and American Type Culture Collection (ATCC) microbes of *Neisseria gonorrhoea*, *Gardenerella vaginalis*, *Haemophilus ducreyi*, *Candida albicans* and *Escherichia coli* were used in this study. Clinical isolates that had been cultured were obtained from medical microbiology laboratory of Kenyatta National Hospital. The ATCC of *N. gonorrhoeae* ATCC[®] 9793[™], *H. ducreyi* ATCC[®] 33921[™], *G. vaginalis* ATCC[®] 49145[™], *C. albicans* ATCC[®] 753[™],

E.coli ATCC® 25922™ in freeze-dried forms were commercially acquired (10801 University Boulevard, Manassas VA, USA).

5.2.2 Animal selection

Five adult healthy breeder (with at least one offspring in captivity) male baboons (*P. anubis*) with a mean weight 25.1 ± 2.1 kg maintained in captivity at IPR, were used in this study. These baboons were housed in individual cages and kept on a diet of commercially available monkey chow (Unga Feeds Ltd, Nairobi, Kenya) supplemented with fruits and water *ad libitum*. The study protocol was reviewed and approved by the institution's scientific and ethical research committee in accordance with the international guidelines for care and use of research on animals (section 3.2.1).

5.2.3 Smugel and UniPron vaginal gels

Discs impregnated with Smugel (section 3.2.2) and UniPron 0.4, 0.8 and 1.2 gm (section 4.2.2.) were tested against each microbe for antimicrobial activity.

5.3 Sample collection and processing

5.3.1 Microbiological sample processing

Clinical isolates were sub cultured on appropriate culture media as follows; *N. gonorrhoea* and *H. ducreyi*, chocolate blood agar; *G. vaginalis*, blood agar, *E. coli*, MacConkey and nutrient agar and *C. albicans*, Sabaraoud's Dextrose agar.

The ATCC microbes were first rehydrated to form suspensions using the ATCC reagents that were provided. The suspension was used to inoculate the recommended culture media of the

ATCC microbes as follows: *N. gonorrhoea* and *H. ducreyi*, GC chocolate Agar; *G. vaginalis*, GC medium *C. albicans*, Emmons modification of Sabouraud's medium and *E.coli*, 18 Tryptic Soy Agar. Inoculated culture media plates were incubated under appropriate conditions to allow for growth (section 5.3.1.3).

5.3.1.1 Culture media preparation

Preparation of chocolate blood agar

Composition g/l: Tryptone 14.0, Peptone neutralised 4.5, Yeast extract 4.5, Sodium chloride 5.0, Agar 12.0, pH 7.5±0.2 at 25°C.

Forty grams of the medium was suspended in one litre of distilled water and boiled to dissolve completely. Sterilization was done by autoclaving at 121°C for 15 minutes. It was cooled to 50°C and 7% sterile sheep blood (section 3.2.3.4) added and heated to about 80°C to form the chocolate agar.

Preparation of blood agar

Composition g/l: Tryptone 14.0, Peptone neutralised 4.5, Yeast extract 4.5, Sodium chloride 5.0, Agar 12.0, pH 7.5±0.2 at 25°C.

Forty grams of the medium was suspended in one litre of distilled water and boiled to dissolve completely. Sterilization was done by autoclaving at 121°C for 15 minutes. It was cooled to 50°C and 7% sterile sheep blood (section 3.2.3.4) added.

Preparation of MacConkey agar

(Section 3.2.3.5)

Preparation of Sabouraud's dextrose agar

(Section 3.2.3.5)

Preparation of nutrient agar

Composition g/l: Beef extract 3.0, Peptone 5.0, Agar 15.0, pH 6.8 ± 0.2

Twenty three grams of the medium was added to one litre distilled water and mixed. The solution was heated with frequent agitation and boiled to completely dissolve the powder. Sterilization was done by autoclaving at 121°C for 15 minutes.

Preparation of GC chocolate agar

The agar was made from GC agar base (BD 228950), 2% bovine haemoglobin and IsoVitaleX Enrichment. Haemoglobin provides hemin (X factor) required for growth of *Haemophilus* and enhanced growth of *Neisseria*. IsoVitaleX Enrichment provides V factor (nicotinamide adenine dinucleotide, NAD) for *Haemophilus* species and vitamins, amino acids, coenzymes, dextrose, ferric ion and other factors which improve the growth of pathogenic *Neisseria*.

GC Agar Base

Composition g/l; Proteose Peptone No. 3 15.0, Corn starch 1.0, Dipotassium phosphate 4.0, Potassium dihydrogen phosphate 1.0, Sodium chloride 5.0, Agar 10.0, pH: 7.2 ± 0.2 at 25°C .

Preparation of GC agar

About seven (7.2) gm of GC base medium was suspended in 100 ml distilled water and mixed. The solution was heated with frequent agitation and boiled to completely dissolve the powder. Sterilization was done by autoclaving at 121°C for 15 minutes and cooled to $45\text{-}50^{\circ}\text{C}$

IsoVitaleX™ Enrichment (BD 211876)

Composition Per Litre: Adenine 1.0 gm, *p*-Aminobenzoic acid 13.0 mg, L-Cysteine hydrochloride 25.9 gm, L-Cystine 1.1 gm, Nicotinamide adenine dinucleotide 0.25 gm, Ferric nitrate 0.02 gm, L-Glutamine 10.0 gm, Guanine hydrochloride 0.03 gm, Thiamine hydrochloride 3.0 mg, Vitamin B12 0.01 gm, Dextrose 100.0 gm, Thiamine pyrophosphate 0.1 gm.

About seven (7.2) gm of GC base medium was suspended in 100 mL distilled water. The solution was heated with frequent agitation and boiled to completely dissolve the powder. Sterilization was done by autoclaving at 121° C for 15 minutes and cooled to 45-50°C. Aseptically, 100 ml of hemoglobin 2% and 2 ml IsoVitale X Enrichment were added and mixed.

Preparation of Emmons modification of Sabouraud's medium

Composition g/l: Neopeptone 10.0g, Dextrose 20.0g, Agar 20g, pH 7.0±0.2.

Fifty grams of the medium was suspended in one litre of distilled water and mixed. The solution was heated with frequent agitation and boiled to completely dissolve the powder. Sterilization was done by autoclaving at 121° C for 15 minutes and cooled to 45-50°C.

Preparation of 18 Tryptic Soy Agar ATCC

Composition g/l: Tryptone 17.0, Soytone 3.0, Dextrose 2.5, Sodium chloride 5.0, Potassium dihydrogen phosphate 2.5, Agar 15. pH 7.3 ± 0.2 at 25°C.

Forty grams of the medium was suspended in one litre distilled water. The solution was heated with frequent agitation and boiled to completely dissolve the powder. Sterilization was done by autoclaving at 121° C for 15 minutes and cooled to 45-50°C.

5.3.1.2 Microbe cultures and disc-diffusion sensitivity test

Clinical isolates were sub cultured on appropriate culture media (Section 5.3.1). Suspensions of freeze-dried forms of the ATCC microbes were prepared and used to inoculate appropriate culture media (section 3.3.1). Both sets of inoculated culture media were incubated at the recommended conditions for growth to occur (section 5.3.1.2). A suspension of each microbe equivalent to a McFarland No. 2 standard was prepared in 2 ml normal saline. A sterile swab was dipped in the suspension and excess fluid drained by rotating the swab against the inside of the container. The swab containing the culture suspension of bacteria to be tested was spread uniformly across the entire agar plate. The same suspension of each microbe with a fresh sterile swab was used to inoculate all the media plates. A filter-paper disc, impregnated with either Smugel, Unipron 0.4 gm, 0.8 gm or 1.2 gm was placed on the surface of the inoculated agar. Each plate contained four discs of the test product. Using a flame-sterilized forceps, each disc was gently pressed to the agar to ensure that the disc was attached to the agar. The plates were then incubated under appropriate conditions (section 5.3.1.2) to allow for growth and the sensitivity of UniPron on clinical isolates was compared with the standard reference ATCC microbes. The inhibition zones around the discs were measured. The quality of growth was noted and zone sizes measured in millimetres.

5.3.1.3 Incubation of cultures

N. gonorrhoea and *G. vaginalis* were incubated at 37° C with 5% CO₂ for 48 hours, *H. ducreyi* at 30° C with 5% CO₂ for 48 hours, *E. coli* at 37° C for 24 hours and *C. albicans* at 35° C for 48 hours under aerobic atmosphere.

5.3.2 The baboon semen

5.3.2.1 Semen collection and processing

Freshly ejaculated baboon semen was obtained by electrostimulation of healthy sexually mature male animals and collected directly into sterile plastic tubes (BD, Erembodegem-Dorp, Belgium) and transported immediately into the laboratory. During electrostimulation, the baboons were sedated by intramuscular injection with a mixture of Ketamine and xylazine (section 3.2.1). Electrostimulation was done using a rechargeable rectal probe (Standard Precision Electronics, Inc., Littleton, CO, USA) with amplitude of 20-30 mA. The samples were allowed to liquefy at 37° C for 45 min and semen characteristics and analysis were performed according to the normal criteria as per WHO (WHO, 2010) guidelines. Analysis of the ejaculate of all the five animals was done based on the volume, pH, sperm motility, concentration and morphology before treatment with UniPron. The pH of the semen was measured within 1 hour of collection using a pH indicator paper (Merck, Darmstadt, Germany) and the volume was read directly against the graduated marks in these tubes. After liquefaction, 10µl of the semen supernatant was placed on Neubauer chamber and covered with a cover slip to assess motility. Spermatozoa were observed at ×320 magnification under a microscope (Axiovert 10, model 1-216-66-EL01-002; Zeiss; Gottingen, West Germany) and motility was assessed. Spermatozoa were classified as either motile or immotile. For determination of sperm concentration, an aliquot of each sample was observed at ×320 magnification under a microscope and a dilution of 1:10 was made to ensure easy evaluation of sperm concentration. Then a drop of formaldehyde was added to the diluted sperm to immobilize them for counting. Ten microliters of the dilution was loaded on each side of the Neubauer cell-counting chamber and the number of free sperm was counted in the middle 25 squares. An average was calculated and divided by the dilution factor (WHO, 2010). A

further aliquot of 10 μ of semen sample from each animal was fixed in ethanol and stained using papanicolaou stain (section 5.3.2.2) for sperm morphology determination. The morphologically normal and abnormal sperm were recorded as a percentage of the total number of counted spermatozoa. Abnormal sperm morphology was scored by using the categories described by WHO (WHO, 2010). Based on this, sperm were scored as abnormal if they had abnormal heads; (large, small, tapering, elongated, amorphous, pyriform, vacuolated or double heads, or any combination of these; neck or midpiece defects; bent or abnormally thin or thick, and tail defects; broken, irregular width or coiled/bent tails, or any combination of these. Each animal was electrostimulated three times and was given one week rest before the next electrostimulation. One hundred spermatozoa were counted in each smear at a magnification of $\times 1000$ under light microscopy (002-10247, N.V-S.A, Axioskop; Carl Zeiss, Gottingen, West Germany) and percentage normal and abnormal morphology calculated.

5.3.2.2 Papanicolaou staining procedure for sperm morphology

Sequentially, the ethanol fixed smears were immersed in;

| | |
|------------------------|----------|
| Ethanol 80% (v/v) | 30 sec |
| Ethanol 50% (v/v) | 30 sec |
| Purified water | 30 sec |
| Harris's Haematoxylin | 4 min |
| Purified water | 30 sec |
| Acidic ethanol | 4-8 dips |
| Running cold tap water | 5 min |
| Ethanol 50% (v/v) | 30 sec |

| | |
|--------------------|-----------------|
| Ethanol 80% (v/v) | 30 sec |
| Ethanol 95% (v/v) | At least 15 min |
| G-6 orange stain | 1 min |
| Ethanol 95% (v/v) | 30 sec |
| Ethanol 95% (v/v) | 30 sec |
| Ethanol 95% (v/v) | 30 sec |
| EA-50 green stain | 1 min |
| Ethanol 95% (v/v) | 30 sec |
| Ethanol 95% (v/v) | 30 sec |
| Ethanol 100% (v/v) | 15 sec |
| Ethanol 100% (v/v) | 15 sec |

5.3.2.3 Treatment of baboon Semen with UniPron

Baboon semen samples were pooled, washed three times in PBS, pH 7.2 by centrifugation for 30 sec at 700g. PBS washed sperm were resuspended in 5ml of Ham F-10 (L-glutamine and 25 mM HEPES, Sigma) medium containing 3% bovine serum albumin, centrifuged at 1500 rpm at 37°C for 10 minutes and the supernatant discarded. The sperm pellet was overlaid with 1 ml of the medium in a 15 ml sterile culture tube inclined at an angle at 37° C for 1 hr. Aliquots of 100 µl of the top layer of the medium containing motile sperm were mixed with UniPron 0.4 gm volume by volume. Immediately, an aliquot of the mixture was loaded on to the improved Neubauer haemocytometer and covered with a cover slip to assess motility. Spermatozoa were observed at ×320 magnification under a microscope (Axiovert 10, model 1-216-66-EL01-002; Zeiss; Gottingen, West Germany). Spermatozoa were classified as motile or immotile. This

procedure was repeated three times and the mean sperm percentage immotility at every point was determined.

5.3.2.4 Data analysis

The data were analysed as described in section 3.2.3.15.

5.4 Results

5.4.1 Antibacterial activity of UniPron *in vitro*

Antimicrobial activity was determined on both clinical and ATCC microbes by the use of disc diffusion method. The ATCC microbes included *N. gonorrhoea* ATCC[®]9793[™], *H. ducreyi* ATCC[®]33921[™], *G. vaginalis* ATCC[®]49145[™], *C. albicans* ATCC[®]753[™] and *E. coli* ATCC[®]25922[™]. None of the tested microbes were inhibited by the placebo gel, Smugel. UniPron had antimicrobial activity on *N. gonorrhoea*, *H. ducreyi*, *G. vaginalis*, *C. albicans* and *E. coli*, with larger inhibition zones of 18 mm, 21 mm, 18 mm, 23 mm, 26 mm diameter, respectively for clinical isolate microbes; 13 mm, 15 mm, 14 mm, 18 mm, and 19 mm diameter, respectively for ATCC microbes being observed in cultures treated with UniPron 1.2 gm. The clinical isolate microbes showed higher sensitivity than the ATCC microbes across the three UniPron gel arms. *C. albicans* and *E. coli* were found to be most sensitive to UniPron gel treatment (Table 5.1).

| Microorganism | Inhibition zone diameter (mm) | | | | | | | |
|----------------------|-------------------------------|------|----------|------|-------------|------|----------|------|
| | Placebo gel | | | | UniPron gel | | | |
| | 0.4 gm | | 0.8 gm | | 1.2 gm | | | |
| | Clinical | ATCC | Clinical | ATCC | Clinical | ATCC | Clinical | ATCC |
| <i>N. gonorrhoea</i> | – | – | 14.5 | 9.5 | 15 | 11 | 18 | 13 |
| <i>H. ducreyi</i> | – | – | 14.5 | 11 | 17.5 | 13 | 21 | 15 |
| <i>G. vaginalis</i> | – | – | 13 | 9 | 14.5 | 11.5 | 18 | 14 |
| <i>C. albicans</i> | – | – | 14 | 11.5 | 19 | 18 | 23 | 18 |
| <i>E.coli</i> | – | – | 18 | 13 | 20.5 | 16 | 26 | 19 |

Table 5.1. Zones of inhibition. Zones of inhibition for clinical and ATCC microbe cultures treated with Smugel and UniPron vaginalgels *in vitro*. *(-) No inhibition zone observed.

5.4.2 Spermicidal activity of UniPron *in vitro*

Parameters of freshly ejaculated baboon semen for all the animals were established after every electrostimulation. Sperm quality was determined by assessing baboon spermatology values namely volume, pH, concentration, motility and morphology. The lowest semen volume obtained was 0.35 ± 0.2 ml and the pH of all the semen was alkaline ranging from 7.2 ± 0.26 to 7.6 ± 0.29 . Sperm concentration ranged from 52.41 ± 11.41 million/ml to 56.62 ± 12.35 million/ml. Percentage sperm motility was more than 60% in each animal and sperm morphology was normal in more than 83% of sperm cells (Table 5.2). Abnormalities that were observed in baboon sperm cells included elongated head (Fig 5.1 B), bent neck (Fig 5.1C) and bent neck and coiled tail (Fig 5.1 D).

UniPron was found to be highly spermicidal. When UniPron 0.4 gm was added to 100 μ l of semen volume by volume and mixed, the percentage immotility of baboon sperm was observed to increase as the volume of UniPron increased. At a volume of 10 μ l, 20% of sperm cells were immobilized, compared to 56% and 84% that were immobilized at volumes of 20 μ l and 30 μ l, respectively. At 40 μ l, UniPron irreversibly immobilized 100% baboon sperm (Fig 5.2) within 15 seconds. The resultant mixture became more viscous with a resultant pH of 3.6. Addition of UniPron 0.8 gm and 1.2 gm caused complete sperm immobilization within 10 seconds.

| Animal Number | Semen volume (ml) | Semen pH | Sperm | | |
|---------------|----------------------|----------|-------------------------------|--------------|---------------------|
| | | | concentration (million/ml) | Motility (%) | Normal Sperm (%) |
| Pan 3156 | 0.4±0.1 | 7.3±0.1 | 53.88±9.43 | 68.23±8.52 | 89.67±2.36 |
| Pan 2794 | 0.5±0.15 | 7.5±0.32 | 55.12±7.82 | 62.33±10.12 | 88.42±3.84 |
| Pan 3142 | 0.35±0.2 | 7.2±0.26 | 52.41±11.41 | 62.65±7.23 | 86.72±3.24 |
| Pan 2974 | 0.45±0.1 | 7.6±0.29 | 56.32±8.11 | 69.22±12.11 | 83.84±6.62 |
| Pan 2696 | 0.5±0.1 | 7.6±0.15 | 56.62±12.35 | 67.37±7.92 | 86.22±4.41 |

Table 5.2. Semen parameters of electrostimulated healthy sexually mature male baboons ($\bar{x} \pm SD$).

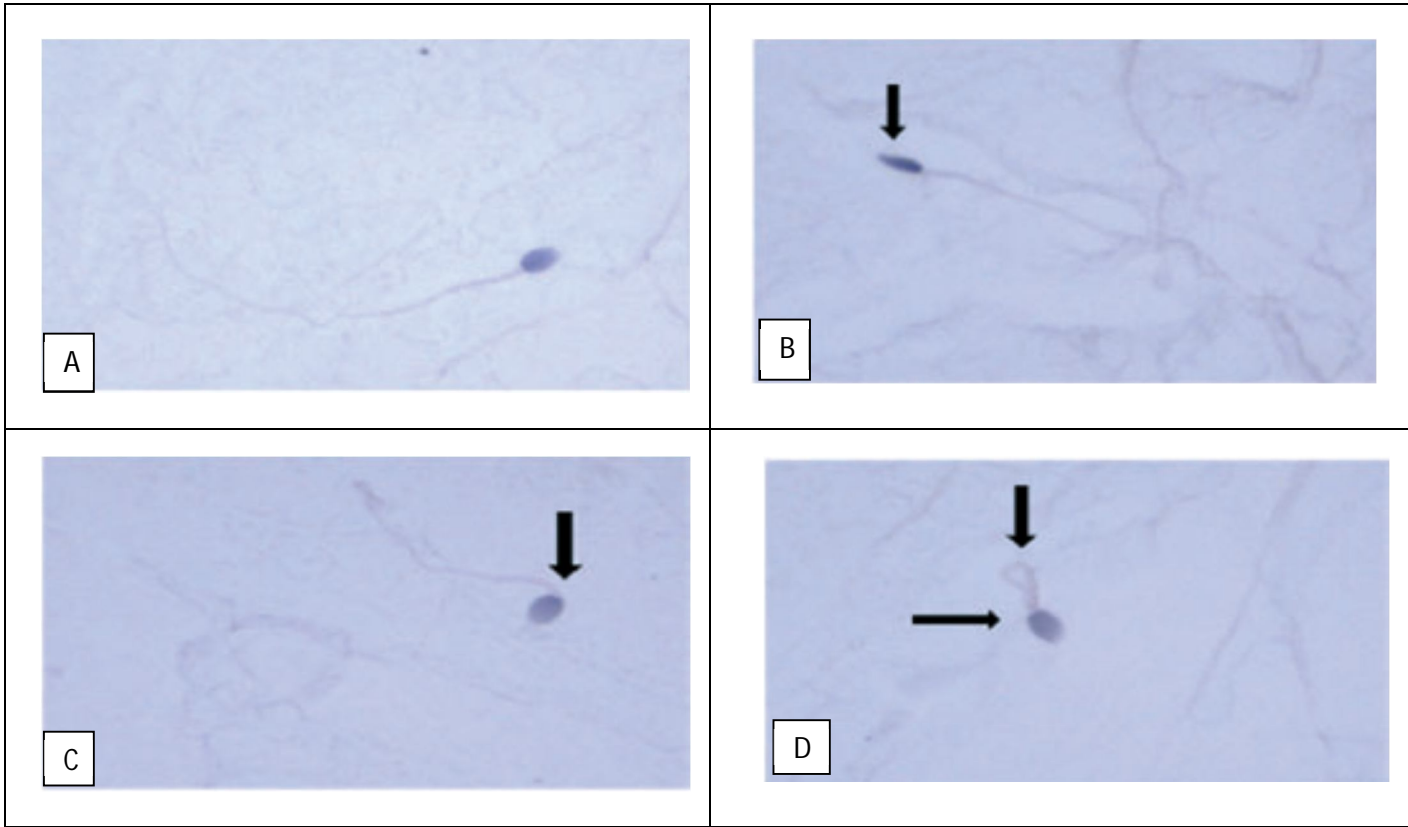


Figure 5.1. Baboon spermatozoon. (A) normal, (B) abnormal with elongated head, (C) abnormal with bent neck, (D) abnormal with bent neck and coiled tail, (Magnification $\times 400$).

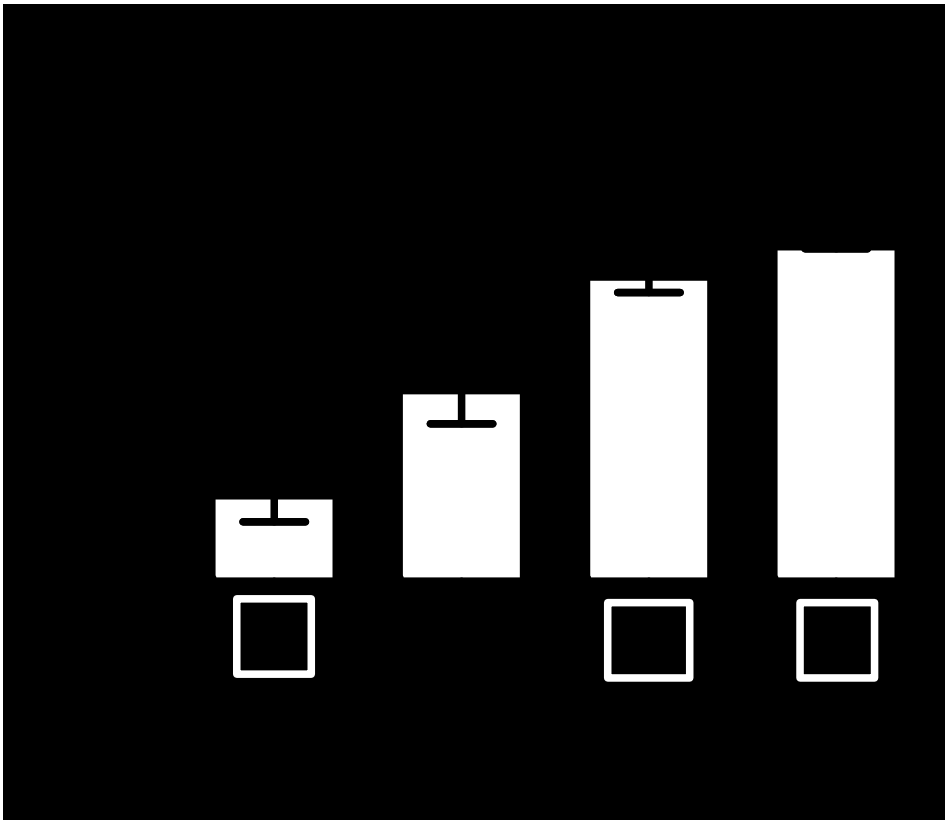


Figure 5.2. Effect of UniPron 0.4 gm on baboon sperm motility. At 40 μ l, all sperm cells are immobilized.

5.5 Discussion

A significant number of women, especially in developing countries, need protection against STIs, and more accessible family planning methods to prevent unwanted pregnancies (Friend & Doncel, 2010). It is likely that women's needs for vaginal microbicide and contraceptive products will be best met if dual function types of products with varying use requirements are available. Some women will prefer a coitally dependent method while others a continuous-use method and these needs will change across a woman's sexual life (Nel *et al.*, 2012). Antimicrobial sensitivity test using the disc agar-diffusion procedure, which is one of the most widely used method to determine susceptibility of microorganisms to antimicrobial agents (William, 1990), was performed for both clinical isolates and ATCC microbes of *N. gonorrhoea*, *H. ducreyi*, *G. vaginalis*, *C. albicans* and *E. coli*. The ATCC microbes tested for susceptibility to UniPron included *N. gonorrhoea* ATCC[®] 9793[™], *H. ducreyi* ATCC[®] 33921[™], *G. vaginalis* ATCC[®] 49145[™], *C. albicans* ATCC[®] 753[™] and *E. coli* ATCC[®] 25922[™]. The placebo gel exhibited no inhibition of reproduction of all the microbes tested on the surface of the solid medium. Inhibition of growth, characterized by presence of inhibition zones (Table 5.1) of the microbes was observed around all the UniPron impregnated discs suggesting the possibility of diffusion of the antimicrobial agent into the medium from the filter paper disc inhibiting reproduction around the disc. In cultures of *N. gonorrhoea*, *H. ducreyi* and *G. vaginalis* of both clinical and ATCC microbes treated with UniPron 0.4 gm, a few colonies were observed to have grown within the inhibition zone. The results indicated that though inhibitory, these three microbes are less sensitive to UniPron 0.4 gm. In addition, UniPron exhibited higher microbicidal activity against clinical isolates than ATCC microbes across the three gel treatment arms, but the differences were not statistically significant ($P > 0.05$). However the compound proved to have antimicrobial activity against *N. gonorrhoea*, *H. ducreyi*, *G. vaginalis*, *C. albicans*

and *E. coli* (Table 5.1) (Obiero *et al.*, 2013d). Due to shared routes of infection, HIV-infected persons are frequently co-infected with other STIs. Studies have reported that STI pathogens increase susceptibility to HIV infection (Chun *et al.*, 2013). While *E. coli* is not an STI pathogen, its overgrowth in the vagina characterizes a disturbed and abnormal vaginal flora, which is an established risk factor in acquisition of STIs including HIV (Atasili *et al.*, 2008 van de Wijgert *et al.*, 2008, Rose *et al.*, 2012). Several *in vitro* and *in vivo* studies have reported inhibition of STI pathogens including *N. gonorrhoea*, *Treponema pallidum*, *H. ducreyi*, *Chlamydia trachomatis*, herpes virus simplex type 2, human papillomavirus and bacteria associated with BV with acidic pH (Zeitlin *et al.*, 2001; Spencer *et al.*, 2004; Tuyama *et al.*, 2006). The inhibition observed in microbe cultures treated with UniPron was therefore attributable to its low pH.

In addition to microbicidal activity, this study assessed the spermicidal activity of UniPron on baboon semen *in vitro*. Freshly ejaculated baboon semen obtained by electrostimulation which is an acceptable method of semen collection from non-human primates (Amboka & Mwethera, 2003; Nyachieo *et al.*, 2012), was used to determine sperm quality. The spermatology values assessed included; semen volume (0.35-0.5 ml), semen pH (7.2-7.6), sperm motility (62.33-69.22%), concentration (53.88-56.63 million/ml) and morphology (83.22-89.67% normal) (Table 5.2). Morphological abnormalities that were observed in baboon sperm cells included elongated head (Fig 5.1 B), bent neck (Fig 5.1C) and bent neck and coiled tail (Fig 5.1 D). These data were comparable to what has previously been reported in fertile baboons (Amboka & Mwethera 2003, Nyachieo *et al.*, 2012). The data are also comparable to (Wildt, 1986; Cooper *et al.*, 2010) or higher than that obtained in human sperm donors with reference to percentage sperm motility, normal morphology and concentration. A decline in these semen parameters has been observed among fertile men in the past 50 years (Auger *et al.*, 1995; Benshushan *et al.*, 1997). The effect

of UniPron on sperm was examined by mixing the product with ejaculated baboon spermatozoa *in vitro*.

UniPron significantly reduced the mean number of progressively motile sperm. As the volume of UniPron increased, percentage sperm immotility increased. At a concentration of 40% UniPron (v/v), 100% sperm immotility was observed (Fig 5.2) within 15 seconds. In addition, when mixed with semen, the viscosity of UniPron created a matrix that impeded sperm mobility. This observation was comparable to other acidic buffers such as Acidform (Mayer *et al.*, 2001; Amara *et al.*, 2004) which has been marketed as a personal lubricant (Amphora™ gel; Evofem Inc., San Diego, CA) and is currently being evaluated for contraception in Phase III trial (ClinicalTrials.gov Identifier: NCT01306331). Acidform and Buffergel are two candidate microbicides that have shown *in vitro* spermicidal and antibacterial activity against certain STI pathogens such as *N. gonorrhoea* and *C. trachomatis* (Mayer *et al.*, 2001; Zeitlin *et al.*, 2001). Both Acidform (Garg *et al.*, 2001) and Buffergel (Mayer *et al.*, 2001; van De Wijgert *et al.*, 2001) are acidic buffers with a pH of 3.5 and 3.9 respectively. Rapid immobilization of sperm cells following UniPron administration could therefore be due to the rapid lowering of the pH and subsequent viscosity of the resulting mixture.

UniPron is an acidic buffer that works by lowering the pH to enhance the normal vaginal defence mechanism. It maintained a low pH of 3.6 in the presence of relatively alkaline baboon semen when mixed in equal volume. This acidic environment is also inhospitable to sperm as they are immobilized at a pH of 5.0 while partial immobilization occurs at a pH between 5.0 and 6.0 (Garg *et al.*, 2001). The speed of sperm immobilization is correlated with increasing acidity (Olmsted *et al.*, 2000). For example, at pH 5.0, sperm are completely immobilized within 5

minutes; at pH 4.0, sperm are immobilized within 30 seconds. Likewise, rates at which sperm are both killed is linearly proportional to pH. However, spermatozoa can regain motility when pH is increased to normal highlighting the need for an acidifying agent to also have strong acid buffering properties (Makler *et al.*, 1981; Olmsted *et al.*, 2000). It is these acid-buffering qualities that make UniPron an effective spermicide. Acidifying agents may work as microbicides and spermicides by enhancing the normal vaginal defences. Like Buffergel and Acidform (Mayer *et al.*, 2001; Zeitlin *et al.*, 2001; Spencer *et al.*, 2004) which are acidic buffers, UniPron has shown *in vitro* spermicidal and microbicidal activity against certain STI pathogens. This gel was designed to prevent disease transmission and conception by promoting acidity as acidic environment is inhospitable to acid sensitive microbes and spermatozoa.

5.6 Conclusion

Women at risk for unintended pregnancies are by definition also at risk of STIs and women who are at risk of one STI are also likely to be at risk of several different STIs. Simplifying and extending the dosing regimen, decoupling prophylaxis from coital acts and developing female controlled protection products are all innovative strategies and approaches to increasing the use of these much needed vaginal products. Such products are likely to have a synergistic impact on the epidemics of STIs and unintended pregnancies. Several current prevention approaches are promising, and the search for new more effective interventions need to be enhanced. These results demonstrate that UniPron is a potential microbicidal contraceptive vaginal product that warrants further evaluation for both STI prevention and conception. Dual protection vaginal products capable of preventing STI transmission while simultaneously providing contraception will empower women to control their fertility and reproductive health.

CHAPTER 6:

DURATION OF UNIPRON EFFECTIVENESS IN THE BABOON VAGINA AND AS A REVERSIBLE NON- HORMONAL CONTRACEPTIVE IN THE BABOON MODEL

6.1 Introduction

Increasing the level of contraceptive uptake among women of children bearing age is an important component of many national population and developmental programs in sub-Saharan Africa. However, the prevalence use of these methods is still low despite the efforts of many governmental and non-governmental agencies. Barriers to the use of modern contraceptives by women might inhibit the uptake of methods for preventing unwanted pregnancies. Abortions arising from unwanted pregnancies constitute a major reproductive health problem. Since abortion is illegal under most conditions particularly in sub-Saharan Africa, an increase in the incidence of unwanted pregnancy is likely to result in a rise in the incidence of unsafe abortions. This in turn, is likely to raise the proportion of women with abortion-related morbidity and mortality. The increasing incidence of complications from unsafe abortions can have a substantial impact on the region's fragile health system (Asekun-Olarinmoye *et al.*, 2013). The safety of hormonal contraceptives and fear of side effects have been reported as prominent perceived barriers to contraceptive use (Casterline & Sinding, 2000; Nagase *et al.*, 2003; Aryeetey *et al.*, 2010; Severy & Newcomer, 2005). Several epidemiological studies indicate that systemic hormonal contraception, particularly progesterone-containing injectables, may be associated with an increased risk of both HIV acquisition and transmission (Martin *et al.*, 1998; Lavreys *et al.*, 2004; Heffron *et al.*, 2012). Moreover, nonoxynol-9 (N-9), approved in the United States as a vaginal contraceptive, provides no protection against HIV or other sexually transmitted infections and frequent use was shown to be associated with an increased risk of

acquisition of STIs including HIV (Winkinson *et al* 2002; van Damme *et al* 2002; Obiero *et al.*, 2012). Focus towards achievement of universal access to reproductive health remains a high priority. One of the targets of the Millennium Development Goals (MDGs) which aims to improve maternal health indicates that there is some effort to expand access to effective contraceptive methods (UN-MDGs, 2012; Singh & Darrock, 2012).

As contraceptive prevalence has increased, the unmet need for family planning - defined as the percentage of women aged 15 to 49, married or in union, who report the desire to delay or avoid pregnancy but are not using any form of contraception - has declined overall. Worldwide, unmet need for family planning dropped from 15% in 1990 to 12% in 2011, driven by progress in developing regions. Current levels of unmet need range from a low of 4% in Eastern Asia to a high of 25% in Oceania and sub-Saharan Africa. This translates into more than 140 million women (married or in union) who would like to delay or avoid pregnancy, but are not using contraception. By 2015, total demand for family planning among married women in these regions is projected to grow to more than 900 million, mostly due to population growth. This is one indication of the unfinished agenda in reproductive health and the scale of efforts needed to keep pace with the demand for contraceptives, especially more effective modern methods (Alkema *et al.*, 2013; UN-MDGs report, 2013). Thus, the development of safe and effective alternative contraceptives is a major global health priority. This study aimed to assess the effectiveness of UniPron as a reversible non-hormonal contraceptive in the baboon model.

6.2 Materials and methods

6.2.1 Animal selection

Fifteen sexually mature female baboons and five adult healthy breeder (with at least one offspring in captivity) male baboons (*P. anubis*) maintained in captivity at the Institute of Primate Research (IPR), Kenya, were used in this study. The baboons were kept on a diet of commercially available chow (Unga feeds Ltd, Nairobi, Kenya) supplemented with fruits and water *ad libitum*. The study protocol was reviewed and approved by the institution's scientific and ethical research committee in accordance with the international guidelines for care and use of research animals (section 3.2.1).

6.2.2 UniPron administration and vaginal pH monitoring

To establish the duration that the low pH is maintained in the baboon vagina after administration of UniPron, five sexually mature cycling female baboons in the follicular phase of the menstrual cycle were used. Each anesthetized baboon was initially placed on an examination table and vaginal pH measured prior administration of 15 ml of UniPron into the vagina. The animals were monitored on an examination table until they sat upright when they were immediately put in the restraining chair for further assessment. A restraining chair designed specifically for baboon's size and comfort (Albatros, France) was used to restrain all the animals after immobilization for precise monitoring of vaginal pH changes after restoration of consciousness from anaesthesia. Thereafter, the pH was measured at hourly intervals for 6 hours (section 3.2.3.1). The baboons were restrained in the chair for a maximum of 7 hours according to the National Research Council (NRC) guidelines of Kenya for physical restraint (NRC, 1996). All the baboons were

administered a 5% Glucose intravenous infusion to maintain blood sugar levels and prevent development of hypoglycaemia.

6.2.3 Vaginal UniPron administration and time mating

Five sexually mature male and ten female baboons were used in this study to assess the contraceptive effectiveness of UniPron. The females were of proven fertility (defined by previous nipples). Male baboons of proven fertility were mated with UniPron treated or untreated females of proven fertility during the fertile stages. Each female baboon's menstrual cycle was monitored by visual assessment of perineal tissue tumescence and detection of menstrual blood to allow for timed experimentation. Co-housing of the male and female baboons was done daily during ovulatory cycle. The female baboons were housed in individual cages placed adjacent to that of one of the male baboons on the project. The cages were separated by a wide wire mesh dividers which allowed for fingertip access (first stage of tactile contact). Following positive compatible observations with wide mesh, animals were allowed further contact through grooming bars, which allow arms and legs to reach into the neighbouring cage. Following successful grooming contact observations, all cage dividers were opened to allow for a timed co-housing session of male and female baboons. At the end of each timed session, the animals were separated by closing of the cage dividers. If aggressive behaviour was noted to dominate a compatibility session, the session was ended and male baboon returned to its separate home cage.

Prior to co-housing sessions the female baboons were randomized into two groups of five animals each. They were sedated (section 3.2.1) and vaginal pH measured (section 3.2.3.1) on

the first day of co-housing. Thereafter, 15 ml of UniPron 0.4 gm was administered into the vagina of the five experimental animals. No treatment was given to the control group. This was done daily to both groups of animals during the ovulatory stage and then the animals were time mated immediately afterwards every day until deflation of the sex skin. To reverse the effect of sedation and allow the female baboons to be active, both groups of animals were given intramuscular injection of an antisedan (Atipamezole, Orion Pharma, Finland) (Langoi *et al.*, 2009). Each female was time mated with a male of proven fertility during the period of maximum tumescence-ovulation period (Skaikh *et al.*, 1982) and successful mating was confirmed by observation. At the conclusion of each timed session which lasted for 3 hours, cage dividers were closed to separate the female baboons from the males. After 4 complete menstrual cycles, administration of UniPron was stopped in the treatment group and mating continued for another 4 complete cycles to determine the reversibility effect of UniPron. The control animals were time mated as the experimental animals within the fertile periods. Pregnancy was confirmed by ultrasound via a transabdominal probe (Pie Medical, 402150, 240 Parus Vet, Basi Unit, Pie Medical Equipment B. V, Maastricht Nertherlands) and palpation.

6.2.4 Data analysis

The data were analysed as described in section 3.2.3.15

6.3 Results

6.3.1 Monitoring of vaginal pH

Baseline vaginal pH of the five animals at 0 hour was observed to have a mean of 5.72 ± 0.47 before UniPron application. Administration of UniPron into the baboon vagina significantly lowered the pH to 3.6 ± 0.45 within 1hr ($P < 0.05$). The mean vaginal pH 2 and 3 hours post UniPron application was 3.78 ± 0.058 and 4.36 ± 0.65 , respectively. The vaginal pH was significantly lower 3 hours after UniPron application. Thereafter the pH returned to normal (Figure 6.1). All the animals were in the follicular phase of the menstrual cycle (Figure 6.2b).

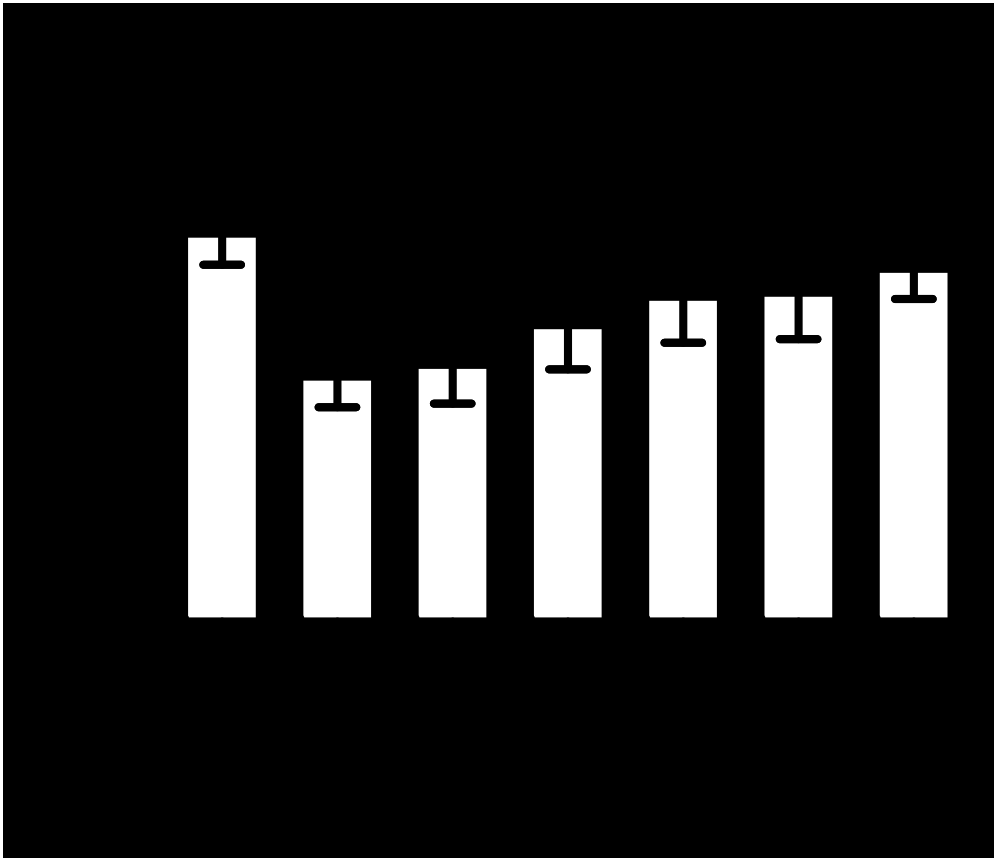


Figure 6.1. Baboon vaginal pH hourly after UniPron administration ($\bar{x} \pm SD$). 0 hour was the mean baseline vaginal pH before UniPron administration. (n=5)

6.3.2 Administration of vaginal UniPron and co-housing

Baboon vaginal pH assessed before co-housing showed significant inter individual variation and ranged from 4.3 to 6.7 (Figure 6.1). Co-housing sessions were carried out daily during follicular phase of the menstrual cycle for approximately 3 hours after UniPron application. Coital activity was reliably observed in the majority of cohousing sessions during follicular phase of the menstrual cycle (Figure 6.2b). Incidents of mutual grooming, females presenting and male mountings (coitus) were observed. Generally, multiple episodes of mounting occurred, with the majority of activity occurring within the first 30 minutes of co-housing the animals. On average five mounts per co-housing session were observed daily within the first 30 minutes. Visual evidence of male ejaculation was also noted, as was the female's menstrual cycle phase. All the control animals conceived within the first 2 cycles. UniPron completely prevented pregnancy in baboons. None of the experimental animals conceived within 4 cycles of mating but fertility was restored in all these animals when administration of UniPron was stopped (Table 6.1). No treatment-related abnormalities were observed in menstrual cycle length.

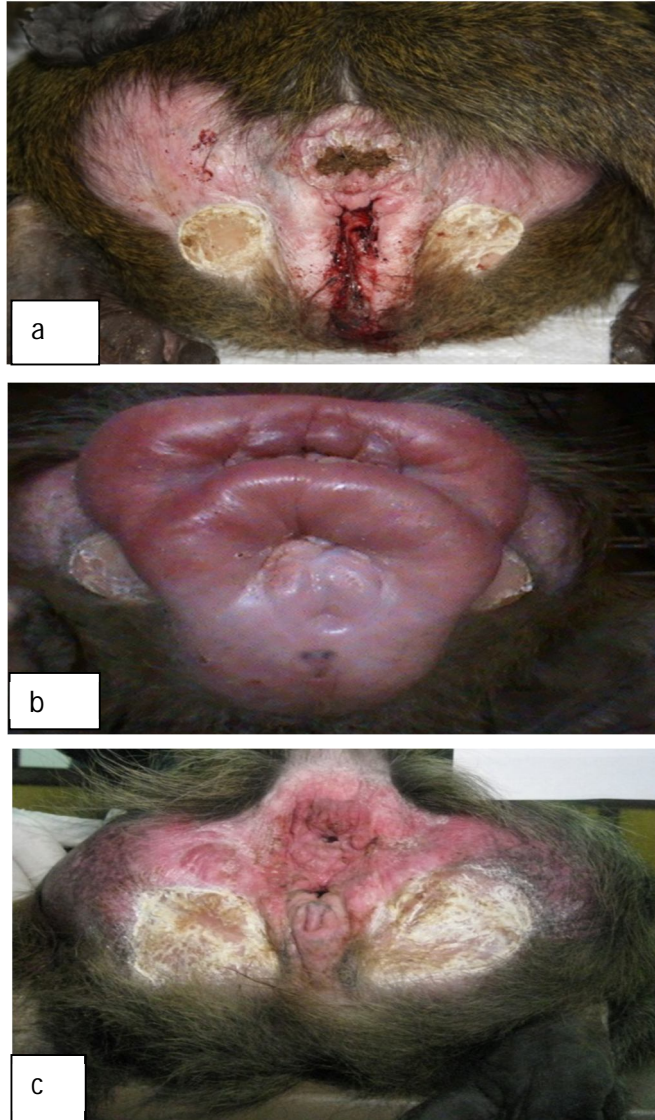


Figure 6.2. Baboon sex skin appearances during different phases of the ovulatory cycle. (a) Menstrual phase characterized with flow of menstrual blood; (b) Follicular phase (maximum tumescence) characterized by bright red-pink inflation, no wrinkles; (c) Luteal phase characterized by flat dry skin and wrinkles.

| | Animal number | Conception | Cycle of conception | Reversal of fertility (Conception) | Cycle of conception |
|--------------|---------------|------------|---------------------|------------------------------------|---------------------|
| Experimental | PAN 2729 | - | | + | 1 |
| | PAN 3010 | - | | + | 2 |
| | PAN 2727 | - | | + | 4 |
| | PAN 2734 | - | | + | 3 |
| | PAN 2728 | - | | + | 3 |
| Control | PAN 2950 | + | 1 | | |
| | PAN 2856 | + | 1 | | |
| | PAN 2782 | + | 1 | | |
| | PAN 2989 | + | 2 | | |
| | PAN 2612 | + | 2 | | |

Table 6.1. Effect of administration of 15 ml of 0.4g UniPron on baboon fertility. ('+'): Conception ('-'): No conception

6.4 Discussion

Despite currently available prevention strategies, there is an unacceptably high rate of unintended or mistimed pregnancies (Buckheit *et al.*, 2010). This study evaluated the possibility and feasibility of olive baboon as the model to study the potential effects and mechanism of action of UniPron on human reproduction. The vagina is normally too acidic for spermatozoa to survive. Baseline vaginal pH of the five baboons used to assess duration of effectiveness (Fig 6.1) was observed to have a means of 5.72 ± 0.47 before UniPron application. UniPron significantly lowered the vaginal pH ($P < 0.05$) and this low pH was maintained for about three hours after which it returned to normal. This suggests that the duration of protective effects of UniPron against conception may last for three hours.

The high levels of oestrogen in reproductive age women cause large amounts of glycogen to be deposited in the vaginal epithelium (Paavonen, 1983) and it is subsequently metabolized primarily by bacterial populations to produce organic acids (Boskey *et al.*, 1999). Several studies have reported that the healthy human vagina in reproductive aged women is acidic, with a pH ranging from 3.5 to 4.5, primarily because of lactic acid and this creates a restrictive environment that precludes the growth of many pathogenic organisms and immobilizes sperm (Garg *et al.*, 2001, Boskey *et al.*, 2001; Pavlova *et al.*, 2002). Due to physiological similarities with women the baseline acidic vaginal pH observed in baboons could be due to high levels of estrogen during the follicular phase that cause large amounts of glycogen to be deposited in the vaginal epithelium (Paavonen, 1983) and is subsequently metabolized primarily by bacterial populations to produce organic acids (Boskey *et al.*, 1999).

The baboon vaginal pH ranged from 4.5 to 7 (Fig 6.1, Table 6.1) and was comparable to other data reported in baboons (pH 5.0-6.0) (Obiero *et al* 2008), (pH 5.0-7.0) (Rivera *et al.*, 2011), (4.5-7.0) (Obiero *et al* 2013b). The baboon vaginal tract pH though acidic, is higher than that normally encountered in women (pH<5) (Miller, 1994, Miller *et al.*, 1994, Tevi-Benissan *et al.*, 1997). Whether this is a result of host physiology or bacterial activities is unknown. Previous studies in humans, have interpreted this elevated pH values to indicate a disease state, namely bacterial vaginosis (van De Wijnert *et al.*, 2000; Schwebke, 2001; Zhou *et al.*, 2004). However, recent studies have reported higher median vaginal pH values in normal Hispanic (pH 5.0±0.59) and black (4.74.7 ±1.04) compared with Asian (pH 4.4+ 0.50) and white women (pH 4.2+ 0.3) (Ravel *et al.*, 2011). High vaginal pH reflected the higher prevalence of communities not dominated by *Lactobacillus* species in the two ethnic groups which formed a large heterogeneous group of organisms yet these women had no symptoms of BV. Elevated vaginal pH ≥ 4.5 and vaginal communities not dominated by lactobacillus but anaerobic Gram positive bacteria does not necessarily reflect a disease state as interpreted in many previous studies, but it could be a characteristic of vaginal microbial communities normal to women in some ethnic groups.

The incidence of vaginal bacterial communities with high proportions of phylotypes related to strictly anaerobic bacteria and not dominated by species of *Lactobacillus*, hence less production of lactic acid, are thus common and appear normal in black and Hispanic women (Ravel *et al.*, 2011), hence a highly significant effect of ethnicity on the frequency of bacterial community types among women in different racial groups. These findings challenge the common wisdom that the occurrence of high numbers of lactobacilli and a vaginal pH of <4.5 are synonymous

with “normal” and “healthy” vagina (Zhou *et al.*, 2004; Ravel *et al.*, 2011; Gajer *et al.*, 2012). They also suggest that the baboon vaginal pH is comparable to the elevated vaginal pH in normal women with no symptoms of BV. In the absence of symptomology, the elevated vaginal pH might be considered normal and healthy and these different pH values could reflect differences in the composition of vaginal secretions or in the activities of vaginal bacteria or both.

For contraceptive effectiveness, female baboons were co-housed for time mating with the males during the follicular stage of the menstrual cycle (Figure 6.2b). It is not safe to house previously sedated female animal while in recovery phase with a fully alert male as it requires several hours to fully recover from the effects of sedation. To reverse the effect of sedation, both groups of animals were given intramuscular injection of an antisedan (Atipamezole, Orion Pharma, Finland) as previously described (Langoi *et al.*, 2009). Each female was time mated with a male of proven fertility during the period of maximum tumescence-ovulation period according to the method by Shaikh *et al.*, (1982) which lasted between four and seven days depending on the cyclicity of the female baboon. Successful mating was confirmed by observation and was noted to occur during follicular phase of the ovulatory cycle only. This phase was characterised by the swelling and reddening of the sexual skin around the perineum (Figure 6.2). Studies have reported that cyclic changes observed in the appearance of the sexual skin usually reflect cyclic fluctuations in secretion of ovarian hormones during the female cycle (Girolami & Bielert, 1987) and markedly influence the attractivity of females to males (Bielert & Girolami, 1998).

The average five mounts within the first 30 minutes per co-housing session observed were considered successful in that coital activity. However, the co-housing sessions were continued for three hours, the duration that UniPron was found to significantly lower the baboon vaginal

pH. These observations confirm that compatible baboons will copulate during follicular stage of the female's menstrual cycle.

Menstrual cycle was monitored by visual assessment of the sex skin and three phases of the cycle were observed namely; menstrual, follicular and luteal which were characterised by flow of the menstrual blood, bright red-pink swelling and flat wrinkled sex skin respectively (Figure 6.2). Studies have reported that during during the follicular phase of the menstrual cycle, the olive baboon anogenital area increases in turgescence due to oestrogenic stimulation (Dixon, 1998). Ovulation most commonly occurs during the last few days of maximal tumescence (Hendrickx & Kraemer, 1969; Koyama *et al.*, 2009), typically two days before the swelling subsides (Daspre *et al.*, 2009; Bercovitct, 1987; Shaikh *et al.*, 1982). In a number of primate species, males tend to mate during the period when ovulation is most likely (Garcia *et al.*, 2009, Higham *et al.*, 2009a) suggesting that they are able to make mating decisions based on the discrimination of fertility differences within the female reproductive cycle. In several primate species, the sexual swelling is one of the most conspicuous cues of fertility that could be used by males to allocate their mating activities (Dixon 2012). In primate species with a conspicuous sexual swelling whose size is correlated with the underlying probability of ovulation, such as baboons (Daspre *et al.*, 2009) female receptive behaviour can be predicted to be more likely linked to the ovarian cycle stage. These signals seem to be related to sexual hormones such as oestrogen and progesterone levels (Higham *et al.*, 2012). Adult males were observed to be able to discriminate intra-cycle differences in fertility and showed signs of aggression towards the females while not in the ovulatory stage. They displayed more sexual behaviour and tended to direct more ejaculatory mounts toward the females during the expected time of ovulation. In

addition, mating times were purposefully scheduled to coincide with ovulation to optimize breeding (Nadler & Rosenblum, 1973; Anderson & Erwin, 1975).

During intravaginal treatment with UniPron, no conception occurred in the UniPron treated animals, except when treatment was stopped and animals time mated. All control animals conceived within the first two cycles (Table 6.1). Intravaginal administration of 15 ml of UniPron was observed to block conception in baboons (Obiero *et al.*, 2008, Obiero *et al.*, 2010; Obiero *et al.*, 2013a; and 2013d). Since an acidic pH is inhospitable to sperm (Brown *et al.*, 2004), UniPron, a compound with a highly buffered low pH of 3.47 may have acted by preserving the acidic vaginal microenvironment in the baboons after ejaculation, resulting in irreversible immobilization of the sperms cells. During sexual intercourse, semen, which is alkaline, neutralizes the acidity of the vagina, making it more likely that spermatozoa are activated. As a vaginal defence enhancer, UniPron boosts body's natural defences by rapidly acidifying the ejaculate, reinforcing the natural acidity of the vagina that inactivates sperm. To protect spermatozoa from the vaginal environment, semen contains a variety of buffering proteins and the buffering quality of the ejaculate effectively neutralizes the vaginal defences for pathogens. The rationale for using an acidifying agent is to maintain an acidic pH in the vagina that will resist the buffering effect of semen and work as a spermicide and microbicide. However, spermatozoa can regain motility when pH is increased to normal (Olmsted *et al.* 2000; Makler *et al.*, 1981), highlighting the need for an acidifying agent to also have strong acid buffering properties. It is exactly these acid-buffering qualities that make UniPron an effective spermicide. The maintenance of an acidic vaginal pH counters the buffering capacity of semen and makes the gel spermicidal. This mechanism of action is comparable to other acidic vaginal buffers such as Acidform with a pH of 3.5 and is currently being sold as an over the counter

vaginal lubricating gel and also being developed as a vaginal contraceptive gel and Buffergel with a pH of 3.9. Both products have been shown to maintain low vaginal pH in the presence of relatively alkaline semen (Williams *et al.*, 2007).

6.5 Conclusion

Inclusion of sexual activity in the baboon model for topical vaginal contraceptive effectiveness evaluation closely mimics intended human use and provides a rigorous effectiveness assessment. This study demonstrates that it is feasible to incorporate sexual activity in the baboon model for candidate contraceptive products effectiveness assessment.

UniPron an acid-buffering product that inactivates spermatozoa, was found to be an effective and reversible topical non-hormonal contraceptive in the baboon model, suggesting that a safe, effective and reversible female contraception, which is non immunological and non-hormonal, is an attainable goal in humans. Since acidity immobilizes sperm with sufficient rapidity, a product that maintains vaginal acidity despite the deposit of an alkaline ejaculate might provide effective contraception. Further studies should be conducted to assess the effects of UniPron on human sperm function *in vitro* and its safety as a vaginal product in women.

CHAPTER 7: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General discussion

To alleviate dryness and discomfort during intercourse lubricants such as petroleum jelly, saliva, oil and water are commonly used (Galo *et al.*, 2010). These products have no published information on product-specific vaginal tolerance or irritation potency despite their frequent use by women. The use of oil based lubricants has been established to weaken the integrity of latex condom (Voeller *et al.*, 1989). Compared with KY Jelly, the most commonly used commercial lubricant; Smugel was not associated with local or systemic effects. However, some variation in % isolation of vaginal microflora was observed, but this could not be attributed the use of any of the gels as a similar trend was observed in base line data. Smugel, being water based not only has advantage over oil based lubricant, but also over the over presently marketed lubricating gels that have no information on their safety profile (Cunha *et al.*, 2014) and are only currently being characterized.

High rates of unintended pregnancies and STIs has led to increased interest in female controlled dual methods of STI prevention that also provide contraception. It is essential that any potentially harmful effects of vaginal microbicial contraceptive products be evaluated. Like the Smugel gel, the use of UniPron gel was not associated with local or systemic effects and variation in % isolation of vaginal microflora was also observed. The mechanism of action UniPron is based on it highly buffered low pH that works to enhance the normal vaginal defence mechanim. UniPron gel was found to inhibit growth of some STI pathogens and of baboon sperm cells in vitro. The mechanism of action of UniPron gel is comparable to other acidic vaginal buffers such as Acidform with a pH of 3.5 which currently being sold as an over the

counter vaginal lubricating gel and also being developed as a vaginal contraceptive gel and Buffergel with a pH of 3.9. Both products have been shown to maintain low vaginal pH in the presence of relatively alkaline semen (Williams *et al.*, 2007 Keller *et al.*, 2012).

7.2 Conclusions

- a) Both Smugel and UniPron gels were not associated with alteration of baboon vaginal pH, flora, clinical chemistry profile and vaginal and cervical mucosal irritation.
- b) The variation observed in % isolation of vaginal microflora could have been due to a number of factors including:
 - Host specific relationships
 - Composition of vaginal secretions and receptors on vaginal epithelial cells
 - Vaginal tract tolerance to a variety of different of different stains of bacteria, a feature that might contribute to survival of primate species
- c) UniPron enhanced low vaginal pH that was maintained for approximately three hours
- d) UniPron was found to inhibit growth STI pathogens *in vitro* and therefore a potential antimicrobial product
- e) UniPron was found to be effective as a non-hormonal contraceptive vaginal contraceptive in the baboon model

7.3 Research recommendations

Animal models should continue to play an important role in pre-clinical testing, with emphasis on safety, pharmacokinetics and efficacy testing. The positive safety and toxicity results of these studies indicate the need to move ahead with additional testing. Studies investigating the safety and effectiveness of UniPron as microbicide contraceptive in the baboon model are promising, but further trials are needed which include:

- a) Expanded safety studies of thorough investigation of the changes in inflammatory cells and cytokines following repeated Smugel and UniPron applications in the baboon vagina
- b) Development of Smugel as an over the counter personal vaginal lubricating gel
- c) Effect of UniPron on human sperm function *in vitro*.
- d) Safety of UniPron as a vaginal product in women
- e) Validation and standardization of the baboon as an animal model for testing vaginal microbicide contraceptive products.

REFERENCES

Abdool Karim Q, Abdool Karim SS, Frohlich JA, Grobler AC, Baxter C, MansoorLE, Kharsany ABM, Sibeko S, Mlisana KP, Omar, Gengiah TN, Maarschalk S, Arulappan N, Mlotshwa M, Morris L & Taylor D. (2010). Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science*. **329**: (5996):1168-74.

Achilles AP, Shete PB, Whaley KJ, Moench TR & Cone RA (2002). Microbicide efficacy and toxicity tests in a mouse model for vaginal transmission of *Chlamydia trachomatis*. *Sex Transm Dis*. **29**: 655-64.

Ahmed S, Li Q, Liu & Tsui AO. (2012). Maternal deaths averted by contraceptive use: an analysis of 172 countries. *Lancet*. **380**: 111–25.

Alkema L, KantorovaV, Menozzi C & Biddlecom A. (2013). National, regional and global rates and trends in contraceptive prevalence and unmet need for family planning between 1990 and 2015: a systematic and comprehensive analysis. *Lancet*. **381**: 1642-52.

Amaral E, Perdigao A, Souza M, Mauck C, Waller D, Zaneveld L & Faúndes A. (2004). Postcoital testing after the use of a bio-adhesive acid buffering gel (Acidform) and a 2% Nonoxynol- 9 product. *Contraception*. **70**: 492-7.

Amboka JN & Mwethera PG. (2003). Characterization of semen from olive baboons. *J Med Primatol*. **32**: 325–9.

Anderson B & Erwin J. (1975). A comparison of two timed-mating strategies for breeding pigtail macaques (*Macaca nemestrina*). *Theriogenology*. **4**:153-6.

Anton G, Rid J, Mylonas I, Friese K & Weissenbacher ER. (2008). Evidence of a TH1-shift of local vaginal inflammatory response during bacterial vaginosis. *Infection*. **36**: 147-52.

Antonio MA, Hawes SE & Hillier SL. (1999). The identification of vaginal *Lactobacillus* species and the demographic and microbiologic characteristics of women colonized by these species. *J Infect Dis*. **180**:1950-6.

Aryeetey R, Kotoh AM & Hindin MJ. (2010). Knowledge, perceptions and ever use of modern contraception among women in the Ga East District, Ghana. *Afr J Reprod Health*. **14**: 26-31.

Asekun-Olarinmoye O, Adebimpe WO, Bamidele JO, Odu OO, Asekun-Olarinmoye IO & Ojofeitimi EO. (2013). Barriers to use of modern contraceptives among women in an inner city area of Osogbo metropolis, Osun State, Nigeria. *Int J Women's Health*. **5**: 647-55.

Ashish J, Lokesh K, Bhavana K, Monika S, Aastha P, Vikas V, Vikas S, Vishal S, Tara R & Vishnu L. S. (2013). Combining a synthetic spermicide with a natural trichomonacide for safe, prophylactic contraception. *Hum. Reprod*. doi: 10.1093/humrep/det423.

Atashili J, Poole C, Ndumbe PM, Adimora AA & Smith JS. (2008). Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies. *AIDS*. **22**:(12):1493-501.

Auger J, Kunstmann JM, Czyglik & Jouannet P. (1995). Decline in semen quality among fertile men in Paris during the past 20 years. *N Engl J Med.* **332**: (5):281-5.

Ayehunie S, Cannon C, Lamore S, Kubilus J, Anderson DJ, Pudney J & Klausner M. (2006). Organotypic human vaginal-ectocervical tissue model for irritation studies of spermicides, microbicides, and feminine-care products. *Toxicol In Vitro.* **20**: (5): 689-98.

Bachmann GA & Nevadunsky NS. (2000). Diagnosis and treatment of atrophic vaginitis. *Am Fam Physician.* **61**: (10): 3090-6.

Baeten JM, Benki S, Chohan V, Lavreys L, McClelland RS, Mandaliya K, Ndinya-Achola JO, Jaoko W & Overbaugh J.(2007). Hormonal contraceptive use, herpes simplex virus infection, and risk of HIV-1 acquisition among Kenyan women. *AIDS.* **21**: 1771-7.

Baeten J, Hassan W, Chohan V, Richardson B, Mandaliya K, Ndinya-Achola JO, Jaoko W & McClelland RS. (2009). Prospective study of correlates of vaginal Lactobacillus colonization among high risk HIV-1 seronegative women. *Sex Transm Infect.* **85**: (5):348-53

Ballagh SA (2005). Vaginal hormone therapy for urogenital and menopausal symptoms. *Semin Reprod Med.* **23**: (2):126-40.

Ballagh SA, Bracheb V, Mauck C, Callahanc MM, Cochonb L, Wheellessd A & Moenche TR. (2008). A Phase I study of the functional performance, safety and acceptability of the BufferGel. *Contraception.* **77**:130-7

Bambra, C. & Chai, D. (1993). Veterinary management and research Technics for reproductive studies in baboons. Practical approach. *IPR Publication*. Nairobi, 10-15.

Barlow DH, Samsioe G & van Geelen JM. (1997). A study of European womens' experience of the problems of urogenital ageing and its management. *Maturitas*. **27**: (3):239-47.

Begay O, Jean-Pierre N, Abraham CJ, Chudolij A, Seidor, Rodriguez A, Ford, BE Henderson M, Katz D, Zydowsky T, Robbiani M & Ferná'ndez-Romero JA. (2011). Identification of personal lubricants that can cause rectal epithelial cell damage and enhance HIV type 1 replication in vitro. *AIDS Res Hum Retroviruses*. **27**: (9):1019-24.

Benshushan A, Shoshani O, Paltiel O, Schenker JG & Lewin A. (1997). Is there really a decrease in sperm parameters among healthy young men? A survey of sperm donations during 15 years. *J Assist Reprod Genet*. **14**: (6):347-53.

Bercovitch FB. (1987). Female weight and reproductive condition in a population of olive baboons (*Papio anubis*). *Am J Primatol*. **12**: 189-95.

Bing MA, Forney LJ & Ravel J. (2012). Vaginal Microbiome: Rethinking Health and Disease. *Annu Rev Microbiol*. **66**:371-89.

Bielert, C. & Girolami, L. (1986). Experimental assessments of behavioural and anatomical components of female chacma baboon (*Papio ursinus*) sexual attractiveness. *Psychoneuroendocrinology*. **11**: 75-90.

Boris S & Barbes C. (2000). Role played by lactobacilli in controlling the population of vaginal pathogens. *Microbes Infect.* **2**: (5):543-6.

Bornman MS, van Vuuren M, Meltzer DGA, van der Merwe CA & Rensburg SJR (1988). Quality of semen obtained from electroejaculation from chacma baboons (*Papio ursinus*). *J Med Primatol.* **17**: 57-6.

Boskey ER, Telsch KM, Whaley KJ, Moench TR & Cone RA. (1999). Acid production by vaginal flora in vitro is consistent with the rate and extent of vaginal acidification. *Infect Immun.* **67**: 5170-5

Boskey ER, Cone RA, Whaley KJ & Moench TR. (2001). Origin of vaginal acidity; high D/L lactate ratio is consistent with bacteria being the primary. *Hum Reprod.***16**: 1809-13.

Brown H. (2004). Marvellous microbicides. Intravaginal gels could save millions of lives, but first someone has to prove that they work. *Lancet.* **363**: (9414):1042-3.

Buckheit RW Jr, Watson KM, Morrow KM & Ham AS. (2010). Development of topical microbicides to prevent the sexual transmission of HIV. *Antiviral Research.* **85**:142-58.

Canning D & Schultz TP. (2012).The economic consequences of reproductive health and family planning. *Lancet.* **380**: 165-71.

Centers for Disease Control and Prevention ,(2010).Nationally Notifiable Infectious Conditions.<http://www.cdc.gov/ncphi/diss/nndss/phs/infdis2010.htm>

Chan DJ. (2005). Factors affecting sexual transmission of HIV-1: Current evidence and implications for prevention. *Curr HIV Res.* **3**:223-41.

Cherpes TL, Meyn LA, Krohn MA, Lurie JG & Hillier SL. (2003). Association between acquisition of herpes simplex virus type 2 in women and bacterial vaginosis. *Clin Infect Dis.* **37**: 319-25

Chun HM, Carpenter RJ, Macalino G & Crum-Cianflon NF. (2013). The role of sexually transmitted infections in HIV-1 progression: A comprehensive review of the literature. *J sex trans dis.* <http://dx.doi.org/10.1155/2013/176459>

Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, Haugen TB, Kruger T, Wang C, Mbizvo MT & Vogelsong KM. (2010). World Health Organization reference values for human semen characteristics. *Hum Reprod Update.* **16**: (3):231-45.

Cleland J, Conde-Agudelo A, Peterson HJH & Ross J, Tsui A. (2012). Contraception and health. *Lancet.* **380**: 149–56.

Coleman JS, Hitti J, Bukusi EA, Mwachari C, Muliro A, Nguti R, Gausman R, Jensen S, Patton D, Lockhart D, Coombs R & Cohen CR. (2007). Infectious correlates of HIV-1 shedding in the female upper and lower genital tracts. *AIDS.* **21**:755-9.

Cost M, Dezzutti CS, Clark MR, Friend DR, Akil A & Rohan LC. (2012). Characterization of UC781-tenofovircombination gel products for HIV-1 infection prevention in an ex vivo ectocervical model. *Antimicrob Agents Chemother.* **56**:3058–66.

Creanga AA, Gillepsie D, Karklins S & Tsui AO. (2011). Low use of contraceptive among poor women in Africa: An equity issue. *Bull World Health Organ.* **89**: 258-266.

Crossby RA, Holtgrave DR, DiClemente RJ, Wingood GM & Gayle JA. (2003). Social capital as a predictor of adolescents' sexual risk behavior: a state-level exploratory study. *AIDS Behav.* **7**: (3): 245-252.

Cunha AR, Machado RM, Palmeira-de-Oliveira A, Martinez-de-Oliveira J, Neves J & Palmeira-de-Oliveira R. (2014). Characterization of commercially available vaginal lubricants. A safety perspective. *Pharmaceutics* **2014**, 6, 530-542

Dancet EA, Brännström M, Brasky K, Chai D, Chan AW, Conn PM, Else J, Falconer H, Fazleabas AT, Farah IO, Goddeeris BM, Golos TG, Hau J, Hearn JP, Kariuki TM, Kyama CM, Lebovic DI, Mwenda JM, Ndung'u J, Nyachio A, Parker J, Slayden OD, Stouffer RL, Strauss JF, Taylor HS, Vanderpoel S, Westergaard JG, Zelinski M & D'Hooghe TM. (2013). The role of scientists and clinicians in raising public support for animal research in reproductive biology and medicine. *Biol Reprod.* **88**:(2):33.

Daspre A, Heistermann M, Hodges JK, Lee PC & Rosetta L. (2009). Signals of female reproductive quality and fertility in colony-living baboons (*Papio h anubis*) in relation to ensuring paternal investment. *Am J Primatol.* 71:529-38.

Debski R, Kotarski J, Paszkowski T, Pawelczyk L, Skrzypulec V & Tomaszewski J; Polish Gynecological Society. (2009). The statement of Polish Gynecological Society Experts on oral use of contraceptive 75 microg desogestrel minipill in different clinical casus – state of art in 2008. *Ginekol Pol.* **80**: 63-75.

Debski R, Karowicz-Bilińska A, Kotarski J, Kurzawa R, Nowak-Markwitz E, Pawelczyk L, Paszkowski T, Poreba R & Spaczyński M. (2010). The statement of Polish Gynecological Society Expertson use of transdermal contraceptive. *Ginekol Pol.* **81**: 69-73.

D’Cruz OJ & Uckun FM. (2004). Clinical development of microbicides for the prevention of HIV infection. *Curr Pharm Design.* **10**: (3):315-36.

Decrion AZ, Dichamp I, Varin A & Herbein G. (2005). HIV and inflammation. *Curr HIV Res.* **3**:243-59.

Dethlefsen L, McFall-Ngai M & Relman DA.(2007). An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature.* **449**: 811-18.

Dew JE, Wren BG & Eden JA. (2003). A cohort study of topical vaginal estrogen therapy in women previously treated for breast cancer. *Climacteric.* **6**: (1):45-52.

Dezzutti CS, Brown ER, Moncla B, Russo J, Cost M, Wang L, Uranker K, Kunjara Na Ayudhya RP, Pryke K, Pickett J, Leblanc MA & Rohan LC. (2012a). Is wetter better? An evaluation of over-the-counter personal lubricants for safety and anti-HIV-1 activity. *PLoS One*. **7**:e48328

Dezzutti CS, Shetler C, Mahalingam A, Ugaonkar SR, Gwozdz G, Buckheit KW & Buckheit RW Jr. (2012b). Safety and efficacy of tenofovir/IQP-0528 combination gels-a dual compartment microbicide for HIV-1 prevention. *Antiviral Res.* **96**:221–5.

D'Hooghe TM, Bambara CS, Hill JA & Koninckx PR. (1996). Effect of endometriosis on white blood cell sub populations in the peripheral blood and peritoneal of baboons. *Human Redrod.* **1**:1736-1740

D'Hooghe TM, Nyachio A, Chai DC, Kyama CM, Spiessens C & Mwenda JM. (2008). Reproductive research in non-human primates at Institute of Primate Research in Nairobi, Kenya (WHO Collaborating Center): a platform for the development of clinical infertility services? *ESHRE Monogr.* 102 107doi:10.1093/humrep/den164.

D'Hooghe TM, Pudney J, & Hill JA. (2000). Immunobiology of the reproductive tract in a female baboon. *Am J Primatol.* **53**:47-54.

D'hooge TM, Nugent NP, Cuneo S, Chai DC, Deer F, Debrock S, Kyama CM, Mihlyi A & Mwenda JM. (2006). Recombinant human TNFRSF1A(r-hTBP1) inhibits the development of endometriosis in baboons : a prospective, randomized placebo- and drug-controlled study. *Biol Reprod.* **74**:(1):131-6.

Dixson AF. (1998). Primate Sexuality: comparative studies of the prosimians, monkeys, apes and human beings. New York: Oxford University Press. 560 p.

Dixson AF. (2012). Primate sexuality: comparative studies of the prosimians, monkeys, apes, and humans. 2nd edition. New York: Oxford University Press. 808 p.

Donders GG, Bosmans F, Dekeermaecker A, Vaereken A & Van B (2000). Pathogenesis of abnormal vaginal bacterial flora. *Am J Obste Gynecol.* **182**: 872-8.

Dunn KM, Jordan K, Croft PR & Assendelft WJ. (2002). Systematic review of sexual problems: epidemiology and methodology. *J Sex Marital Ther.* **28**: (5):399-422.

Elias CJ & Heise LL. (1994). Challenges for the development of female-controlled vaginal microbicides. *AIDS.* **8**: (1):1-9.

Eschenbach DA, Thwin SS, Patton DL, Hooton TM, Stapleton AE, Agnew K, Winter C, Meier A & Stamm WE. (2000). Influence of the normal menstrual cycle on vaginal tissue, discharge, and microflora. *Clin Infect Dis.* **30**: 901-7.

Ezzati M, Lopez AD, Rodgers A, VanderHoorn S & Murray CJ. (2002). Comparative risk assessment collaborating group: Selected major risk factors and global and regional burden of disease. *Lancet*. **360**:1347-60.

Farage M & Maibach H. (2006). Lifetime changes in the vulva and vagina. *Arch Gynecol Obstet*. **273**: 195–202.

Farage MA, Maibach HI. (2011). Morphology and physiological changes of genital skin and mucosa. *Curr Probl Dermatol*. **40**: 9-19.

Fichorova RN, Yamamoto HS, Delaney ML, Andrew B, Onderdonk AB & Gustavo F. Doncel GF. (2011). Novel vaginal microflora colonization model providing new insight into microbicide mechanism of action. *mBio*. **2**:(6):e00168-11.

Fichtenberg MC, Jennings MJ, Glass AT & Ellen MJ. (2010). Neighborhood socioeconomic environment and sexual network position. *J Urban Health: Bulletin of the New York Academy of Medicine*. **87**:2

Fihn SD, Latham RH, Roberts P, Running K & Stamm WE. (1986). Association between diaphragm use and urinary tract infection. *JAMA*. **254**:240-5.

Forney LJ, Foster JA & Ledger W. (2006). “The vaginal flora of healthy women is not always dominated by *Lactobacillus* species,” *J Infect Dis*. **194**: (10):1468-9.

Frankel SS, Wenig BM, Burke AP, Mannan P, Thompson LD, Abbondanzo SL, Nelson AM, Pope M & Steinman RM. (1996). Replication of HIV-1 in dendritic cell-derived syncytia at the mucosal surface of the adenoid. *Science*. **272**: (5258):115-7.

Friend DR & Doncel GF. (2010). Combining prevention of HIV-1, other sexually transmitted infections and unintended pregnancies: Development of dual-protection technologies. *Antiviral Res.* 88 Suppl 1:S47-54.

Gallo MF, Sharma A, Bukusi EA, BNjoroge, Nguti R, Jamieson DJ, Bell AJ & Eschenbach DA. (2010). Intravaginal practices among female sex workers in Kibera, Kenya. *Sex Transm Infect.* **86**: 318-22.

Gajer P, Brotman RM, Bai G, Sakamoto J, Schutte UM, Zhong X, Koenig SS, Fu L, Ma ZS, Zhou X, Abdo Z, Forney LJ & Ravel J. (2012). Temporal dynamics of the human vaginal microbiota. *Sci Transl. Med.* **4**: 132-52.

Garcia C, Shimizu & Huffman M. (2009). Relationship between sexual interactions and the timing of the fertile phase in captive female Japanese macaques (*Macaca fuscata*). *Am J Primatol.* **71**: 868–79.

Garg S, Anderson RA, Chany CJ, 2nd, Waller DP, Diao XH, Vermani K & Zaneveld LJD. (2001). Properties of a new acid-buffering bioadhesive vaginal formulation (ACIDFORM). *Contraception*. **64**: 67-75.

Giorgi A, Torriani S, Dellaglio, F, Bo G, Stola E & Bernuzzi, L. (1987). Identification of vaginal lactobacilli from asymptomatic women. *Microbiologica*. **10**: 377-84.

Girolami L. & Bielert C. (1987). Female perineal swelling and its effects on male sexual arousal: an apparent sexual releaser in the chacma baboon (*Papio ursinus*). *Int J Prim*. **8**: 651-61.

Golderngerg RL, Andrew WW, Yuan AC, Mackay HT & St. Louis ME. (1997). Sexually transmitted diseases and adverse and adverse outcomes of pregnancy. *Clin Perinatol*. **24**:23-41.

Goldstein I. (2010). Recognizing and treating urogenital atrophy in postmenopausal women. *J Womens Health*. **19**: (3):425-32.

Goldstein I & Alexander JL. (2005). Practical aspects in the management of vaginal atrophy and sexual dysfunction in perimenopausal and postmenopausal women. *J Sex Med*. **2**:154-65.

Grammen C¹, Ariën KK, Venkatraj M, Joossens J, Van der Veken P, Heeres J, Lewi PJ, Haenen S, Augustyns K, Vanham G, Augustijns P & Brouwers J. (2014). Development and in vitro evaluation of a vaginal microbicide gel formulation for UAMC01398, a novel diarylthiazine NNRTI against HIV-1. *Antiviral Res*. **101**:113-21.

Gravett MG, Nelson HP, DeRouen T, Critchlow C, Eschenbach DA & Holmes KK. (1986). Independent association of bacterial vaginosis and *Chlamydia trachomatis* infection with adverse pregnancy outcome. *JAMA*. **256**: 1899-903.

Gray RH, LI X, Wawer MJ, Serwadda D, Sewankambo NK, Wabwire-Mangen F, Lutalo T, Kiwanuka N, Kigozi G, Nalugoda F, Meehan MP, Robb M & Quinn TC. (2004). Determinants of HIV-1 load in subjects with early and later HIV infections, in a general population cohort of Rakai, Uganda. *J Infect Dis*. **187**: 1513-21.

Gupta K, Stapleton AF, Hooton TM, Roberts PL, Fennell CL & Stamm WF. (1998). Inverse association of H₂O₂-producing lactobacilli and vaginal *Escherichia coli* colonization in women with recurrent urinary infections. *J Infect Dis*. **18**: 446-50.

Haddad LB. (2009). Unsafe abortion: unnecessary maternal mortality. *Rev Obstet Gynecol*. **2**:122-6.

Haggerty CL, Hillier SL, Bass DC & Ness RB. (2004). Bacterial vaginosis and anaerobic bacteria are associated with endometritis. *Clin Infect Dis*. **39**: 990-5.

Harwell JI, Moench T, Mayer KH, Chapman S, Rodriguez I & Cu-Uvin S. (2003). A pilot study of treatment of bacterial vaginosis with a buffering vaginal microbicide. *J Women's Health*. **12**: 255-9.

Hendrickx AG & Kraemer DC. (1969). Observations on the menstrual cycle, optimal mating time and pre-implantation embryos of the baboon, *Papio anubis* and *Papio cynocephalus*. *J Reprod Fertil.* **6**:119-28.

Heffron R, Donnell D, Rees H, Celum C, Mugo NWere E, de Bruyn G, Nakku-Joloba E, Ngure K, Kiarie J, Coombs RW & Baeten JM. (2012). Use of hormonal contraceptives and risk of HIV-1 transmission: a prospective cohort study. *Lancet Infectious Dis.* **12**: 19-26.

Hendrickx AG & Kraemer DC. (1969). Observations on the menstrual cycle, optimal mating time and pre-implantation embryos of the baboon, *Papio anubis* and *Papio cynocephalus*. *J Reprod Fertil.* **6**:119-28.

Higham JP, Semple S, MacLarnon A, Heistermann M & Ross C. (2009a). Female reproductive signaling, and male mating behavior, in the olive baboon. *Horm Behav.* **55**: 60-7.

Higham JP, Heistermann M, Saggau C, Agil M, Perwitasari-Farajallah D & Engelhardt A. (2012). Sexual signaling in the crested macaque and the evolution of primate fertility signals. *BMC Evol Biol.* **12**:89.

Hilber AM, Francis SC, Chersich M, Scott P, Redmond S, Bender N, Miotti P, Temmerman M & Low N. (2010). Intravaginal practices, vaginal infections and HIV acquisition: systematic review and meta-analysis. *PLoS One.* **5**: (2):e9119.

Hillier SL. (1993). Diagnostic microbiology of bacterial vaginosis. *Am J Obstet Gynecol.* **169**: 455-9.

Hillier SL, Nugent RP, Eschenbach DA, Krohn MA, Gibbs RS, Martin DH, Cotch MF, Edelman R, Pastorek JG & Rao AV. (1995). Association between bacterial vaginosis and preterm delivery of a low birth weight infant. *N Engl J Med.* **333**: 1737-42.

Historical Demographic Data (2013). <http://www.populstat.info/Africa/kenyac.htm>

Hladik F, Sakchalathorn P, Ballweber L, Lentz G, Fialkow M, Eschenbach D & McElrath MJ. (2007). Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. *Immunity.* **26**: 257–270.

Hoffman S, Morrow KM, Mantell JE, Rosen RK, Carballo-Diéguez A & Gai F. (2010). Covert use, vaginal lubrication, and sexual pleasure: a qualitative study of urban U.S. Women in a vaginal microbicide clinical trial. *Arch Sex Behav.* **39**: (3):748-60.

Holtgrave DR, DiClemente RJ, Wingood GM & Gayle JA. (2003). Social capital as a predictor of adolescents' sexual risk behavior: a state-level exploratory study. *AIDS Behav.* **7**: (3): 245-52.

Hooton TM, Hillier S & Johnson C. (1991). *Escherichia coli* bacteriuria and contraceptive method. *JAMA.* **265**: 64-9.

Hu J, Gardner MB & Miller CJ. (2000). Simian immunodeficiency virus rapidly penetrates the cervicovaginal mucosa after intravaginal inoculation and infects intraepithelial dendritic cells.

J Virol. **74**: (13): 6087-95.

Isaacs CE, Rohan L, Xu W, Jia JH, Mietzner T & Hillier S. (2006). Inactivation of herpes simplex virus clinical isolates by using a combination microbicide. *Antimicrob Agents Chemother.* **50**: (3):1063–6.

Jacobson DL, Peralta L, Farmer M, Graham NM, Gaydos C & Zenilman J. (2000). Relationship of hormonal contraception and cervical entropy as measured by computerized planimetry to chlamydial infection in adolescents. *Sex Transm Dis.* **27**: (6):313-9.

Kaewsrichan J, Peeyananjarassri K & Kongprasertkit J. (2006). Selection and identification of anaerobic lactobacilli producing inhibitory compounds against vaginal pathogens.

FEMS Immunol Med Microbiol. **48**: 75-83.

Kaushic C, Ashka AA, Reid LA & Rosenthal KL. (2003). Progesterone increases susceptibility and decreases immune responses to genital herpes infection. *J Virol.* **77**: 4558-65.

Keller MJ, Carpenter CA, Lo Y, Einstein MH, Liu C, Fredricks DN & Herold BC. (2012). Phase I randomized safety study of twice daily dosing of acidform vaginal gel: Candidate antimicrobial contraceptive. *PLOS One* **7**:10:e46901.

Khanna KV, Whaley KJ, Zetlin L, Moench TR, Mehrazar K, Cone RA, Liao Z, Hildreth JE, Hoen TE, Shultz L & Markham RB. (2002). Epidemiology and clinical significance of cervical erosion in women attending a family planning clinic. *J Clin Invest.* **109**: 205-11.

Klebanoff S, Hillier S L, Eschenbach DA, & Waltersdorff WM. (1991). Control of microbial flora of the vagina by H₂O₂-generating lactibacilli. *J Infect Dis.* **164**: 94-100.

Kyama CM, Falconer H, Cuneo S, Chai D, Mihalyi A, Mwenda J & D'Hooghe T. (2014). Menstrual endometrial supernatant may induce stromal endometriosis in baboons. *Front Biosci (Schol Ed).* **1**: (6):16-28.

Koyama T, De La Pena A & Hagino N. (1977). Plasma estrogen, progestin, and luteinizing hormone during the normal menstrual cycle in the baboon: role of luteinizing hormone. *Am J Obstet Gynecol.* **127**: 67–71.

Laga M, Diallo MO, & Buvé A. (1994). “Interrelationship of STD and HIV: Where Are We Now?” *AIDS.* **8**: (Suppl.): S119-24.

Langoi DL, Mwethera PG, K.S.P. Abelson KSP, Farah IO & H.E. Carlsson. (2009). Reversal of Ketamine/Xylazine combination anesthesia by Atipamezole in olive baboons (*Papio anubis*). *J Med Primatol.* **38**: 404-10.

Larsen B. (1993). Vaginal flora in health and disease. *Clin Obstet Gynecol.* **36**: 107–21.

Larsen B & Monif GR. (2001). "Understanding the bacterial flora of the female genital tract," *Clin Infect Dis.* **32**: (4): e69-77.

Lavreys L, Baeten JM, Martin HL Jr, Overbaugh J, Mandaliya K, Ndinya-Achola J & Kreiss JK. (2004). Hormonal contraception and risk of HIV-1 acquisition: results of a 10-year prospective study. *AIDS.* **18**: 695-7.

Levison ME, Corman LC, Carrington ER, & D. Kaye D. (1977). Quantitative microflora of the vagina. *Am J Obstet Gynecol.* **127**: (1):80-5.

Looker J, Garnett G & Schmid G. (2008). An estimate of the global prevalence and incidence of herpes simplex virus type 2 infection. *Bull World Health Organi.* **86**: 805-12.

Low N, Broutet N, Adu-Sarkodie Y, Barton P, Hossain M & Hawkes S. (2006). Global control of sexually transmitted infections. *Lancet.* **368**: 2001-16.

Lynch C. (2009). Vaginal estrogen therapy for the treatment of atrophic vaginitis. *J Womens Health.* **18**: (10):1595-606.

Ma Z, Lu FX, Torten M & Miller CJ. (2001). The number and distribution of immune cells in the cervicovaginal mucosa remain constant throughout the menstrual cycle. *Clin Immunol.* **100**: (2):244-9.

Mahmoud EA, Svensson LO, Olsson SE & Mardh PA. (1995). Antichlamydial activity of vaginal secretion. *Am J Obstet Gynecol.* **172**:1268-72.

Makler A, David R, Blumenfeld Z & Better OS. (1981). Factors affecting sperm motility.VII. Sperm viability as affected by change of pH and osmolarity of semen and urine specimens. *Fertil Steril.* **36**: 507-11.

Marrazzo JM, Koutsky LA, Eschenbach DA, Agnew K, Stine K, & S. L. Hillier (2002). “Characterization of vaginal flora and bacterial vaginosis in women who have sex with women,” *J Infect Dis.* **85**: (9):1307-13.

Martin HA, Hull TH, Preston-Whyte E, Brigitte Bagno G, Smit J, Wacharasin C & Widyantoro N. (2010). A cross cultural study of vaginal practices and sexuality: implications for sexual health. *Soc Sci Med.* **70**: (3): 392-400.

Martin HL, Richardson BA Nyange PM, Lavreys L, Hillier SL, Chohan B, Mandaliya K, Ndinya-Achola JO, Bwayo J & Kreiss J. (1999). Vaginal lactobacilli, microbial flora and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. *J Infect Dis.* **180**: 1863-8.

Martin HL, Nyange PM, Richardson BA, LavreysL, Mandaliya K, Jackson DJ, Ndinya-Achola JO & Kreiss J. (1998). Hormonal contraception, sexually transmitted diseases, and risk of heterosexual transmission of human immunodeficiency virus type 1. *J infect Dis.* **178**: 1053-9.

Marx PA, Spira AI, Gettie A, Dailey PJ, Veazey RS, Lackner AA, Miller CJ, Claypool LE, Ho DD & Alexander NJ. (1996). Progesterone implants enhance SIV vaginal transmission and early virus load. *Nat Med.* **2**: 1084-9.

Mastorakos G, Koliopoulos C, Deligeoroglou E, Diamanti-Kandarakis E & Creastas G. (2006). Effects of two forms of combined oral contraceptives on carbohydrate metabolism in adolescents with polycystic ovary syndrome. *Fertil Steril.* **85**: (2):420-7

Mastromarino P, Brigidi P, Macchia S, Maggi L, Pirovano F, Trichieri V, Conte U & Matteuzzi, D. (2002). Characterization and selection of vaginal Lactobacillus strains for the preparation of vaginal tablets. *J Applied Microbiol.* **93**: (5): 884-93.

Matson PA, Adler NE, Millstein SG, Tschann JM & Ellen JM. (2011). Developmental changes in condom use among urban adolescent females: Influence of partner context. *J Adolesc Health.* **48**: (4); 386-90

Mayaud P & Mabey D. (2004). Approaches to the control of sexually transmitted infections in developing countries: old problems and modern challenges. *SexTrans Infect.* **80**: 174-82.

Mayaud P & McCormick D. (2001). The changing face of HIV and AIDS. *Br Med Bull.* **58**: 129-153.

Mayer KH, Peipert J, Fleming T, Fullem A, Moench T, Cu-Uvin S, Bentley M, Chesney M & Rosenberg Z. (2001). Safety and tolerability of BufferGel, a novel vaginal microbicide, in women in the United States. *Clin Infect Dis.* **32**: 476-82.

Mburu N, Obiero JA, Waititu K, Mwaura BN, Orawo JO, Farah IO & Mwethera PG. (2009). Safety studies of a recently developed microbicial contraceptive gel (UniPron) in female baboons (*Papio anubis*). *Afri J Reprod Health.* **13**: (4):95-104.

McGowan I, Gomez K, Bruder K, Febo I, Chen BA, Richardson BA, Husnik M, Livant E, Price C & Jacobson C. (2011). Phase 1 randomized trial of the vaginal safety and acceptability of SPL7013 gel (VivaGel) in sexually active young women (MTN-004). *AIDS.* **25**: (8):1057-64.

McGowan I. (2012). Rectal microbicide development. *Curr Opin HIV AIDS.* **7**:(6):526-33.

Medard LM & Ostrowska L. (2010). Hormonal (levonorgestrel) emergency contraception--effectiveness and mechanism of action. *Ginekol Pol.* **81**:(7):532-6.

Meis PJ, Goldenberg RL, Mercer B, Moawad A, Das A, McNellis D, Johnson F, Iams JD, Thom E & Andrews WW. (1995). The preterm prediction Study: significance of vaginal infections. *Am J Obstet Gynecol.* **173**: 1231-5.

Miller CJ. (1994). Animal models of viral sexually transmitted diseases. *Am J Reprod Immunol.* **31**: 52–63.

Miller CJ, Alexander NJ, Vogel P, Anderson J & Marx PA. (1992). Mechanism of genital transmission of SIV: a hypothesis based on transmission studies and the location of SIV in the genital tract of chronically infected female rhesus macaques. *J Med Primatol.* **21**: (2-3): 64-8.

Miller CJ, Marthas M, Torten J, Alexander NJ, Moore JP, Doncel GF & Hendrickx AG. (1994). Intravaginal inoculation of rhesus macaques with cell-free simian immunodeficiency virus results in persistent or transient viremia. *J Virol.* **68**: 6391-400.

Minnis AM & Padian NS. (2005). Effectiveness of female controlled barrier methods in preventing sexually transmitted infections and HIV: current evidence and future research directions. *Sex Trans Infect.* **81**:193-200.

Mohllajee AP, Curtis, KM, Martins SL & Peterson HB. (2006). Hormonal contraceptive use and risk of sexually transmitted infections: a systematic review. *Contraception.* **73**:154-65

Morrison CS, Bright P, Wong E, Kwok C, Yacobson I, Gaydos CA, Tucker HT & Blumenthal PD. (2004). Hormonal contraception use, cervical ectopy, and the acquisition of cervical infections. *Sex Transm Dis.* **31**:561-7.

Mortimer D & Mortimer ST. (1999). Laboratory investigation of the infertile male. In: A Textbook of In Vitro Fertilization and Assisted Reproduction, 2nd edn. Brinden (ed.). Cambridge: Parthenon publishing group, 53–81.

Moss JA, Malone AM, Smith TJ, Kennedy S, Kopin E, Nguyen C, Gilman J, Butkyavichene I, Vincent KL, Motamedi M, Friend DR, Clark MR & Baum MM. (2012). Simultaneous delivery of tenofovir and acyclovir via an intravaginal ring. *Antimicrob Agents Chemother.* **56**: 875-82.

Nachtigall LE. (1994). Comparative study: Replens versus local estrogen in menopausal women. *Fertil Steril.* **61**: (1):178-80.

Nadler RD & Rosenblum LA. (1973). Sexual behavior of male pigtail macaques in the laboratory. *Bra in Behav Evol.* **7**: 18-33.

Nagase T, Kunii O, Wakai S & Khaleel A. (2003). Obstacles to modern contraceptive use among married women in southern urban Maldives. *Contraception.* **68**: (2):125-34.

National Research Council. (1996). Guide for the care and use of laboratory animals. Washington DC: National Academy Press. 9–11.

NCT01306331. Study of the Contraceptive Efficacy and Safety of Amphora Gel Compared to Conceptrol Vaginal Gel.<http://www.Clinicaltrials.gov/show/NCT01306331>.

Nel AM, Mitchnick LB, Risha P, MuungoLT & Norick PM. (2011). Acceptability of vaginal film, soft-gel capsule, and tablet as potential microbicide delivery methods among African women. *J Womens Health (Larchmt)*. **20**: (8):1207-14.

Ness RB, Kip KE, Hillier SL, Super DE, Stam CA, Sweet RL, Rice P, & Rtcher HE. (2005). A cluster analysis of bacterial vaginosis-associated microflora microflora and pelvic inflammatory disease. *Am J Epidemiol*. **162**: 585-90.

Nyachio A, Spiessens1C, Chai CC, Kiulia MN, Jason M. Mwenda JM & D'Hooghe TM. (2012). Baboon spermatology: basic assessment and reproducibility in olive baboons (*Papio anubis*). *J Med Primatol*. **41**:297-303.

Oberg K, Fugl-Meyer AR & Fugl-Meyer KS. (2004). On categorization and quantification of women's sexual dysfunctions: an epidemiological approach. *Int J Impot Res*. **16**: (3):261-9.

Obiero JA, Maureen NM, Ndung'u BM, Waititu KK, Mulei I, Farah IO, & Mwethera PG. (2008). UniPron is a fully effective Non-hormonal reversible contraceptive in baboon model (*Papio anubis*). *J Reprod & Contracep*. **19**: (2): 107-18.

Obiero JA, Mburu N, Mwaura B, Waititu KK, Mulei I, Farah IO & Mwethera PG. (2010).

Development of a safe microbicide contraceptive, unipron, to prevent pregnancies and sexually transmitted infections. In the proceedings of the 18th Institute of Primate Research International Biomedical Conference, Page 14, 6th-8th July 2010, Kenya Institute of Education (KIE), Nairobi, Kenya.

Obiero J, Mwethera PG & Wiysonge CS. (2012a). Topical microbicides for prevention of sexually transmitted infections (2012a). *Cochrane Database System Rev.* Issue 6. Art. No.: CD007961. DOI: 10.1002/14651858.CD007961.pub2.

Obiero J, Mwethera PG, Hussey GD & Wiysonge CS. (2012b). Vaginal microbicides for reducing the risk of sexual acquisition of HIV infection in women: systematic review and meta-analysis. *BMC Infectious Diseases*. **12**:289 (<http://www.biomedcentral.com/1471-2334/12/289>)

Obiero JA, Kunyera R, Waititu K, Mulei I, Farah IO, Kavoo L, Jaoko W & Mwethera PG. (2012c). A comparative study of Smugel and KY Jelly vaginal lubricating gels. Oral presentation: In the proceedings of the 36th Kenya Obstetrical and Gynaecological Society (KOGS) Annual Conference, Page 38, 15th-17th February 2012, Safari Park Hotel, Nairobi, Kenya.

Obiero JA, Kunyera R, Waititu K, Mulei I, Farah IO, Kavoo L, Jaoko W & Mwethera PG. (2012d). A comparative study of Smugel and KY Jelly vaginal lubricating gels. In the proceedings of the 1st National Science, Technology and Innovation (Organised by Ministry of Higher Education, Science and Technology & the National Council for Science and Technology), Abstract No. 23, Page 37, 7th-11th May, Kenyatta International Conference Centre (KICC), Nairobi, Kenya.

Obiero JA, Mulei I, Farah IO, Linge K, Jaoko W & Mwethera PG. (2013a). Safety and contraceptive studies of UniPron. In the proceedings of the University of Nairobi/Kenyatta National Hospital International Scientific Conference, Abstract No. 22, Page 15-16, 19th-21st June, Kenyatta National Hospital, Nairobi, Kenya.

Obiero JA, Kunyera R, Waititu K, Mulei I, Farah IO, Kavoo L, Jaoko W & Mwethera PG. (2013b). A comparative study of Smugel and KY Jelly vaginal lubricating gels. *J Reprod Contracept.* **24:** (2) 76-87.

Obiero JA, Mwethera PG & Wiysonge CS. (2013c). Topical microbicides for prevention of sexually transmitted infection. In the proceedings of the African Cochrane Indaba Conference, Abstract No.P06, Page 33, 6th-8th May, Lagoon Beach Hotel, Cape Town, South Africa.

Obiero JA, Mulei I, Farah IO, Linge K, Jaoko W & Mwethera PG. (2013d). Safety and contraceptive studies of UniPron. In the proceedings of the University of Nairobi/Kenyatta National Hospital International Scientific Conference, Abstract No. 22, Page 15-16, 19th-21st June, Kenyatta National Hospital, Nairobi, Kenya.

Obiero JA, Mulei I, Farah IO, Linge K, Jaoko W & Mwethera PG. (2013e). Microbicidal and contraceptive studies of UniPron vaginal gel. In the proceedings of the 1st International Research and Innovation Conference of Mount Kenya University, Page 41, 28th-30th August, Safari Park Hotel, Nairobi, Kenya.

O'Connor TJ, Kinchington D, Kangro HO & Jeffries DJ. (1995). The activity of candidate virucidal agents, low pH and genital secretions against HIV-1 in vitro. *Int J STD AIDS*. **6**:267-72.

Olmsted SS, Dubin NH, Cone RA & Moench TR. (2000). The rate at which human sperm are immobilized and killed by mild acidity. *Fertil Steril*. **73**: 687-93.

Paavonen J. (1983). Physiology and ecology of the vagina. *J Infect Dis*. **40**: 31-5.

Pavlova S, Kilic AO & Kilic SS. (2002). ``Genetic diversity of vaginal lactobacilli from women in different countries based on 16S rgene sequencesRNA''. *Gynecol*. **173**: 1231-5

Palamuleni ME. (2013). Socio-economic and demographic factors affecting contraceptive use in Malawi. *Afr J Reprod Health*. **17**: (3): 91-104.

Palmer AR & Likis FE. (2003). Lactational atrophic vaginitis. *J Midwifery Womens Health*. **48**: (4):282-4.

Pandit L & Ouslander JG. (1997). Postmenopausal vaginal atrophy and atrophic vaginitis. *Am J Med Sci.* **314**: (4):228-31

Pettit RK, McAllister SC & Hamer TA. (1999). Response of gonococcal clinical isolates to acidic conditions. *J Med Microbiol.* **48**: 149-56.

Potts M. (1994). The urgent need for a vaginal microbicide in the prevention of HIV transmission. *Am J Public Health.* **84**: (6):890-1.

Potts M, Halperin DT, Kirby D, Swidler A, Marseille E, Klausner JD, Hearst N, Wamai RG, Kahn GJ & Walsh J. (2008). Reassessing HIV Prevention. *Science.* **320**: (5877):749–55.

Randrianasolo B, Swezey T, Van Damme K, Khan MR, Ravelomanana N, Lovaniaina Rabenja N, Raharinivo M, Bell AJ, Jamieson D & Behets F. (2008). Barriers to the use of modern contraceptives and implications for woman-controlled prevention of sexually transmitted infections in Madagascar. *J Bio Soc Sci.* **40**: (6):879-93.

Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, Karlebach S, Gorle R, Russell J, Tacket CO, Brotman RM, Davis CC, Ault K, Peralta L & Forney LJ. (2011). Vaginal Microbiome of reproductive-age women. *Proc Natl Acad Sci, U S A.* **108**: Suppl 1:4680-7.

Redondo-Lopez V, Cook RL, & Sobel JD. (1990). “Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora,” *Rev Infect Dis.* **12**: (5): 856-72.

Reid G, McGroart JA, Tomeczek L, & Bruce AW. (1996). "Identification and plasmid profiles of Lactobacillus species from the vagina of 100 healthy women. " *FEMS Immunol Med Microbiol.* **15:** (1):23–6.

Richardson BA, Martin HL Jr, Stevens CE, Hillier SL, Mwatha AK, Chohan BH, Nyange PM, Mandaliya K, Ndinya-Achola J & Kreiss JK. (1998). Use of nonoxynol-9 and changes in vaginal lactobacilli. *J Infect Dis.* **178:** (2):441-5.

Rivera AJ, Stumpf RM, Wilson B, Leigh S & Salyers AA. (2010). Baboon vaginal microbiota: an overlooked aspect of primate physiology. *Am J Primatol.* **72:** (6):467-74.

Rodriguez JM, Collins MD, Sjoden B & Falsen E. (1999). Characterisation of a novel Atopobium isolate from the human vagina: description of Atopobium vaginae sp. nov. *Bacteriol.* **49:** 1573-6.

Rose WA, McGowin CL, Spagnuolo RA, Eaves-Pyles TD, Popov VL & Pyles RB. (2012). Commensal bacteria modulate innate immune responses of vaginal epithelial cell multilayer cultures. *PLoS One.* **7:** (3):e32728.

Rosenberg ZF & Devlin B. (2012). Future strategies in microbicide development. *Best Pract Res Clin Obstet Gynaecol.* **26:** (4):503-13.

Sarkar NN. (2008). Barriers to condom use. *Eur J Contracept Reprod Health Care*. **13**: (2):114-22.

Schwebke JR. (2000). Assymptomatic bacterial vaginosisl response to therapy. *Am J Obstet Gynecol*. **183**: 1434-9

Sérgio A, Teixeira M & Taquette SR. (2010). Violence and unsafe sexual practices in adolescents under 15 years of age. *Assoc Med Bras*. **56**: (4):440-6.

Severy LJ & Newcomer S. (2005). Critical issues in contraceptive and STI acceptability research. *J Soc Issues*. **61**: (1):45–65.

Shattock RJ & Moore JP. (2003). Inhibiting sexual transmission of HIV-1 infection. *Nat Rev Microbiol*. **1**:(1): 25-37.

Shaikh AA, Celaya CL, Gomez I & Shaikh SA. (1982). Temporal relationship of hormonal peaks to ovulation and sex skin deturgescence in the baboon. *Primates*. **23**: 444-52.

Shepherd J, Picot J, Jones J, Kavanagh J, Harden A, Barnett-Page E, Clegg A, Hartwell D, Frampton G & Price A. (2012). An economic model of school-based behavioral interventions to prevent sexually transmitted infections. *Int J Technol Assess Health Care*. **28**: (4); 407–14.

Shust GF, Cho S, Kim M, Madan RP, Guzman EM, Pollack M, Epstein J, Cohen HW, Keller MJ & Herold BC. (2010). Female genital tract secretions inhibit herpes simplex virus infection: correlation with soluble mucosal immune mediators and impact of hormonal contraception. *Am J Reprod Immunol.* **63**: 110-9.

Sibbald B. (2004). Emergency contraception could lower abortion rate. *CMAJ.* 13:1903.

Siddique SA. (2003). Vaginal anatomy and physiology. *J Pelvic Surgery.* **9**: 263-72.

Siegfried N, Muller M, Deeks JJ & Volmink J. (2009). Male circumcision for prevention of heterosexual acquisition of HIV in men. *Cochrane Database System Rev.* Issue 2 Art. No.: CD003362. DOI:10.1002/14651858.CD003362.pub2.

Singh S, Wulf D, Hussain R, Bankole A, & Sedgh G. (2009). *Abortion Worldwide: A Decade of Uneven Progress*, Guttmacher Institute, New York, NY, USA.

Singh S & Darroch JE. (2012). *Adding it up: costs and benefits of contraceptive services-estimates for 2012*. New York: Guttmacher Institute and United Nations Population Fund, 2012.

Sobel JD. (2000). Bacterial vaginosis. *Annu Rev Med.* (2000). **51**:349-56.

Sokal DC, Karim QA, Sibeko S, Yende-Zuma N, Mansoor LE, Baxter C, Grobler A, Frolich J, Kharsany AB, Miya N, Mlisana K, Maarshalk S & Karim SS. (2013). Safety of tenofovir gel, a vaginal microbicide, in South African women: results of the CAPRISA 004 Trial. *Antivir Ther.* **18**: (3):301-10.

Spear GT, St. John E & Zariffard MR. (2007). Bacterial vaginosis and human immunodeficiency virus infection. *AIDS Res Ther.* 4:25

Spencer SE, Valentin-Bon IE, Whaley K & Jerse AE. (2004). Inhibition of *Neisseria gonorrhoeae* genital tract infection by leading-candidate topical microbicides in a mouse model. *J Infect Dis.* **189**: 410-9.

Spurbeck RR & Arvidson CG. (2008). Inhibition of *Neisseria gonorrhoea* epithelial cell interactions by vaginal *Lactobacillus* species. *Infect Immun.* **76**: 3124-30.

Spurbeck RR & Arvidson CG. (2011). Lactobacilli at the front line of defence against vaginally acquired infections. *Future Microbiol.* **6**: 567-82.

Stafford MK, Ward W, Flanagan A, Rosenstein IJ Taylor-Robinson D, Smith JR, Weber J & Kitchen VS. (1998). Safety study of nonoxynol-9 as a vaginal microbicide: evidence of adverse effects. *J Acquir Immune Defic Syndr Hum Retrovirol.* **17**: 327-31.

Stapleton A, Moseleu D, Stamm & WE. (1991). Urovirulence determinant in *Escherichia coli* isolates causing first-episode and recurrent cystitis in women. *J Infect Dis.* **163**:773-9.

Stevens VC. (1997). Some reproductive studies in the baboon. *Human Reprod Update*. **3**: 533-40.

Stephen D, Fihn E, Boyko H, Esther HN, Chin-Li C, Grafton RJ, Marcia H, Uarbro P, Delia S & Andy S. (1996). Association between use of spermicide-coated condoms. *Am J Epidemiol*. **144**: (5):512-20.

Stuart JE, Secura GM, Zhao Q, Pittman ME & Peipert JF. (2013). Factors associated with 12-month discontinuation among contraceptive pill, patch, and ring users. *Obstet Gynecol*. **121**: 330-6.

Suckling J, Lethaby A & Kennedy R. (2006). Local oestrogen for vaginal atrophy in postmenopausal women. *Cochrane Database Syst Rev*. 4:CD001500.

Sundquist A, Bigdeli S, Jalili R, Druzin ML, Walker S, Pullen KM, El-Sayed YY, Taslimi MM, Batzoglou S & Ronaghi M. (2007). Bacterial flora typing with targeted chip-based pyrosequencing. *BMC Microbiol*. 7:128.

Szlendak-Sauer K, Radowicki S & Skórzewska K. (2009). The impact of a new low dose oral contraceptive containing drospirenone on lipid profile, carbohydrate metabolism and hepatic function. *Ginekol Pol*. **80**: (2):99-102.

Tevi-Benissan C, Belec L, Levy M, Schneider-Fauveau V, Si Mohamed A, Hallouin MC, Matta M & Gresenguet G. (1997). In vivo semen-associated pH neutralization of cervicovaginal secretions. *Clin Diagn Lab Immunol.* **4**: 367-74.

Thurman AR, Clark MR & Doncel GF. (2011). Multipurpose prevention technologies: Biomedical tools to prevent HIV-1, HSV-2, and unintended pregnancies. *Infect Dis Obstet Gynecol.* **2011**:1-10.

Tsai CA, Hung JK & Weiser DS. (2012). Is food insecurity associated with HIV risk? Cross-sectional evidence from sexually active women in Brazil. *PLoS Med.* **9**: (4) e1001203

Truvona N, Tsai L, Tung S, Schneider E, Harouse J, Gettie A, Simon V, Blanchard J & Cheng-Mayer C. (2006). Progestin-based contraceptive suppresses cellular immune responses in SHIV-infected rhesus macaques. *Virology.* **352**:169-77.

Tuyama AC, Cheshenko N, Carlucci MJ, Li JH, Goldberg CL, Waller DP, Anderson RA, Profy AT, Klotman ME, Keller MJ & Herold BC. (2006). ACIDFORM inactivates herpes simplex virus and prevents genital herpes in a mouse model: optimal candidate for microbicide combinations. *J Infect Dis.* **194**: 795–803.

Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggert CM, Knight R & Gordon JL. (2007). The Human Microbiome Project. *Nature.* **449**: 408-10.

Uckun FM, D'Cruz OJ. (1999). Prophylactic contraceptives for HIV/AIDS. *Human Reprod Update*, **5**: (5): 506-14.

UNAIDS. (2010). Joint united nations programme on HIV/AIDS. Report on global AIDS epidemic.

UNAIDS. (2010). UNAIDS Best Practice collection. Gender and HIV/AIDS: taking stock of research and programmes UNICEF. (2008). Poverty drives Kenyan girls into sex work [.http://www.unicef.org/infobycountry/kenya_46571.html](http://www.unicef.org/infobycountry/kenya_46571.html)

UNAIDS: (2010). Global report, UNAIDS report on the global AIDS epidemic UNAIDS/10.11E / JC1958E. Joint United Nations Programme on HIV/AIDS.

UNAIDS. (2010). Global Report: UNAIDS Report on the Global AIDS Epidemic 2010. 2010. http://data.unaids.org/pub/Report/2009/JC1700_Epi_Update_2009_en.pdf (accessed 8 Jul 2011).

UNAIDS. (2012) *Global Report. UNAIDS report on the global AIDS epidemic*. Geneva:

UNAIDS; 2012. Available from:

http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2012/gr2012/20121120_UNAIDS_Global_Report_2012_en.pdf. Accessed August 24, 2013.

United Nations. The millennium development goals report. (2012). New York: United Nations, 2012.

United Nations. The Millennium Development Goals Report. (2013).

(<http://www.un.org/millenniumgoals/pdf/report-2013/mdg-report-2013-english.pdf>)

United Nations Report (2013). United nations department of economic and social affairs/population division world population to 2300. www.unpopulation.org.

United Nations Report. (2013). “World Population Prospect.” <http://hdr.undp.org/en/2013-report>

UNFPA. 2013, available at <http://www.unfpa.org/rh/> (1 July 2013, date last accessed).

Ursell LK, Gunawardana M, Chang S, Mullen M, Moss JA, Herold BC, Keller MJ, McDonald D, González A, Knight R & Baum MM. (2013). Comparison of the vaginal microbial communities in women with recurrent genital HSV receiving acyclovir intravaginal rings. *Antiviral Res.* **19**: (102C):87-94.

Van Damme L, Ramjee G, Alary M, Vuylsteke B, Chandeying V, Rees H, Pachara Sirivongrangson P, Mukenge-Tshibaka L, Ettiègne-Traoré V, Uaheowitchai C, Abdool-Karim SS, Mâsse B, Perriens J & Laga M. (2002). Effectiveness of COL-1492, a nonoxynol-9 vaginal gel, on HIV-1 transmission in female sex workers: a randomised controlled trial. *Lancet.* **360**: 971-7.

Van Der Pol B, Kwok C, Pierre-Louis B, Rinaldi A, Salata RA, Chen PL, van de Wijgert J, Mmiro F, Mugerwa R, Chipato T & Morrison CS. (2008). Trichomonas vaginalis infection and human immunodeficiency virus acquisition in African women. *J Infect Dis.* **197**: (4):548-54.

van de Wijgert JH, Morrison CS, Cornelisse PGMunjoma M, Moncada J, Awio P, Wang J, Van der Pol B, Chipato T, Salata RA & Padian NS. (2008). Bacterial vaginosis and vaginal yeast, but not vaginal cleansing, increase HIV-1 acquisition in African women. *J Acquir Immune Defic Syndr.* **48**:203-10.

van de Wijgert JH, Chirenje ZM, Iliff V, Mbizvo MT, Mason PR, Gwanzura L, Shiboski S & Padian NS. (2000). Effect of intravaginal practices on the vaginal and cervical mucosa of Zimbabwean women. *J Acquir Immune Defic Syndr.* **24**: (1):62-7.

van De Wijgert J, Fullem A, Kelly C, Mehendale S, Ruggao S, Kumwenda N, Chirenje Z, Joshi S, Taha T, Padian N, Bollinger R & Nelson K. (2001). Phase 1 trial of the topical microbicide BufferGel: safety results from four international sites. *J Acquir Immune Defic Syndr.* **26**: 21-7.

van de Wijgert JH, Mason PR, Gwanzura L, Mbizvo MT Chirenje ZM, Iliff V, Shiboski S & Padian NS. (2000). Intravaginal practices, vaginal flora disturbances, and acquisition of sexually transmitted diseases in Zimbabwean women. *J Infect Dis.* **181**: 587-94.

van der Linde I. (2013). HIV/AIDS in South Africa: At last the glass is half full. In the proceedings of the 6th South African AIDS Conference; June 18–21; 2013; Durban, South Africa.

Voeller B, Coulson AH, Bernstein GS & Nakamura RM. (1989). Mineral oil lubricants cause rapid deterioration of latex condoms. *Contraception*. **39**: (1):95-102.

Van Der Pol B, Kwok C, Pierre-Louis B, Rinaldi A, Salata RA, Chen PL, van de Wijgert J, Mmiro F, Mugerwa R, Chipato T & Morrison CS. (2008). Trichomonas vaginalis infection and human immunodeficiency virus acquisition in African women. *J Infect Dis*. **197**: (4):548-54.

Veazey SR. (2008). Microbicide safety/Efficacy studies in animals – macaques and small animal models. *Curr Opin HIV AIDS*. **3**: (5):567-73.

Veazey RS, Shattock RJ, Klasse PJ & Moore JP. (2012). Animal models for microbicide studies. *Curr HIV Res*. **10**: (1):79-87.

Voeller B, Coulson AH, Bernstein GS & Nakamura RM. (1989). Mineral oil lubricants cause rapid deterioration of latex condoms. *Contraception*. **39**: (1):95-102.

Vogenthaler SN, Kushel BM, Craig H, Frongillo Jr AE, Riley DE, Bangsberg RD & Weiser DS. (2013). Food Insecurity and Risky Sexual Behaviors Among Homeless and Marginally Housed HIV-Infected Individuals in San Francisco. *AIDS Behav*. **17**: (5): 1688-93.

Voravuthikunchai SP, Bilaso S & Supamala O. (2006). Antagonistic activity against pathogenic bacteria by human vaginal lactobacilli. *Anaerobe*. **12**: 221-6.

Vrbikova J & Cibula D. (2005). Combined oral contraceptive in the treatment of polycystic ovary syndrome. *Hum Reprod Update*. **11**:2 77-91.

Warlde DA. (2000). Stability of ecosystem properties in response to above-ground functional group richness and composition. *Oikos*. **89**: 11-23.

Wasserheit JN. (1989). The Significance and scope of reproductive tract infections among third world women. *Suppl Int J Gynecol Obstet*. **3**: 145-68.

Wiesenfeld HC, Hillier SL, Krohn MA, Landers DV & Sweet RL. (2003). Bacterial vaginosis is a strong predictor of Neisseria gonorrhoea and Chlamydia trachomatis infection. *Clin Infect Dis*. **36**: 663–8.

Wildt DE. (1986). Spermatozoa. Collection, evaluation, metabolism, freezing, and artificial insemination. In: Comparative Primate Biology. Vol. **4**. Dukelow , Erwin (eds). New York: Alan R. Liss, 171–93.

Wilkinson D, Ramjee G, Tholandi M & Rutherford G. (2002a). Nonoxynol-9 for preventing vaginal acquisition of sexually transmitted infections by women from men. *Cochrane Database System Rev*. Issue 1. Art. No.: CD003939. DOI: 10.1002/14651858.Cd003939.

Wilkinson D, Tholandi M, Ramjee G & Rutherford G. (2006). Nonoxonyl-9 spermicide for prevention of vaginally acquired HIV and other sexually transmitted infections: systematic review and meta-analysis of randomised controlled trials including more than 5000 women. *The Lancet Infect.* **2**: (10):613-7.

Williams JD. (1990). Prospects for standardisation of methods and guidelines for disc susceptibility testing. *Eur J Clin Microbiol Infect Dis.* **9**: (7):496-501.

Williams DL, Newman DR, Ballagh SA, Creinin MD, Barnhart K, Weiner DH, Bell AJ & Jamieson DJ. (2007). Phase I safety trial of two vaginal microbicide gels (Acidform or BufferGel) used with a diaphragm compared to KY jelly used with a diaphragm. *Sex Transm Dis.* **34**: 977-84.

Wiysonge CS, Kongnyuy EJ, Shey M, Muula AS, Navti OB, Akl EA & Lo YR. (2011). Male circumcision for prevention of homosexual acquisition of HIV in men. *Cochrane Database System Rev.* Issue 6 Art. No.: CD007496. DOI: 10.1002/14651858.CD007496.pub2.

World Health Organization. (2001). Global Prevalence and Incidence of Selected Curable Sexually Transmitted Infections. 2001. http://whqlibdoc.who.int/hq/2001/WHO_HIV_AIDS_2001.02.pdf (accessed 8 Jul 2011).

World Health Organization U, UNFPA and the World Bank. (2007). Maternal mortality in 2005: Estimates developed by WHO, UNICEF, UNFPA and the World Bank. 2007.

World Health Organization. (2007). Figure 3-1 Comparing typical effectiveness of contraceptive *Methods*. Geneva: World Health Organization; 2007. Available from:

<http://www.contraceptivetechnology.org/table.html>. Accessed August 24, 2013.

World Health Organization. (2008). Global incidence and prevalence of selected curable sexually transmitted infections – 2008; available at http://apps.who.int/iris/bitstream/10665/75181/1/9789241503839_eng.pdf (1 July 2013, date last accessed).

World Health Organization Bulletin. (2010). Bulletin of the World Health Organization (2010).

88: 243-243.doi: 10.2471

World Health Organization. (2010). Sexually transmitted infections. Factsheet no. 110. Geneva:

Available from: <http://www.who.int/mediacentre/factsheets/fs110/en/index.html>.

/BLT.10.077446

World Health Organization. (2010). WHO Laboratory manual for the examination of human semen and sperm-cervical mucus interaction.

World Health Organization. (2011). World Health Organization. Global prevalence and incidence of selected curable sexually transmitted infections.

2001. http://whqlibdoc.who.int/hq/2001/WHO_HIV_AIDS_2001.02.pdf (accessed 8 Jul 2011).

World Health Organization. (2013). The importance of a renewed commitment to STI prevention and control in achieving global sexual and reproductive health. WHO/RHR/13.02

Wykrzykowska JJ, Pauley DR, Lackner AA & Simon MA.(1996). Evaluation of anti-human antibodies for immunocytochemistry on archival non- human primate tissues. *J Med Primatol.* **25**:71-7.

Zeitlin L, Hoen TE, Achilles SL, Hegarty TA, Jerse AE, Kreider JW, Olmsted SS, Whaley KJ, Cone RA & Moench TR.(2001). Tests of BufferGel for contraception and prevention of sexually transmitted diseases in animal models. *Sex Transm Dis.* **28**: 417-23.

Zheng HY, Alcorn TM & Cohen MS. (1994). Effects of H₂O₂-producing lactobacilli on *Neisseria gonorrhoeae* growth and catalase activity. *J Infect Dis.* **170**: 1209-15.

Zhou X, Bent SJ, Schneider MG, Davis CC, Islam MR & Forney LJ. (2004). Characterization of vaginal microbial communities in adult healthy women using cultivation-independent methods. *Microbiology.***150**: 2565-73.

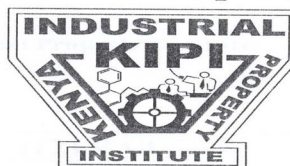
Zhou X, Brown CG, Abdo Z, Davis CC, Hansmann MA, Joyce P, Foster JA & Forney LJ. (2007). Difference in the composition of vaginal microbial communities found in healthy Caucasian and Black women. *ISME J.* **1**: 121-33.

Zhou X, Melanie A. Hansmann, Catherine C. Davis, Haruo Suzuki, Celeste J. Brown, Ursel Schutte Jacob D. Pierson & Larry JF. (2010). The vaginal bacterial communities of Japanese women resemble those of women in other racial groups. *FEMS Immunol Med Microbiol.* **58**: 169-81.

APPENDICES

Appendix 1. Patent Certificate

Kenya Industrial Property Institute



The Industrial Property Act, 2001

CERTIFICATE

OF GRANT OF A PATENT

It is hereby certified that a patent with patent number KE 218 has been granted to: DR MWETHERA PETER GICHUHI of POBOX 11706 TOM MBOY A STREET NAIROBI KENYA in respect of an invention disclosed in an application number *KIPI* 2005 000452 having a date of filing of 13/05/2005 and, being an invention titled DEVELOPMENT OF A VIGINAL SPERMICIDE AND MICROBICIDE TO PREVENT PREGNANCY AND TRANSMISSION OF HIV/AIDS

Dated at Nairobi this 15th day of June, 2006.

Prof. James O. Odek
Managing Director,
Kenya Industrial Property Institute.

Appendix 2. Proposal ethical approval form



INSTITUTE OF PRIMATE RESEARCH
WHO COLLABORATING CENTRE



INSTITUTIONAL REVIEW COMMITTEE (IRC)

FINAL PROPOSAL APPROVAL FORM

Our ref: **IRC/06/10**
Dear **Dr. Peter Mwethera**

It is my pleasure to inform you that your proposal entitled "*Development of a microbicial contraceptive (Unipron), for prevention of unwanted pregnancies and sexually transmitted infections using a baboon (Papio anubis) model*", in collaboration with *Prof. Walter Jaoko, University of Nairobi, Department of Medical Microbiology*, has been reviewed by the Institutional Scientific and Review Committee (IRC). The proposal was reviewed on the scientific merit and ethical considerations on the use of animals for research purposes. The committee is guided by the Institutional guidelines (e.g. S.O.Ps) as well as International regulations, including those of WHO, NIH, PVEN and Helsinki Convention on the humane treatment of animals for scientific purposes and GLP.

This proposal has been approved and you are bound by the IPR Intellectual Property Policy.

Signed  Chairman IRC: DR PETER G. MWETHERA

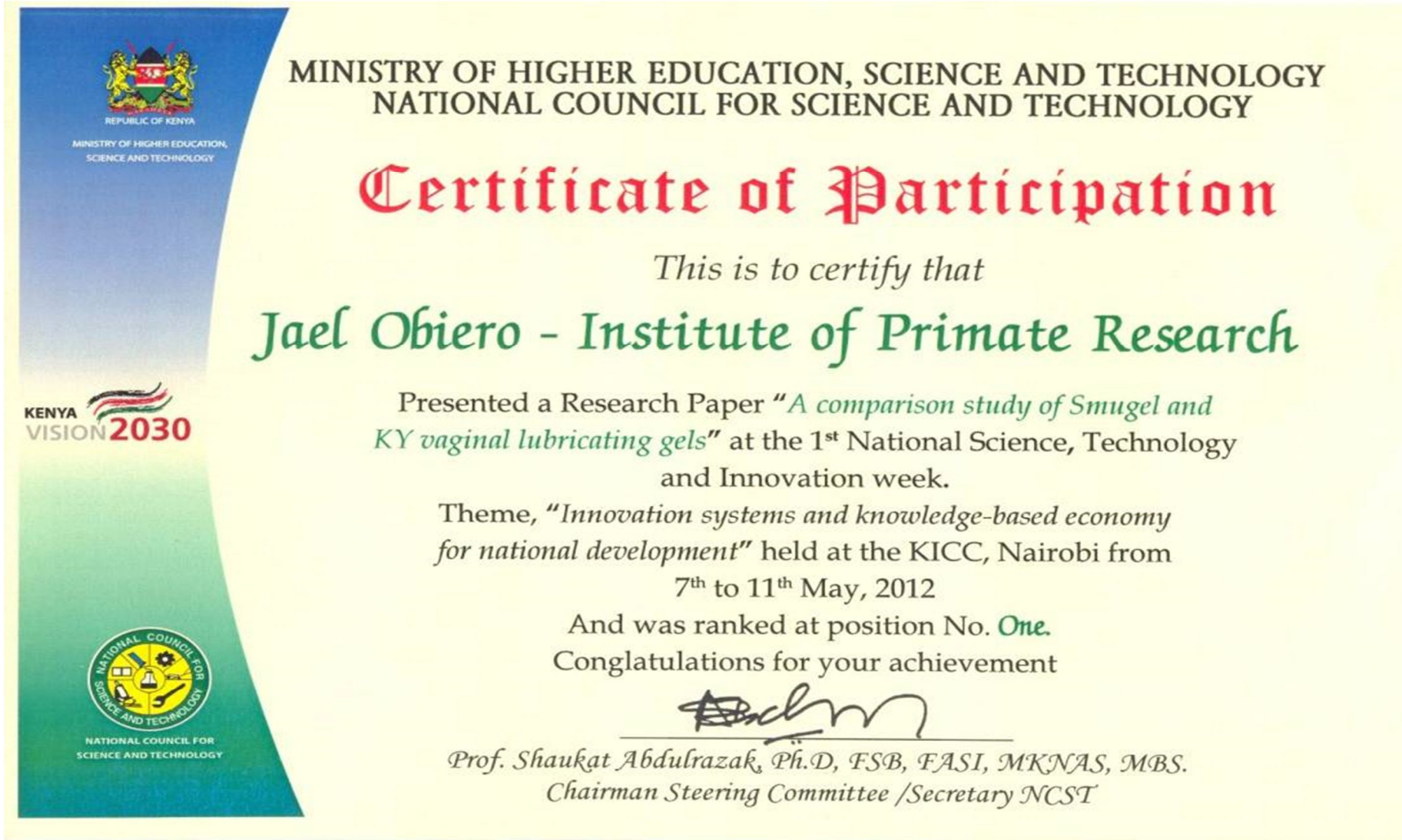
Signed  Secretary IRC: Dr. Hastings Ozwara

Date: 12th July 2010

INSTITUTE OF PRIMATE RESEARCH
INSTITUTIONAL REVIEW COMMITTEE
P. O. Box 24481-00502 KAREN
NAIROBI - KENYA
APPROVED...12th July 2010...

Appendix 3. Awards

i. National Council for Science and Technology



ii. The Kenya Public Service Award



iii. The African Union Award



Appendix 4. Publications

A. Presentations at scientific conferences and meetings

Obiero JA, Linge K, Jaoko W, Mwethera PG. (2013). Baboon vaginal flora. In the proceeding of The African Microbiome Conference, 27th - 29th November, International Livestock Research Institute (ILRI), Nairobi, Kenya.

Obiero JA, Kunyera R, Waititu K, Mulei I, Farah IO, Kavoo L, Jaoko W, Mwethera PG. (2013). Development of a Microbicidal Contraceptive Gel (UniPron) to Prevent Pregnancy and Acquisition of Sexually Transmitted Infections. In the proceeding of the 19th International Institute of Primate Research, Page 50, 11th-13th September, Southern Sun Mayfair Hotel, Nairobi, Kenya.

Obiero JA, Mulei I, Farah IO, Linge K, Jaoko W & Mwethera PG. (2013). Microbicidal and contraceptive studies of UniPron vaginal gel. In the proceedings of the 1st International Research and Innovation Conference of Mount Kenya University, Page 41, 28th-30th August, Safari Park Hotel, Nairobi, Kenya.

Obiero JA, Mulei I, Farah IO, Linge K, Jaoko W, Peter G. Mwethera. (2013). Safety and contraceptive studies of UniPron. In the proceedings of the University of Nairobi/Kenyatta National Hospital International Scientific Conference, Abstract No.22, Page 15-16, 19th-21st June 2013, Kenyatta National Hospital, Nairobi, Kenya.

Obiero JA, Kunyera R, Waititu K, Mulei I, Farah IO, Kavoo L, Jaoko W, Mwethera PG (2012). A comparative study of Smugel and KY Jelly vaginal lubricating gels. In the proceeding of the 1st Kenya Society for Immunology Symposium, Abstract No. ID06, Page 12, 24th-25th May, Kenya Medical Research Institute (KEMRI), Nairobi, Kenya.

Obiero JA, Mwethera PG, Wiysonge CS. (2013). Topical microbicides for prevention of sexually transmitted infection. In the proceedings of the African Cochrane Indaba Conference, Abstract No. P06, Page 33, 6th-8th may, Lagoon Beach Hotel, Cape Town, South Africa

Obiero JA, Kunyera R, Waititu K, Mulei I, Farah IO, Kavoo L, Jaoko W, Mwethera PG (2012). A comparative study of Smugel and KY Jelly vaginal lubricating gels. In the proceedings of the 1st National Science, Technology and Innovation (Organised by Ministry of Higher Education, Science and Technology & the National Council for Science and Technology), Abstract No. 23, Page 37, 7th-11th May, Kenyatta International Conference Centre (KICC), Nairobi, Kenya.

Obiero JA, Kunyera R, Waititu K, Mulei I, Farah IO, Kavoo L, Jaoko W, Mwethera PG.(2012). A comparative study of Smugel and KY Jelly vaginal lubricating gels. In the proceedings of the 36th Kenya Obstetrical and Gynaecological Society (KOGS) Annual Conference, Page 38, 15th-17th February 2012, Safari Park Hotel, Nairobi, Kenya.

Obiero J, Mwethera PG, Wiysonge CS. (2012). Vaginal microbicides for prevention of HIV and other sexually transmitted infections: systematic review and meta-analysis.: In the proceedings of the 36th KOGS Annual Conference, Page 27-28, 15th-17th February 2012, Safari Park Hotel, Nairobi, Kenya.

Mburu N, **Obiero JA**, Waititu K, Mwaura BN, Orawo JO, Farah IO and Mwethera PG. (2010). Safety Studies of a Recently Developed Microbicidal Contraceptive Gel (UniPron) in Female Baboons (*Papio anubis*). Poster presentation: In the proceedings of Microbicides Conference, Page 105, 22nd-25th May Pittsburg, Pennsylvania, USA.

Obiero J.A., Mburu, N., Mwaura, B., Waititu, K., Mulei, I., Farah, I., Mwethera, P.G. (2010). Development of a safe microbicidal contraceptive, unipron, to prevent pregnancies and sexually transmitted infections. In the proceedings of the 18th IPR International Biomedical Conference, Page 14, 6th-8th July Kenya Institute of Education (KIE), Nairobi, Kenya.

Mwethera P. G., **Obiero J.A.**, Mburu M. N., Mwaura B, Waititu K, Langoi D., Farah I. Development of human vaginal spermicide and microbicide (UniPron) to prevent pregnancy and HIV/AIDS. Oral in proceedings of KOGS 32nd Annual Conference, 16th-18th February, 2008, Intercontinental Hotel, Nairobi, Kenya

Obiero J.A., Mburu M. N., Mwaura B, Waititu K, Langoi D., Farah I Mwethera P. G. Development of human vaginal spermicide and microbicide (UniPron) to prevent pregnancy and HIV/AIDS (safety studies). in proceedings of KOGS 32nd Annual Conference, 16th-18th February, 2008, Intercontinental Hotel, Nairobi, Kenya

B. Abstracts of papers published in peer reviewed Journals a review protocol and a book title

- i. Gregor Reid, Nicholas Nduti, Wilbert Sybesma, Remco Kort, Tobias R Kollmann, Rod Adam, Hamadi Boga, Eric M Brown, Alexandra Einerhand, Hani El-Nezami, Gregory B Gloor, Irene I Kavere, Johanna Lindahl, Ameer Manges, Wondu Mamo, Rocio Martin, Amy McMillan, **Jael Obiero**, Pamela A Ochieng', Arnold Onyango, Stephen Rulisa, Eeva Salminen, Seppo Salminen, Antony Sije, Jonathan R Swann, William van Treuren, Daniel Waweru and Steve J Kemp. Harnessing microbiome and probiotic research in sub-Saharan Africa: recommendations from an African workshop. *Microbiome*. 2014, 2:12 (<http://www.microbiomejournal.com/content/2/1/12>).
- ii. **Jael A. Obiero**, Robert kunyera, Kenneth K. waititu, Isaac mulei, Idle O. Farah, Kavoo Linge, Walter Jaoko, Peter G. Mwethera. A Comparative Study of Smugel and KY Jelly Vaginal Lubricating Gels *Journal of Reproduction & Contraception* 2013 Jun.; 24(2):76-87.

- iii. **Jael Obiero**, Peter G Mwethera, Gregory D Hussey, and Charles S Wiysonge. Vaginal microbicides for reducing the risk of sexual acquisition of HIV infection in women:systematic review and meta-analysis.*BMC Infectious Diseases*. 2012, 12:289
- iv. **Obiero J**, Mwethera PG, Wiysonge CS. Topical microbicides for prevention of sexually transmittedinfections.*Cochrane Database of Systematic Reviews*.2012, Issue 6. Art. No.: CD007961. DOI: 10.1002/14651858.CD007961.pub2.
- v. Mburu N, **Obiero JA**, Waititu K, Mwaura BN, Orawo JO, Farah IO and Mwethera PG.Safety Studies of a Recently Developed Microbicial Contraceptive Gel (UniPron) in Female Baboons (*Papioanubis*).*African Journal of Reproductive Health*, 2009,13(4)95-104.
- vi. **Jael A. Obiero**, Maureen N. Mburu, Benson M. Ndung'u, Kenneth K. Waititu, Isaac Mulei, Idle O. Farah, Peter G. Mwethera. UniPron is A Fully Effective Non-hormonal Reversible Contraceptive in Baboon Model (*Papio anubis*).*Journal of Reproduction & Contraception* 2008, 19(2):107-118.
- vii. **Obiero J**, Wiysonge CS, Mwethera PG. Topical microbicides for prevention of sexually transmitted infections. *Cochrane Database of Systematic Reviews* 2009, Issue 3. Art. No.: CD007961. DOI: 10.1002/14651858.CD007961.
- viii. **Obiero J.** (2012); *Book title*: Menstrual cycle stage and baboon vaginal flora (*Papio* species): LAMBERT Academic Publishing (LAP): 978-3-8383-5934-2