Improving choice and use of contraception

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Declaration

This thesis has been composed entirely by myself and the work within it is my own unless acknowledged.

The work within this thesis was carried out under the umbrella of the Contraceptive Development Network (CDN). The CDN was an international research network comprising five centres (Edinburgh, Cape Town, Nigeria, Shanghai, and Hong Kong), set up in 1995, and directed by Professor David Baird. The network was jointly funded by a grant from the Medical Research Council (MRC) and the Department for International Development (DfID).

I worked as part of the multidisciplinary team, at the coordinating centre, based in the University of Edinburgh from August 2003 to April 2005. Being the last research fellow to work at the CDN I gained a lot from the experience and expertise of those around me who, the unit having been multiply tested prior to my arrival.

My contribution to each of the studies included in this thesis is as follows:

EC and unintended pregnancy study

Professor Glasier was the primary investigator and conceived the study. I wrote the protocol and applied for ethical approval. I designed the questionnaire and piloted it. I developed the Gantt chart and project managed the study (which had numerous sites within Lothian) with support from Ann Kerr. I conducted the analysis with statistical support from Dr Rob Elton. Professor Glasier, Dr Elton and myself worked together to agree on the interpretation of the analyses. I wrote the first draft of the paper and worked with Professor Glasier to produce the paper in its final format.

Implanon® study

Professor Glasier conceived the study. I wrote the protocol, designed the questionnaire and undertook the study. I retrieved the information from the notes where possible, sent the letters and telephoned Implanon® users as necessary. I created the dataset, did the data entry and conducted the analysis and interpretation. I wrote the first draft of the paper and once Professor Glasier had done the second draft we worked together to produce the paper in its final format.

Depo-provera® study

Professor Glasier conceived the study. I wrote the protocol, designed the questionnaire and conducted the first half of the study. I supervised Charlotte Henderson with respect to the protocol and questionnaire content for the second half of the study. I created the dataset for the whole study. I did the data entry for the first questionnaire. The second questionnaire did not require manual data entry. I undertook data analysis and interpretation, wrote the first draft and post Professor Glasier's revisions worked with Professor Glasier to produce the final paper.

Multi-centre mifepristone study

I joined the CDN in August 2004. At this time the protocol had been written, ethical approval obtained and recruitment was underway in Nigeria, Cape Town and Hong Kong. My primary role was to recruit women and project manage the Edinburgh arm of the study. I was responsible for recruitment, carrying out ultrasounds, biopsies, data collection and generally looking after the women volunteers for the duration of the study.

I attended and contributed to the monthly review meetings as well as the two multi-centre meetings where I collated data from all sites and delivered presentations on the current state of play for the study overall.

I carried out the more basic data analysis and Dr Rob Elton undertook the more complex aspects of statistical analysis. We reviewed the analyses during our monthly meetings and agreed interpretation as a team.
I wrote the first draft of the paper. Professor Baird edited that and wrote the second draft. I was responsible for all the tables and figures. We worked together to produce the final paper which was then reviewed by all the authors prior to submission.

Mifepristone and protection against STI's and HIV

I helped to complete this study. When I joined the CDN this study was already underway and five women had been recruited. I recruited the final three women, carried out the screens, the ultrasound examinations, endometrial biopsies and general care for these women with support from Sister Ann Kerr. Of the six vaginal biopsies I observed two, was supervised for one and carried out the remainder.
With respect to analysis I carried out vaginal thickness measurements for all participants. I optimised the immunohistochemistry protocols with Dr Teresa Henderson and I carried out the final protocols. I learned how to optimise the RNA extraction protocol with Dr Henderson but the final protocol was carried out by Dr Henderson.
We discussed the analyses during monthly CDN meetings and came to a joint decision regarding interpretation.
I produced all the figures bar figure 4 (see original paper).
I wrote a draft (introduction, methods and results) of which my methods and results sections were incorporated into the final paper.

This work has not been submitted for any other degree or professional qualification.

Fatim Lakha

October 2011

Abbreviations

AIDS	Acquired immune deficiency syndrome
ABC	Avidin biotin complex
AR	Androgen receptor
AUC	Area under the curve
BMI	Body mass index
BMD	Bone mineral density
cDNA	Complementary deoxyribonucleic acid
CI	Confidence interval
COC	Combined oral contraceptive pill
CGD	Cystic glandular dysplasia
Cr	Creatinine
DAB	3,3'-Diaminobenzadine substrate reagent
DEXA	Duel energy x-ray absorptiometry
DHS	Demographic and Health Surveys
DMPA	Depot medroxyprogesterone acetate/ Depo-Provera®
DMPA-IM	Intramuscular Depo-provera®
DMPA-SC	Subcutaneous depo-provera®
EC	Emergency contraception
E1G	Estrone glucuronide
E2	Oestrodiol
ER	Oestrogen receptor
ERa	Oestrogen receptor alpha
ERß	Oestrogen receptor beta
EVA	Ethinylvinyl acetate
FDA	Food and Drugs Administration
FN	Femoral neck
FSH	Follicle stimulating hormone

GDG	Guideline development group
GnRH	Gonadatrophin releasing hormone
GP	General practitioner
HBD	Human beta defensins
HIV	Human Immunodeficiency Virus
HMO	Health maintenance organisation
IHC	Immunohistochemical
IUD	Intra-uterine device
IUS	Intra-uterine system
LARC	Long-acting reversible contraception
LH	Luteinising hormone
LIF	Leukaemia inhibitory factor
LMUP	London measure of unplanned pregnancy
LNG	Levonorgestrel
LNG-EC	Levonorgestrel emergency contraception
LNG-IUS	Levenorgestrel intra-uterine system (Mirena)
LS	Lumber spine
MDG	Millenium Development Goal
MPA	Medroxyprogesterone acetate
mRNA	Messenger ribonucleic acid
MWRA	Married women of reproductive age
NCD	Non-communicable disease
NGO	Non Governmental Organisation
NHS	National health service
NICE	National Institute for Health and Clinical Excellence
NS	Not significant
OCP	Oral contraceptive pill
OR	Odds ratio
P	Progesterone
PCR	Polymerase chain reaction

PdG	Pregnanediol glucuronide
PGDH	Prostaglandin dehydrogenase
PH3	Phospho-histone H3
POP	Progestogen only pill
PR	Progesterone receptor
PRa	Progesterone receptor alpha
PRß	Progesterone receptor beta
QNA	Question not answered
RH	Reproductive health
RIA	Radioimmunoassay
RNA	Ribonucleic acid
RP	Reference period
RR	Relative risk
RU486	Mifepristone
SD	Standard deviation
SEM	Standard error of the mean
SIV	Simian immunodeficiency virus
SLPI	Secretory leukocyte peptidase inhibitor
SOS	Speed of sound
SPRM	Selective progesterone receptor modulator
STI	Sexually transmitted infection
TH	Total hip
UK	United Kingdom
UPSI	Unprotected sexual intercourse
US/USA	United States of America
WHO	World Health Organisation
WHO-MEC	World Health Organisation Medical Eligibility Criteria

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I would like to thank Professor Anna Glasier, Professor David Baird and Professor Hilary Critchley for awarding me the research fellowship at the contraceptive Development Network, for proposing the projects and for their guidance, comments and support.

I would especially like to thank Anna for her patience over the years and for not giving up on me.

I would also like to thank the administrative team at the Contraceptive Development Network and each of the staff at Dean Terrace Family Planning Clinic for their support and assistance with the projects.

I would like to thank Rob Elton for statistical support, Teresa Henderson for technical assistance and Neil Hollow for endocrine assays.

I would like to thank Evi for proofreading and support over the last few months.

Importantly I would like to thank all the women who participated in the various studies – without them there would be no studies to report.

Finally, and most importantly I would like to thank Rowan, Alyssa, Xavier and Skye for their patience, perspective and encouragement.

Abstract

Background

Almost all women are at risk of unintended pregnancy throughout their reproductive years [1]. In the UK alone, more than 200,000 pregnancies were terminated by induced abortion in 2010 [2,3]. Additionally, a substantial number of births result from unintended pregnancy [4].Family planning is achieved through use of contraceptive methods [5]. Contraceptive prevalence is increasing worldwide, however, some need for contraception remains unmet. Even in industrialised countries where contraception is readily available and use is high, many unintended pregnancies occur. The reason for this is that existing methods are not perfect, and their acceptability is limited by side effects and inconvenience leading to either non-use or incorrect and inconsistent use [6]. Preventing unintended pregnancy requires the number of successful contraceptive users to increase. This, at a minimum, requires the availability of safe, acceptable and effective methods of contraception; access to information, supplies and services; and the motivation and ability (including recognition of risk) to initiate and use contraceptives correctly and consistently [7]. Currently available methods need to be reviewed and where necessary adapted to address users' concerns and preferences in an effort to increase acceptability and hence uptake and adherence. And, most importantly, new methods need to be developed which do not cause the systemic side-effects linked to existing methods and offer additional non-contraceptive health benefits.

Methodology

Using a variety of methodologies we explored three areas. Firstly we sought to establish via a questionnaire survey how many pregnancies ending in either childbirth or abortion are unintended, and what proportion of women use emergency contraception (EC) to try to prevent pregnancy.

Secondly we explored the issue of acceptability of adapted methods of contraception (Implanon® and Depo-provera®) via questionnaire survey. And thirdly we further developed a novel contraceptive, mifepristone by exploring both its effectiveness and its

potential non-contraceptive health benefits (amenorrhoea and protection against STI's including HIV).

Results

Ninety percent of pregnancies which end in induced abortion were clearly unintended, however, of these women only 12% recognised their risk and used EC to try and prevent a pregnancy. Additionally one third of pregnancies which resulted in a birth were not clearly intended.

Both Implanon®, in practice, and subcutaneous depo-provera®, in theory, were found to be acceptable methods of contraception to women. Approximately half (47%) of those who used Implanon® continued to use it for the full duration (>2years 9/12) and one third of all users chose to have another implant when the first one expired. Regarding subcutaneous depo-provera® 67% of current users, 26% of never users and 40% of ex-users said they would seriously consider self-administration of depo-provera® if it were to be licensed.

In investigating mifepristone it was found that there were no pregnancies in 356 months of exposure to mifepristone and more women were amenorrhoic whilst taking mifepristone than POP (49% vs 0% $p<0.001$) and no mifepristone users discontinued for reasons related to bleeding profiles. Additionally no significant changes were found in vaginal thickness or content with use of mifepristone.

Discussion

Unintended pregnancy is common, even among women who choose to continue pregnancy. EC use is low indicating that women are often not aware of their risk. Thus EC is unlikely to reduce unintended pregnancy. Rather, we need to encourage improved use of regular contraception.

Long acting reversible contraceptives are particularly beneficial as they do not require daily intake and hence can be 'forgotten'. Our findings suggest that the long acting reversible methods of contraception (LARC) Implanon® is acceptable to women and its

continuation rates justify its widespread provision. Similarly, the advent of subcutaneous self-administrable Depo-provera® would likely be beneficial and popular with women. Alongside adapting existing methods of contraception there is a need to develop novel methods of contraception such as antigestogens. Our studies of mifepristone show that mifepristone is an effective oral contraceptive pill with a better pattern of menstrual bleeding than an existing POP (levenorgestrel). We also found that in contrast to other oestrogen-free contraceptives, mifepristone is unlikely to be associated with an increased risk of transmission of HIV and other sexually transmitted infections.

Chapter 1: Background

Family planning is one of the twelve pillars of reproductive health as defined by the World Health Organisation (WHO) (Appendix 1) [8]. It allows people to attain their desired number of children and determines the spacing of pregnancies. It is achieved through use of contraceptive methods and the treatment of infertility [5]

Definitions of Reproductive Health and Reproductive Health Care

"Reproductive health (RH) is defined as a state of physical, mental, and social well-being in all matters relating to the reproductive system and its functions and processes, and not merely the absence of disease or infirmity. Reproductive health, therefore, implies that people are able to have a satisfying and safe sex life and that they have the capability to reproduce and the freedom to decide if, when, and how often to do so. Implicit in this last condition is the right of men and women to be informed and to have access to safe, effective, affordable, and acceptable methods of family planning of their choice, as well as other methods of their choice for regulation of fertility that are not against the law. They also need to have the right of access to appropriate health-care services that will enable women to go safely through pregnancy and childbirth and provide couples with the best chance of having a healthy infant." [9]

Accordingly, "reproductive health care is defined as the constellation of methods, techniques, and services that contribute to reproductive health and well-being by preventing and solving reproductive health problems. It also includes sexual health, the purpose of which is the enhancement of life and personal relations, and not merely counselling and care related to reproduction and sexually transmitted infections" [9].

The above definitions of 'reproductive health' and 'reproductive health care' were the result of the 1994 United Nations International Conference on Population and Development. At this conference the majority of participating nations agreed that without the most basic rights for women within the family and society – most of all reproductive rights (including the right to decide, jointly or alone if necessary, on the number of children they were prepared to bear) – meaningful strides in public health, education, the protection of the environment and economic development would lag at best and be impossible at worst [10,11]. This resulted in a decisive shift of national population policies from the demographic, where the central justification for programs was the reduction of environmental, economic and societal pressures, to the quality of life imperative [11,12] and the foundations for the global reproductive health agenda were laid. Whilst there remained much debate as to where the boundaries lay, it seemed that no longer was RH solely about preventing and treating disease, but also about supporting normal functions such as pregnancy and childbirth. Above all, the definition of RH assumed responsibility within it to enhance life-processes and how to nurture them in the face of adversity [13].

Political endorsement

To improve any country's socio-economic welfare, its government has to look to meet and improve its health requirements. In 2000, 189 countries made a promise to free people from extreme poverty and multiple deprivation. This pledge became the eighth Millenium Development Goal (MDG) to be achieved by 2015 (Appendix 2). However, reproductive health was not specifically included as an independent goal or a measurable target. This was despite experts providing evidence for years that investing in reproductive health is integral to directly meeting those MDGs related to health [14,15,16,17,18,19,20,21,22]. It took until September 2005, after much intense advocacy by both non-governmental organisations (NGO's) and leaders in the field of reproductive health, for a specific commitment to be made by World leaders, to integrate

the target of 'achieving universal access to reproductive health by 2015' together with indicators for measuring its progress (Appendix 3), into strategies to attain internationally agreed development goals, including those contained in the Millennium declaration (MDGs) [23]. Examples of goals where it was felt increased attention to sexual and reproductive health would contribute significantly were: MDG1 - to reduce poverty and hunger; MDG 3 – to promote gender equality and empowerment of women and MDG 6 - combating HIV and other diseases [23].
Subsequently most countries now have population policies in place. However, there remains a real need for these to be legitimised and for encouragement from development agencies to implement these policies with conviction and commitment [24]

The importance of Reproductive Health and Health Care

The benefits of helping women and couples access and effectively use contraception include the prevention of negative outcomes such as infant death, low birth weight babies, lower breast feeding rates, and depression associated with unintended pregnancies [25,26,27,28] and on a broader scale can lead to improving women's education and employment opportunities and their participation in social and political domains [26,29]. Couples with the means to control their fertility are usually able to invest more resources in each child. This ultimately raises the standard of health, education and wealth in a population [30]. Thus reproductive health has been identified as both a fundamental human right and also a social and economic imperative [8].

The need for contraceptive research and development

Most countries in the world have some national family planning program and allow NGO's to provide such services. Whilst over time the specific objectives of such programmes have been debated, there is no doubt that they have been successful with a resultant decline in fertility rate throughout much of the world. Between 1950-1955 and 2005-2010, the median level of total fertility in the world (constituted by 196 countries

for whom data was available) fell by half, from 4.95 to 2.52 children per woman and the reduction of fertility was even more marked in some countries or regions [31] (figure 1). However, the world population continues to grow.

Figure 1: World Fertility Rate 1950-2050

Source: UN Population Division

It is known that in 1000AD, just over 1000 years ago, the world population did not exceed 300 million people. It took about 800 years to reach the first billion in 1804, and 123 years to reach the second in 1927 [31]. Succeeding increments of one billion have taken less and less time - 33, 14, 13 and 12 years and the world population is predicted to reach seven billion on the 31st of October, 2011. This is despite the population growth rate having declined from 1.82 in 1950-55 to 1.22 in 2000-2005, and continuing to decline, being 1.15 in 2011 [31,32]. One possible reason for this is that the decline in crude death rate (18.7 in 1950-55 to 8.4 in 2005-2010) and hence the increase in life expectancy (47.7 years for both sexes combined in 1950-55 to 67.9 in 2005-2010) are greater than the decline in the total fertility rate (4.95 in 1950-55 to 2.52 in 2005-2010) [32].

Fertility rates are dropping throughout the world (current [2011] world fertility rate is 2.45 [32]) and women are now spending the majority of their reproductive lives trying to avoid pregnancy [33]. The average reproductive lifespan of a woman is 35.9 years [33]. A woman in a first world country will spend 90% of her reproductive life postponing or avoiding further births. A woman in a developing country will spend slightly less time avoiding pregnancy [34]. This therefore leads to increased potential for unintended pregnancy and hence a greater need for effective fertility control.

The decline in both crude death rates and fertility rates can be attributed to advances in technology and thus, the lesser decline in fertility rates could be said to reflect the inadequate attention and resources afforded to the contraceptive research and development field, as well as possibly a shift in focus of effort from discovery to adaptation [35]. Most governments, including those of poorer developing countries, already have population and family planning policies but are receiving too little encouragement and funding to implement them [24,36].

'Unmet need for contraception' or 'at risk of unintended pregnancy'?

The concept of 'Unmet need' for contraception

The general concept of unmet need was first introduced in the 1960's, when researchers began to demonstrate and measure the discordance between women's desires to limit their births and their actual use of contraception in much of the developing world [37]. In 1988, Westhoff (1995) developed an algorithm for measuring unmet need using data from the Demographic and Health surveys (DHS) [38]. This definition took into account unmet need for contraception to space births and is now considered the standard measure of the level of unmet need for contraception [39,40,41].

Definition of unmet need for contraception

- Is in a marital or consensual union
- Is fecund (i.e. not pregnant, amenorrhoeic or otherwise infecund, according to her own report)
- Does not want to have a child in the next two years, and
- Is not using any contraceptive method, either modern or traditional

In addition, pregnant or amenorrhoeic women in union are considered to have an unmet need if they report that their current or most recent pregnancy was unplanned.

The concept of 'Unintended pregnancy'

Unintended pregnancy is a core concept in understanding, and accurate measurement of, the fertility of populations, fertility-related behaviours and the unmet need for contraception [42].

Definition of unintended pregnancy

An unintended pregnancy is one which occurs among couples who had wanted to delay having a child (mistimed) or who did not want to become pregnant at all (unwanted).

Separate research, from that of measuring unmet need, has been undertaken to estimate the numbers and characteristics of women at risk of unintended pregnancy and in need of contraceptive services and supplies[43,44]. This research has surmised that the definition of 'women at risk of unintended pregnancy' as used in the industrialised world and the definition of 'women with an unmet need for contraception' used in developing countries are essentially fairly similar. The main differentiating factor is related to the

level of contraceptive use. In countries where contraceptive prevalence is low (mostly developing countries) it is more common to measure 'unmet need' and in countries where contraceptive prevalence is high 'unintended pregnancy' is more likely to be used as this includes those pregnancies which occur whilst using a method of contraception. However, critics have pointed out two shortcomings of using the term 'unmet need' nowadays. The first is that data for 'unmet need' often exclude sexually active unmarried women who represent a large and growing segment of the population. The second criticism is that the term 'unmet need' excludes those who are using a method of contraception but are using it incorrectly or inconsistently [45]. Thus I would propose that the concept of being at risk of unintended pregnancy is a much broader one. It encompasses both married and unmarried sexually active women and includes those women who are using a method of contraception but are using it incorrectly or inconsistently. As such it is the preferred measure to use in research undertaken where contraceptive use is high such as the UK and subsequently the reason why I chose to measure unintended pregnancy rather than unmet need in my research [46].

The global prevalence of unintended pregnancy

Almost all women are at risk of unintended pregnancy throughout their reproductive years [1], hence, unintended pregnancy is common. The scale of sexual activity, reproduction and their potential risk at the population level is difficult to visualise.

Tsui et al (2010) surmise that with a majority of the 1.74 billion reproductive age females being sexually active and a probability of conception during unprotected coitus being 3 in 100 [47], each year as many as 720 million conceptions may occur [48]. The majority of conceptions (60-70%) will be spontaneously miscarried, leaving approximately 239 million identified pregnancies, of which 136.2 million will progress to livebirths, 33 million being unwanted [48]. They deduce that another 46 million will

be electively terminated [48]. If their modelling is correct then the conclusion is that 33% of all identified pregnancies are unintended globally.

Prevalence in the developing world

Levels of unintended pregnancy are declining in the developing world but are still very high. In developing countries, the rate of unintended pregnancy declined by 20% between 1995 and 2008 (from 71 to 57 per 1,000 women aged 15-44) [29]. The Guttmacher institute estimated that in 2008, in the developing world, the pregnancy rate was 137 per 1,000 women aged 15-44 and the unintended pregnancy rate was 57 per 1,000, or 82.3 million unintended pregnancies per year [29].

In 2008, annual rates of unintended pregnancy were highest in Africa, at 86/1,000 women aged 15-44. The rate varied from 56 in North Africa to 72 in Southern Africa to 118 in Eastern Africa. Unintended pregnancy were also high in Latin America and the Caribbean (72/1,000) and lower still in Asia (49/1,000).

Prevalence in the United States

The Guttmacher institute has also calculated state-level estimates of unintended pregnancy, in 2001 and 2006, for the United States [49,50]. Finer et al (2011) reported that nearly half (49%) of all pregnancies were unintended in 2006. This was slightly higher than in 2001 (48%). The unintended pregnancy rate rose from 50 per 1,000 women, aged 15-44, in 2001 to 52 per 1,000 women in 2006 [49,50]. The investigators also found that in 2006, in 29 states more than half of pregnancies were unintended and in nine, a consistent upward trend in unintended pregnancy rates, between 2001 and 2006, was apparent. No state had a consistent decline [49].

Prevalence in the United Kingdom

A questionnaire survey of 2000 mothers, randomly selected from birth registrations in 1989 and interviewed six months post childbirth indicated that almost one third of pregnancies which progressed to birth were "unplanned" [4]. Additionally, almost 200,000 pregnancies are terminated every year. However, there have been no estimates of pregnancy intendedness in women in the United Kingdom (UK) since 1989 [4], and consequently one of the aims of this thesis was to quantify pregnancy intention among women who presented over the course of eight months to a large teaching hospital in Edinburgh, UK, for antenatal care or for termination of pregnancy in 2005. The results of this can be found in chapter two.

Consequences of unintended pregnancies

Unintended pregnancies result in either unplanned child-bearing or induced abortion (or miscarriage). Both can have far reaching social, psychological and economic consequences. A reduction in unintended pregnancies reduces the number of events exposed to poor pregnancy outcomes and can make planned events healthier [48]. This is due to there being opportunity for preconceptual care (e.g. folic acid supplementation, smoking cessation advice) and increased likelihood of attending for antenatal care early on [51]. Additionally pregnancies which are planned generally tend to have longer birth intervals and evidence shows a statistical link between longer birth intervals (greater than 30 months) and decreased risk of neonatal, infant and child mortality and of child malnutrition [52]

Unintended childbearing can lead to child health and development issues, relationship instability, and compromises in education and employment that may exacerbate ongoing poverty [26,27,28,48,53]. A study by Schwarz et al (2008) attempted to measure the effects of unintended pregnancy on women's quality of life in the United States (US) in 2008. They surveyed 192 sexually active non-pregnant women and found that 94% of

women felt that an unintended pregnancy would adversely impact on their quality of life [54].

Worldwide, 22% of pregnancies, or about 42 million, are electively aborted, of which 20 million induced abortions happen under unsafe conditions, mostly in the developing world [29]. One quarter of the world's population lives in countries where abortion is prohibited by law (unless to save the mother's life). However, such legislation does not reduce total abortion rates [55], it merely causes women to seek and undergo unsafe abortions. Approximately 67,000 women die every year from unsafe abortions, and thousands more have physical and emotional sequelae [56].

Reducing unintended pregnancy and elective abortions

To attempt to reduce the number of unintended pregnancies we firstly need to understand why they happen, especially, when there are a large number of highly effective contraceptive methods known to us.

As discussed, family planning programmes throughout the world have had undeniable success in utilising a variety of methods of contraception, both hormonal and non-hormonal to reduce fertility rates. Evidence shows that contraception plays a key role in reducing induced abortions [57,58] and can avert as many as 32% of all maternal deaths and nearly 10% of childhood deaths [24]. Women who use any contraceptive method have a lower risk of unintended pregnancy than do sexually active women who use no method [7]. For example, in the United States, women who do not use contraceptives are 6.7 times more likely to have an abortion than women who do [57,59]. In addition, it has been surmised that the number of abortions in Armenia, Kazakhstan, Kyrgizstan, and Uzbekistan could be halved if women who do not use contraceptives or who use traditional methods were to use modern methods [60].

However, using a method of contraception is not sufficient in itself. Three US – state based studies [61,62,63] demonstrated that some contraceptive use or knowledge is advantageous in comparison to non-use in reducing the incidence of unintended pregnancy, but the measured protection was not as high as one might have expected. For example, in one study the adjusted (for age and race/ethnicity) odds ratio, of unintended pregnancy, for women having unprotected sex, compared with those using contraception, was 1.67 (95% CI 1.11- 2.52) [63]. The magnitude of the adjusted odds ratio, whilst statistically significant, is not substantial considering the high efficacy of modern methods of contraception. The reason for this is that the term 'contraceptive prevalence' does not presume correct and consistent use. For example, reported prevalence of condoms does not take into account the often sporadic nature of condom use [48].

Thus, whilst contraceptive methods have the potential to reduce the numbers of unintended pregnancy and elective abortions, to achieve a substantial reduction they must be used both correctly and consistently.

Understanding non-use and poor use of contraceptive methods

Developing World

Of the 82.3 million unintended pregnancies estimated to occur annually in the developing world [29], 82% are due to an unmet need for modern contraception i.e. no method of contraception is being used. The remaining 18% of unintended pregnancies are due to either inconsistent or incorrect use of contraception or to true contraceptive method failure [7,29].

Every year, one in four sexually active women, in the developing world, who want to avoid becoming pregnant has an unmet need for modern contraception. The reasons for this unmet need include lack of access, lack of knowledge, partner opposition, being

postpartum or breastfeeding and method related reasons (fear of side-effects or health risks) to use of modern contraceptive methods [7,39,64]. In Bangladesh, women with good access to high quality family planning services have an abortion rate of 2.3 per 1000 compared with 6.8 for women with poor access [65]. It is estimated that 70% of unmet need could be rectified if appropriate contraceptive methods were made available and that unintended pregnancy could be reduced by as much as 59% if method-related reasons for non-use of modern contraceptives could be overcome [7].

The Industrialised world

In industrialised countries, as opposed to developing countries, prevalence of use of contraception is high. Despite this widespread use of contraception unintended pregnancy is also high and there are 26 million abortions per year [66].

In the USA, modern contraceptives are used by 72% of married women [67], however, 49% of pregnancies are unintended and 54% of women who have an elective abortion were using contraception when they became pregnant [68]. In France, although 97% of women wishing to avoid pregnancy use modern contraceptives, 33% of pregnancies are unintended and of these 50% end in elective abortion [66]. A Swiss study whose aim was to attempt to understand why contraceptive measures are abandoned or not used at all demonstrated that of 103 women attending for elective abortion 98% (101 of the 103 women) had used a modern method of contraception at some point in the past [69]. A Scottish study by Schunmann et al (2006) found that 84% of women requesting an abortion were using contraception about the time they conceived, and only 16% reported non-use [70]. Repeat abortions are also a significant problem [71] and in Scotland between 20 and 25% of women undergoing abortion will have another abortion at some time during their reproductive lives [72]. A UK questionnaire study of 133 women attending for elective abortion revealed that 98% of those having a repeat abortion and

83% of those undergoing a first time abortion were using a recommended method of contraception [73].

Efficacy of modern contraceptive methods is high (table 2: figures for perfect use [74]). Data suggest that true method failure accounts for only 5% of unintended pregnancies occurring when contraception is correctly and consistently being used [74,75,76]. The remaining 95% arise because of non-use or less effective use of modern methods of contraception. This very high failure rate of non-permanent modern methods primarily reflects the difficulty of using them consistently and correctly [77].

Schunmann et al (2006), in their study of 316 women undergoing abortion in Edinburgh, in 2001/2 found that whilst 84% of women were using contraception at the time when pregnancy occurred, 44% admitted , when asked directly, to using their chosen method inconsistently or incorrectly and only 39% claimed perfect use [70].

There are plenty of factors which contribute to why contraception was used inconsistently or incorrectly or was not used at all. These include: lack of awareness, knowledge, access, counselling, support and motivation. These factors have been cited above for the developing world but apply equally, though in different ways, to the industrialised world. For example the studies detailed above demonstrate that lack of awareness and knowledge are not just about knowing of the existence of services and methods but also knowing how to use contraceptives appropriately, knowing one's individual suitability to various methods and being aware of one's need for contraception.

Thus the larger percentage of the problem in industrialised countries lies with compliance and motivation followed by lack of appropriate counselling and support [65,66,68,78,79]. Compliance with a method is heavily reliant on how easy a method is to use and how easy a method is to use is very dependent on the woman's life-style, socioeconomic status, age, and other factors [80]. Consequently compliance is not

always easy and hence the relatively high user-failure rates associated with existing contraceptive methods (apart from a few exceptions, such as sterilisation, implants and intrauterine devices (Appendix 4: figures for typical use [74,81]). One American study found that virtually all pill users (98%) reported having a reminder or routine to help them take their pill every day and yet, 38% reported having missed at least one active pill in the prior three months: eight percent had missed one, 11% two and 19% three or more. Some 71% of those who had missed a pill had simply forgotten to take it [77]. On bivariate analysis inconsistent use was more common amongst those who had had two or more partners in the past year than of those who had only one (58% versus 35%); amongst women who had no children as opposed to those who had two children or more (43% versus 28%); and amongst those who were not completely satisfied with their chosen method as opposed to those who were (48% versus 35%) [77].

Another American study, by Hou et al (2010), used a randomised controlled trial design to estimate whether women receiving daily text-message reminders have increased adherence compared with women not receiving reminders [82]. Pill-taking was tracked for three months by an electronic monitoring device with wireless data collection. The investigators found that the mean number of missed pills per cycle did not differ significantly between the text-message reminder group and control group (4.9±3.0 vs 4.6±3.5 respectively), and that the number of missed pills per cycle increased over the course of the study (P=0.02), but that this pattern did not increase differentially between the groups (P=0.58). The authors concluded that whilst the lack of benefit of the text-message reminders might be due to the frequent use of alternative reminder systems in the control group, the rate of missed pills when measured objectively was still very high in both groups [82].

Similarly of those who report using condoms as their primary method of contraception 61% of users, overall, had not used the method every time they had sex or had put it on after beginning sex at least once in the prior three months [77]. The most common reason for not using a condom consistently was not having one available or not

expecting to have sex (25%). In addition inconsistent use was more common amongst those who were ambivalent about avoiding a pregnancy as opposed to those who thought it was very important (78% versus 58%) [77], and among those who were not completely satisfied with the method than among those who were (66% versus 55%).

Contraceptive choices are very much determined by presumed advantages and disadvantages of the various methods available to each individual user. If women and men are dissatisfied with their method of contraception, their chance of adhering to the behaviours required to use it successfully are reduced [77,83,84].

Whilst reliability and safety are important to women, so are side-effect profiles, be they real or perceived/assumed. The fear of or actual experience of troublesome side-effects (for example: bleeding problems, acne, weight gain) or risks (for example: breast cancer or venous thrombo-embolism with the combined oral contraceptive pill) can lead to a fear to start contraceptive use or early discontinuation. Other reasons include partner influences, cultural values and norms; and problems in the contraceptive care system [85,86].

With discontinuation there is the risk that disillusionment with one method may lead to either use of no method or use of a less effective method. A study by Rosenberg et al (1998) found that six months after a new contraceptive prescription, of those who discontinued (32% of new starts and 16% of switchers) more than four fifths of those who remained at risk of pregnancy either failed to adopt another method or adopted a less effective method [86]. Additionally discontinuation of a method can lead to gaps in use of contraception and this heightens many women's risk of unintended pregnancy. A survey by the Guttmacher Institute found that almost four in ten women for whom avoiding pregnancy was of little or no importance had had at least one month long gap in use of contraception or failed to use any method for a year, compared with fewer than two in ten of those who deemed it very important [85].

The data presented suggests that, both in the developing and industrialised world, increasing uptake of modern methods of contraception requires improving the accessibility and quality of contraception information and services [24,87,88]. However, non-use and ineffective use does not only reflect difficulties in access but also indicates dissatisfaction with available methods. A variety of new methods and administration schedules are needed that address user's concerns and preferences, fit different stages of women's reproductive lives and are compatible with their particular life contexts [7].

Reducing unintended pregnancy

Preventing unintended pregnancy, meeting the standards set in Cairo in 1994 [13,89,90] and achieving the MDGs, requires for the number of successful contraceptive users (implying correct and consistent use) to increase. This, at a minimum, requires the availability of safe, acceptable and effective methods of contraception; access to information, supplies and services; and the motivation and ability to use contraceptives correctly and consistently [7].

For it to be used contraception has to be viewed as an attractive and plausible option. For this methods need to offer a number of things: efficacy, ease of use, safety, convenience, minimal side-effects and easy reversibility. As mentioned earlier, contraceptive choices are very much determined by presumed advantages and disadvantages of the various methods available to each individual user. Non-contraceptive health benefits (e.g amenorrhoea, protection from sexually transmitted infections) can influence continuation rates of contraception.

One study in the USA demonstrated that women who experienced troublesome dysmenorrhoea prior to using the combined oral contraceptive pill (COC) were eight times more likely to continue using the pill than women who did not report dysmenorrhoea [91].

Recent studies have suggested that many women prefer to bleed less often than once a month or not to bleed at all (amenorrhoea) [92,93,94,95,96]. The impact of monthly menstruation may range from a minor inconvenience for some women to a major health concern for those who suffer from menstrual disorders and health conditions that are aggravated during their menstrual cycle [97]. Gynecologists have advocated use of COC in an extended and continuous manner since the 1960's and 70's to treat menstrual disorders, such as menorrhagia and dysmenorrhoea, and gynaecologic disorders such as endometriosis [98]. Being able to offer the choice of amenorrhoea would be advantageous for many women, improving quality of life for those who suffer from menstrual-related disorders and provide greater convenience for women with busy and active lifestyle [97] and thus would likely increase uptake and continuation rates.

Strategy for improving contraceptive choice and use

There is a need for a three tiered strategy, as proposed by Darroch et al (2011), to improve uptake and adherence of contraceptive methods [58].

Women and couples need to receive accurate information about their risk of unintended pregnancy, have access to quality services that offer a range of contraceptive methods, and receive counselling and care that helps them initiate and sustain correct and consistent method use.

Currently available methods need to be reviewed and where necessary adapted to address users' concerns and preferences, fit different stages of women's reproductive lives and be compatible with their particular life contexts in an effort to increase acceptability and hence uptake and adherence.

New methods of contraception need to be discovered and developed which do not cause the systemic side-effects linked to existing methods and offer additional non-contraceptive health benefits.

In the context of this background this **thesis aims** to

- Quantify the incidence of unintended pregnancy in Lothian, Scotland and the outcome of such pregnancies.
- Explore uptake of emergency contraception uptake in women with unintended pregnancy and thus assess awareness of risk of unintended pregnancy.
- Explore continuation rates of a long-acting gestogen implant (Implanon®).
- Assess whether adapting the injectable contraceptive, Depo-Provera® to a form where self-administration is feasible would increase acceptability and improve uptake.
- Compare a novel oestrogen free contraceptive daily pill (mifepristone) with an existing gestogen only daily pill (levonorgestrel) with the aim to further develop mifepristone by assessing efficacy, side-effects and the potential non-contraceptive benefit amenorrhoea.
- Explore a novel oestrogen free contraceptive daily pill's (mifepristone) safety and its potential to protect against sexually transmitted diseases and HIV by studying its effects on ovarian function, endometrium, and vagina.

Appendix 1: Twelve pillars of reproductive health [8]

The status of women

Family planning

Maternal care and safe motherhood

Abortion

Reproductive tract infections and HIV/AIDS

Infertility

Nutrition

Infant and child health

Adolescent reproductive health and sexuality

Sexual behaviour and harmful sexual practices

Environmental and occupational reproductive health

Appendix 2: The Millennium Development Goals

Eradicate extreme poverty and hunger

Achieve universal primary education

Promote gender equality and empower women

Reduce child mortality

Improve maternal health

Combat HIV/AIDS, malaria and other diseases

Ensure environmental sustainability

Develop a global partnership for development

Appendix 3: Millenium Development Goal 5 – Improve maternal health

Target 5A. Reduce by three quarters, between 1990 and 2015, the maternal mortality ratio

Indicators for measuring progress:

5.1 Maternal mortality ratio (UNICEF-WHO)

5.2 Proportion of births attended by skilled health personnel (UNICEF-WHO)

Target 5B. Achieve, by 2015, universal access to reproductive health

Indicators for measuring progress:

5.3 Contraceptive prevalence rate

5.4 Adolescent birth rate

5.5 Antenatal care coverage (at least one visit and at least four visits)

5.6 Unmet need for family planning

Appendix 4: Summary table of contraceptive effectiveness (typical use failure rates) and efficacy (perfect use) plus continuation rates at one year

Percentage of women experiencing an unintended pregnancy during the first year of typical use and the first year of perfect use of contraception and the percentage continuing use at the end of the first year. United States. [74]

-	% of Women Experiencing an Unintended Pregnancy within the First Year of Use		% of Women Continuing Use at One Year [c]
Method (1)	Typical Use [a] (2)	Perfect Use [b] (3)	--- (4)
No method [d]	85	85	-
Spermicides [e]	29	18	42
Withdrawal	27	4	43
Fertility awareness-based methods	25	-	51
--------Standard Days method [f]	-	5	-
--------TwoDay method [f]	-	4	-
--------Ovulation method [f]	-	3	-
Sponge	-	-	-
--------Parous women	32	20	46
--------Nulliparous women	16	9	57
Diaphragm [g]	16	6	57
Condom [h]	-	-	-
--------Female (Reality)	21	5	49
--------Male	15	2	53
Combined pill and progestin-only pill	8	0.3	68
Evra Patch	8	0.3	68
NuvaRing	8	0.3	68

Depo-Provera	3	0.3	56
IUD	-	-	-
--------ParaGard (copper T)	0.8	0.6	78
--------Mirena (LNG-IUS)	0.2	0.2	80
Implanon	0.05	0.05	84
Female Sterilization	0.5	0.5	100
Male Sterilization	0.15	0.10	100

Emergency Contraceptive Pills: Treatment initiated within 72 hours after unprotected intercourse reduces the risk of pregnancy by at least 75% [i].

Lactational Amenorrhea Method: LAM is a highly effective, temporary method of contraception [j].

Explanation of table:

a. Among typical couples who initiate use of a method (not necessarily for the first time), the percentage who experience an accidental pregnancy during the first year if they do not stop use for any other reason. Estimates of the probability of pregnancy during the first year of typical use for spermicides, withdrawal, periodic abstinence, the diaphragm, the male condom, the pill, and Depo-Provera are taken from the 1995 National Survey of Family Growth corrected for underreporting of abortion; see the text for the derivation of estimates for the other methods.

b. Among couples who initiate use of a method (not necessarily for the first time) and who use it perfectly (both consistently and correctly), the percentage who experience an accidental pregnancy during the first year if they do not stop use for any other reason. See the text for the derivation of the estimate for each method.

c. Among couples attempting to avoid pregnancy, the percentage who continue to use a method for 1 year.

d. The percentages becoming pregnant in columns (2) and (3) are based on data from populations where contraception is not used and from women who cease using contraception in order to become pregnant. Among such populations, about 89% become pregnant within 1 year. This estimate was lowered slightly (to 85%) to represent the percentage who would become pregnant within 1 year among women now relying on reversible methods of contraception if they abandoned contraception altogether.

e. Foams, creams, gels, vaginal suppositories, and vaginal film.

f. The Ovulation and TwoDay methods are based on evaluation of cervical mucus. The Standard Days method avoids intercourse on cycle days 8 through 19.

g. With spermicidal cream or jelly.

h. Without spermicides.

i. The treatment schedule is one dose within 120 hours after unprotected intercourse, and a second dose 12 hours after the first dose. Both doses of Plan B can be taken at the same time. Plan B (1 dose is 1 white pill) is the only dedicated product specifically marketed for emergency contraception. The Food and Drug Administration has in addition declared the following 22 brands of oral contraceptives to be safe and effective for emergency contraception: Ogestrel or Ovral (1 dose is 2 white pills), Levlen or Nordette (1 dose is 4 light-orange pills), Cryselle, Levora, Low-Ogestrel, Lo/Ovral or Quasence (1 dose is 4 white pills), Tri-Levlen or Triphasil (1 dose is 4 yellow pills), Jolessa, Portia, Seasonale or Trivora (1 dose is 4 pink pills), Seasonique (1 dose is 4 light-blue-green pills), Empresse (1 dose is 4 orange pills), Alesse, Lessina or Levlite (1 dose is 5 pink pills), Aviane (1 dose is 5 orange pills), and Lutera (1 dose is 5 white pills).

j However, to maintain effective protection against pregnancy, another method of contraception must be used as soon as menstruation resumes, the frequency or duration of breastfeeds is reduced, bottle feeds are introduced, or the baby reaches 6 months of age.

Chapter 2: Quantifying incidence of unintended pregnancy and its outcome, uptake of emergency contraception and assessing awareness of risk of unintended pregnancy among a large cohort of women attending for antenatal care or abortion in Scotland

Background

Unintended pregnancy is common. In the UK, almost 200,000 pregnancies are terminated every year [3,99]. In a questionnaire survey of 2000 mothers who were randomly selected from birth registrations in 1989 and interviewed 6 months after childbirth, almost a third of women said their pregnancies (31·3%) were "unplanned" [4].

Quantifying the level of unintended pregnancy

There have been numerous efforts to measure intendedness of a pregnancy [1,4,49,100]. They varied from studies in which the concept of unintendedness is considered self-evident to studies where sophisticated measurement strategies with multi-dimensional probing questions have been used [101,102]. Large surveys have generally adopted the latter method and the one most widely adopted, for many years, has been the US National Survey of Family Growth which was originally devised in 1973 [102]. However, over time, though items have been added to ensure validity, many of its questions have become outdated and a need was identified for a measure of pregnancy intendedness which accurately reflected the needs and priorities of women in the 21st century [103,104]. This is in keeping with changes in both cultural and social norms such as deferring childbearing to pursue opportunities in education or employment or

having a child outwith marriage. In response to this identified need, and the fact that no new estimates for unintended pregnancy have been produced in the UK since the study by Fleissig et al (1991) detailed above in 1989 [4], Barrett et al (2004) developed and psychometrically evaluated the 'London Measure of unplanned pregnancy' (LMUP) [105]. This is a six item measure of unplanned/unintended pregnancy and psychometric testing (table 1). It demonstrated high internal consistency, high stability and excellent validity. The measure has a number of advantages: it makes no assumptions about the nature of women's relationships; it does not rely on women having fully formed childbearing plans; it does not assume a particular form of family structure; and it is suitable to use regardless of pregnancy outcome. Additionally it does not assume that women have clearly defined intentions and/or behaviours consistent with intentions. Results are on a scale of 0-12 and hence provide more information than previous dichotomous measures [105]. As the authors explain the LMUP's greatest advantage is that it provides a more complex and realistic portrayal of human fertility behaviour than existing measures have to date [105].

Table 1: London Measure of Unplanned Pregnancy (LMUP)

Question	Answer	Score
Q1. At the time of conception	Always used contraception	0
	Inconsistent use	1
	Not using contraception	2
Q2. In terms of becoming a mother	Wrong time	0
	OK but not quite right	1
	Right time	2
Q3. Just before conception	Did not intend to become pregnant	0
	Changing intentions	1
	Intended to get pregnant	2
Q4. Just before conception	Did not want a baby	0
	Mixed feelings about having a baby	1
	Wanted a baby	2
Q5. Before conception	Had never discussed children	0
	Discussed but no firm agreement	1
	Agreed pregnancy with partner	2
Q6. Before conception	No actions	0
	Health preparations (1 action*)	1
	Health preparations (≥2 actions*)	2

Health preparations included: taking folic acid supplements; stopping or reduction of smoking; stopping or reduction of drinking alcohol; healthy eating; seeking medical advice before conception.

Table 1: **Pregnancy intendedness instrument**[4]

Understanding unintended pregnancy

Up to a quarter of pregnancies that end in induced abortion in the UK arise from unprotected sexual intercourse; most of the rest are the result of inconsistent or incorrect use of contraceptives (eg, missed pills) or accidental damage of barrier contraceptives (eg, a burst condom) [70,106].

Once an episode of unprotected sexual intercourse has occurred be it due to non-use, incorrect use or inconsistent use the methods of contraception which can be used are grouped as emergency contraception.

Emergency contraception

The World Health Organisation (WHO) defines 'emergency contraception' (EC) as 'those backup methods for contraceptive emergencies which women can use within the first few days after unprotected intercourse, or in the event of potential contraceptive failure to prevent an unwanted pregnancy' [107].

Methods of EC explored have, to date, included stilbestrol, ethinyl estradiol combined with levonorgestrel LNG (the Yuzpe regimen), danazol, LNG alone, mifepristone, insertion of a copper intrauterine device (IUD) and most recently ulipristal acetate [108,109,110,111,112,113]. Of these methods, levonorgestrel (LNG) EC is the most widely used EC method worldwide today [114].

Levonorgestrel emergency contraception

Pharmacology and metabolism

LNG-EC comprises a 1.5mg tablet. It is rapidly and completely absorbed after oral administration. Bioavailability is approximately 100%. The maximum concentration achieved is 19.1ng/ml and the time to reach this is 1.7 hours [115]. Its half-life is 28hours and it is extensively metabolised to a large number of inactive metabolites [115].

Mode of action

There is much political and ethical controversy surrounding EC regarding its mechanisms of action. It has been demonstrated that LNG-EC acts through an effect on

follicular development to delay or inhibit ovulation [116,117] but appears to have no effect once luteinising hormone has started to increase as the pre-ovulatory surge [118,119]. Thereafter, LNG-EC cannot prevent ovulation and it does not prevent fertilisation or affect the human fallopian tube [114,120]. LNG-EC has no active effect on human sperm function [121,122,123,124] however, in being a progestin, it does alter the cervical mucus, so that it mimics the non-fertile thick, tacky, non-distensible mucus found during the second half of the menstrual cycle, and makes it impenetrable to sperm. LNG-EC has no effect on endometrial development or function [125]. In in-vitro models [126,127], and work in monkeys [128], it has been demonstrated that LNG does not interfere with blastocyst function or implantation.

Safety

LNG-EC is safe for use by all women, including adolescents [129]. Levonorgestrel, the active ingredient in LNG-EC, has been widely used in various formulations for over 30 years and has been extensively studied in women of reproductive age [129]. LNG-EC poses no risk of overdose and no major drug interactions or contraindications exist for LNG-EC [130]. While the WHO recommends a single dose of 1.5mg of levonorgestrel, repeat use doses do not pose any known health risks and no serious adverse outcomes have been reported in this eventuality [131,132].

Risk of Cancer

Current research shows no association with increased risk of cancer [129].

Effect on future fertility

The use of LNG-EC has no effect on future fertility [133,134]. LNG-EC is cleared within a few days and women who have used LNG-EC are at risk of pregnancy from any subsequent acts of unprotected sexual intercourse (UPSI) [129].

Ectopic pregnancy

LNG-EC does not increase the risk of ectopic pregnancy, a potentially dangerous condition in which a fertilised egg implants outside the uterus [135]. A systematic review including data from 136 studies, which followed a defined population of women treated one time with emergency contraceptive pills (either levonorgestrel or mifepristone) and in which the number and location of pregnancies were ascertained found that the rate of ectopic pregnancy when treatment with EC fails does not exceed the rate observed in the general population [136]. The authors concluded that because EC is effective in lowering the risk of pregnancy, their use will reduce the chance that an act of intercourse will result in ectopic pregnancy.

Effect on pregnancy outcomes

LNG-EC does not harm a developing fetus if taken mistakenly early in pregnancy [129]. A study by Zhang et al (2009) found no association between the use of LNG-EC and the risk of major congenital malformations, pregnancy complications or any other adverse pregnancy outcomes [137].

Administration

The World Health Organisation recommends a single dose of 1.5mg LNG-EC orally as soon as possible within 72 hours after unprotected intercourse or known or suspected contraceptive failure [107].

Studies have been undertaken to assess the optimum dosing regimen. Johansson et al (2002) investigated three dosing regimes 0.75mg LNG twice with a 12 hour intervals (regime A), 0.75mg twice with a 24 hour interval (regime B) and 1.5mg in a single dose (regime C) [138]. Maximum LNG concentrations were approximately 27nmol/l for regimens A and B, and close to 40nmol/l for treatment C. The area under the curve was significantly higher for treatment C during the first 12 hours and significantly lower for

treatment B. However, after 48 hours and up to 9 days from onset of treatment, serum LNG levels were similar in all three regimens. They concluded that pharmacologically the regimen A and C were sufficiently similar to justify a clinical comparison. Cheng et al (2008) have undertaken a Cochrane systematic review of interventions for emergency contraception and found that single dose administration has similar clinical effectiveness as the split dose regimen [139]. In view of it being easier to take a single dose this is the administration schedule now recommended.

Piaggio et al (2011) have explored how long after UPSI LNG-EC can be administered and still be effective [140]. Data were analysed from 6794 women participating in four WHO randomised trials receiving 1.5mg LNG-EC in a single dose or split into two doses twelve hours apart. For the four trials combined, odds ratios for pregnancy in the second, third and fourth day with respect to the first day were not significantly different from 1 at the 5% level of significance. On the fifth day, the odds ratio of pregnancy compared to day 1 administration was almost 6. The authors concluded that LNG-EC should be administered as soon as possible after UPSI and that it is uncertain whether administration on day five post UPSI offers any protection [140].

Efficacy

The efficacy of emergency contraception is described in terms of potential pregnancies prevented based on calculating the risk of pregnancy for the day of the cycle on which intercourse occurred [141]. This is difficult to calculate because often it is not known how fertile the user is and information on cycle length, last menstrual period and time of UPSI is unreliable [142]. Additionally, it is difficult to know precisely when in relation to ovulation EC has been given [142].

The WHO fact sheet states that based on reports from four studies including almost 5000 women, LNG-EC used within 5 days after UPSI is thought to reduce a woman's

chance of pregnancy by 60-90% [107]. A randomised controlled trial of LNG-EC versus the Yuzpe regimen of combined oral contraceptives for EC estimated that the proportion of pregnancies prevented was 85% (95%CI 74-93%) [143]. It has to be emphasised that these figures are only estimates and as mentioned above there are problems with the methods used to calculate them hence Raymond et al (2004) used data from two published randomised trials of the levonorgestrel and Yuzpe regimens to calculate minimum effectiveness of the LNG-EC regimen [144]. They conservatively assumed that the Yuzpe regimen was entirely ineffective in these trials and thus estimated that LNG-EC prevented at least 49% of expected pregnancies (95%CI: 17%-69%). The authors concluded that considering physiologic data suggests that the Yuzpe method does, in fact, have some efficacy, the effectiveness of the levonorgestrel regimen is likely to be higher than their minimum estimate [144].

Side-effects

Changes in bleeding pattern have been reported following the use of LNG-EC. A prospective observational study of 544 women who took 1.5mg LNG-EC found that early or timely menses occurred in 69% of women and was late by more than a week in 21%. Normal vaginal bleeding occurred in 57% of women whilst others had intermenstrual bleeding/spotting, premenstrual bleeding/spotting or menorrhagia [145]. Other side-effects that have been reported include nausea and vomiting [145,146] and headache [147].

Economics

EC cost-effectiveness studies weigh the costs of using EC, the likelihood that EC will prevent pregnancy, and the costs of an unintended pregnancy [148,149,150,151]. In addition to payment method, costs can be based on whether a woman obtains EC after unprotected sex versus in advance of need [149,150], and whether she obtains EC from a doctor or clinic versus directly from a pharmacist [148,151,152]. Costs do not, however, include those factors to which a monetary value cannot easily be assigned, such as the psychological consequences of experiencing an unintended pregnancy [153].
A number of studies have compared the costs of EC with an unintended pregnancy and have universally concluded that EC is cost-effective [148,149,150,152,153,154].
Preventing unintended pregnancy in developing countries is particularly imperative as discussed in chapter 1. An analysis comparing the public sector cost of EC with the average medical costs of unintended pregnancy (related to birth, miscarriage, ectopic pregnancy and abortion) found that assuming 85% efficacy, EC is cost-effective in a number of developing countries [151,153].

Access to EC[1]

EC is available in many countries but not all [155]. Ease of access is variable. Although use of a combination of oral contraceptive pills as a substitute for EC has always been possible, in many countries a dedicated product has only recently become available. For example at the time of our study EC was not available in Russia but now LNG-EC is available (labelled Escinor 1.5 and Escapelle) [155]. However, in many countries, such as much of the Middle East and sub-Saharan Africa, a dedicated product remains unavailable [155]. Use of oral contraceptive pills taken in a number to match (roughly) the dose in the yuzpe regimen is common. Where EC is available efforts are being made to improve access. In some countries, such as the UK and the US, it is available over-the-counter, in others women have to see a pharmacist and in others attend a health clinic.

Use of EC [1]

Regardless of improving access, use of EC remains low. In a 2001 survey of 880 female undergraduates in Nigeria, of whom 34% had had an abortion in the past, 58% knew about EC, but only 2% had used it [156]. In a group of 623 women who sought contraception or abortion in India – where an estimated 5-6 million abortions are undertaken every year, most of them illegal – only 6% knew about emergency contraception and none had ever used it [157].

In more developed countries knowledge is greater and whilst more women use it, amongst those undergoing induced abortion use remains low. Among women undergoing abortion, the proportion who said they used emergency contraception to try to prevent the pregnancy was 1.3% in the USA in 2000 [158], 2.9% in Sweden in 2000

[1] excludes IUD's inserted for purpose of emergency contraception

[159], 6.6% in Denmark in 2002 (though 24.1% had used it previously) [160], and 9.2% in France in 2002 [161]. In the UK, use of EC has increased since it was first licensed in 1984 but not proportionately to knowledge. In a questionnaire study of women presenting for abortion in Dundee in 1984, 1% of women had tried to prevent pregnancy with EC [162]. The study was repeated in 1996 and it was found that 7% of women had used EC to try and prevent unintended pregnancy [163]. This was in comparison to the increase from 12% having knowledge of EC in 1984 to 73% in 1996. In 2000, a small study undertaken in Newcastle found that 11% of women presenting for induced abortion had used EC [106]. There are no data available on use of EC among those women who continue with their pregnancy.

Reasons for non-use/ poor use

Lack of knowledge

In many countries, both developing and industrialised, lack of knowledge remains a significant barrier to use. Results from a cross-sectional survey of first year female students in Uganda in 2005 showed that less than half (45.1%) had ever heard about EC [164]. In Nepal, a cross-sectional survey of college students, in 2006, showed that only two thirds had heard of EC [165]. A cross-sectional survey of 14-19 year olds (n=100) in the US found that only 56% had heard of emergency contraception [166]. Even where knowledge of existence of EC is high, knowledge regarding its use is often poor. One study of female college students (n=609) in the US found that whilst 98% had heard of EC, 60% did not believe they could obtain it and 71% had misconceptions regarding the side-effects associated with EC [167].

Poor access

This is not the case in the UK any longer where EC is readily available over-the counter, from pharmacists and health clinics and can be obtained in advance of need. However, in countries where EC is not available at all or can only be accessed by prescription, this

does pose a barrier to use. An online survey of 531 women from 49 states in the United States, aged 14-19 who had engaged in UPSI at a time when they were aware of EC found that only 48% had ever used EC. Those who were able to access EC without a prescription were more likely to use EC within 24 hours (OR2.17; 95% CI 1.06-4.44) [168].

Failure to recognise risk of unintended pregnancy

Several studies have shown that failure to recognise the need to use emergency contraception is common. Even in settings where women have been supplied with EC in advance of need have shown that three out of four women who put themselves at risk of pregnancy did not use EC because they did not recognise – or did not acknowledge – the risk [169,170,171].

We have attempted to quantify pregnancy intention in a study looking at unintended pregnancy and use of emergency contraception amongst a large cohort of women in Scotland (see study below) [172].

Objectives for survey

- Quantify the incidence of unintended pregnancy in Lothian, Scotland and the outcome of such pregnancies
- Explore the uptake of emergency contraception in women with unintended pregnancy and thus assess awareness of risk of unintended pregnancy

Since 1989 there have been no estimates of pregnancy intendedness in the UK [4]. Additionally whilst knowledge of EC has risen dramatically, since being made available in 1984 (12% in 1984 to 73% in 1996) [163], this has not been the case for use of EC (1% in 1984 to 7% in 1996) [162,163] and the most recent data on use appears to be

from 2000 (11%) [106]. Additionally, whilst studies have investigated use of EC in the general population or among women requesting induced abortion, there is no data available on use of EC by women who are continuing with their pregnancy.

In 2004, we undertook a questionnaire survey designed to quantify pregnancy intention and use of emergency contraception among women who presented to a large teaching hospital in Edinburgh, UK, for antenatal care, or to attend the pregnancy support centre (women who have a history or high likelihood of miscarriage), or for termination of pregnancy [172].

Methods

From July 5, 2004, until Feb 28, 2005, all women booking for antenatal care or abortion in the New Royal Infirmary of Edinburgh, were invited to complete a self-administered questionnaire asking about pregnancy intention and use of emergency contraception. We excluded women whose foetus was shown to have died, by ultrasonography, and those who were unable to read or write English well enough to understand or complete the questionnaire. Women who were judged by the nursing staff to be distressed about the clinical consultation were not offered the questionnaire. This judgement applied mainly to women presenting at the abortion clinic.

Before seeking ethics approval for the study, we asked 207 women to read and comment on a draft information sheet and questionnaire. The final questionnaire consisted of 15 questions about age, pregnancy gestation, contraceptive use (including emergency contraception) in the month of conception, and pregnancy intention. Intendedness was measured by using Barrett and colleagues' (2004) instrument (table 1) [105]. For the intendedness score, the answer to each of the six questions was scored from 0 to 2, so the total scores ranged from 0 (least intended) to 12 (most intended). Although Barrett and colleagues (2004) emphasised that there were no cutoff points on the range of scores obtained, they suggested that three score groups were identifiable: 10-12 (planned), 4-9

(ambivalent), and 0-3 (unplanned) [105]. These three groups were used for the purposes of analysis and discussion. Ethics approval for the survey was obtained from the local research ethics committee.

On the basis of an estimated proportion of emergency contraceptive use of at least 10% [106] among women presenting for abortion and our estimate of 2% for women continuing their pregnancies, a sample size of 1000 women undergoing abortion and 4500 booking for antenatal care was needed to ensure that the upper confidence limit for proportion of emergency contraceptive use was only 50% higher than the lower limit. The study was powered to 90% and the confidence intervals set to 95%. Quality control checks were done on 5% of all data entries. Data were analysed by use of Excel (2003) and SPSS (version 12).

Groups were compared by χ^2 tests for binary data, Mann-Whitney tests for intendedness scores, and two-sample t-tests for age and gestation. Ages in different intendedness groups were comparer by one-way ANOVA.

Results

5686 pregnant women attended the hospital during the study period, 5630 of whom were eligible to participate (figure 1).

1285 (78%) of 1645 women attending for abortion were given the questionnaire and it was returned by 1006 (78%). 2905 (93%) of women attending the antenatal clinic and 810 (92%) of those attending pregnancy support centre were given the questionnaire; of those women, 2496 (86%) and 643 (79%), returned the questionnaire, respectively (figure 1). Women attending for abortion were younger than those planning to continue their pregnancies and women seeking abortion or attending the pregnancy support clinic attended hospital at an earlier gestation than those women attending an antenatal clinic (table 2). 3815 women answered all six questions in the intendedness measure, 2908 of them continuing their pregnancy and 907 who requested abortion. 814 (89·7%) of

women who requested abortion had a total score of 3 or less, which suggests that their pregnancies were unintended (table 2). Only two women who wanted an abortion scored 10 or more (intended) and 91 (10·0%) were ambivalent about the intendedness of the pregnancy (score 4–9), (figure 2). Of the women who planned to continue their pregnancies, 250 (8·6%) scored less than 3 (unintended), 1909 (65·6%) scored 10 or more (intended) and 749 (25·8%) had some ambivalence about their intention to conceive (score 4–9) (table 2, figure 2). Intendedness was associated with age in women who chose to continue with their pregnancies; women who had intended to become pregnant were significantly older than those who were ambivalent or had unintended pregnancy. There was no significant association between age and intendedness in women presenting for abortions (table 3). 113 (11·8%) women presenting for abortion used emergency contraception to try to prevent pregnancy whereas only 40 (1·4%) of those continuing with their pregnancies had done so (table 2). Of the women who used emergency contraception, 74 (65%) of those attending for abortion and 20 (50%) of those continuing with their pregnancy said they had used it after every episode of unprotected intercourse during the menstrual cycle in which they got pregnant. Women who used emergency contraception were significantly more likely to score low rather than high on the intendedness scale, both in the abortion group and in those continuing their pregnancies (table 4). In women continuing with their pregnancies, young age was significantly associated with

emergency contraception use ($p<0·0001$). Age was not related to emergency contraception use in women seeking abortion, but in this group, women who presented before 39 days of gestation were significantly more likely to have used emergency contraception than those presenting later.

Figure 1: Study profile

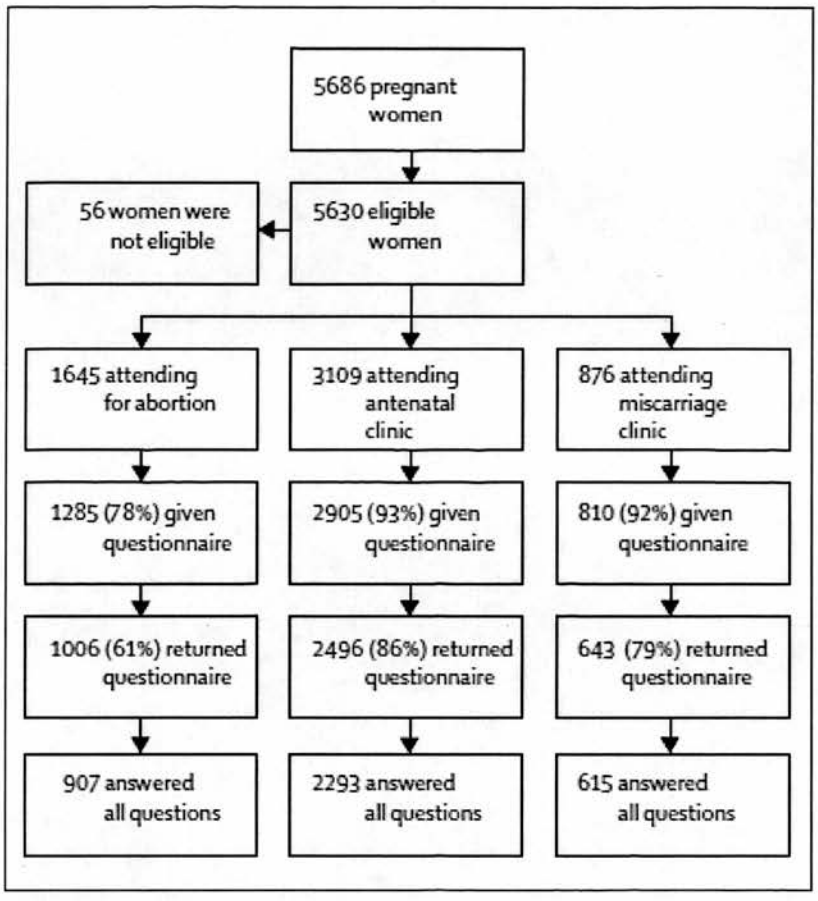


Figure 1: **Study profile**

Table 2: Characteristics of women, use of emergency contraception, and intendedness score

	Seeking abortion	Continuing pregnancy			*	†
		Miscarriage clinic	Antenatal clinic	Total		
Age (years)						
Mean (SD)	25·0 (6·7)	30·3 (6·2)	29·6 (5·8)	29·7 (5·9)	<0·0001	0·002
Range	10–45	14–44	15–44	14–44		
Gestation (days)						
Mean (SD)	56 (19)	58 (15)	92 (17)	87 (21)	<0·0001	<0·0001
Range	7–181	16–129	44–270	16–270		
Total intendedness score, n (%)						
10–12 (intended)	2 (0·2%)	402 (65·4%)	1507 (65·7%)	1909 (65·6%)	<0·0001	0·42
4–9 (ambivalent)	91 (10·0%)	140 (22·8%)	609 (26·6%)	749 (25·8%)		
0–3 (unintended)	814 (89·7%)	73 (11·9%)	177 (7·7%)	250 (8·6%)		
EC used in conception cycle						
Yes	113 (11·8%)	17 (2·7%)	23 (1·0%)	40 (1·4%)	<0·0001	0·002
No	844 (88·2%)	603 (97·3%)	2308 (99·0%)	2911 (98·6%)		
EC after all episodes of unprotected sex ‡	74 (65%)	7 (41%)	13 (56%)	20 (50%)	0·12	0·42

EC=emergency contraception. *Seeking abortion versus total continuing with their pregnancy. †Attending miscarriage clinic versus those attending the antenatal clinic. ‡Percentage is of those answering "Yes" to use of emergency contraception in conception cycle.

Table 2: **Characteristics of women, use of emergency contraception, and intendedness score**

Figure 2: Spread of intendedness scores for women undergoing induced abortion and for all women continuing their pregnancies (including thoe seen in the miscarriage clinic) 0=least intended pregnancies, 12 = most intended.

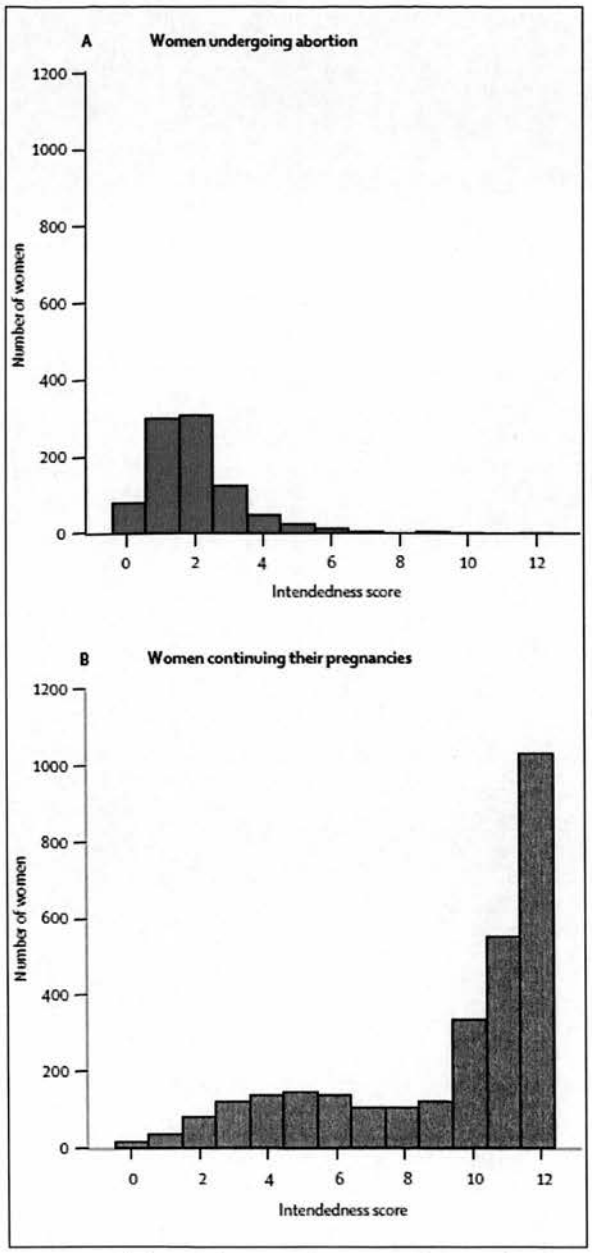


Figure 2: **Spread of intendedness scores for women undergoing induced abortion and for all women continuing their pregnancies (including those seen in the miscarriage clinic).**
0=least intended pregnancies, 12=most intended.

Table 3: Association between mean age (years) and intendedness

	Mean age (years)			
	Seeking abortion	Miscarriage clinic	Antenatal clinic	Continuing pregnancy total
Intended	31·0	31·9	31·0	31·2
Ambivalent	24·9	28·3	27·3	27·5
Unintended	24·8	25·8	25·6	25·7
p	0·28	<0·0001	<0·0001	<0·0001

p values are from one-way ANOVA.

Table 3: **Association between mean age (years) and intendedness**

Table 4: Trends in maternal age, gestational stage, and pregnancy intendedness among women using emergency contraception

	Seeking abortion	Continuing pregnancy		
		Miscarriage clinic	Antenatal clinic	Total
Age (years)				
0–19	19 (9·3%)	1 (3·7%)	6 (3·6%)	7 (3·6%)
20–24	35 (11·1%)	8 (7·8%)	7 (2·3%)	15 (3·7%)
25–29	34 (17·4%)	2 (1·6%)	5 (0·9%)	7 (1·0%)
30–34	10 (8·8%)	4 (2·1%)	2 (0·2%)	6 (0·6%)
≥35	11 (10·4%)	2 (1·2%)	3 (0·7%)	5 (0·8%)
p*	0·64	0·011	0·0001	<0·0001
Gestation (days)				
0–39	21 (22·8%)	2 (5·0%)	0	2 (5·0%)
40–79	74 (10·6%)	9 (2·5%)	2 (0·9%)	11 (1·8%)
80–119	6 (8·7%)	1 (2·5%)	17 (1·0%)	18 (1·0%)
≥120	2 (14·3%)	0	3 (3·8%)	3 (3·8%)
p*	0·015	0·48	0·13	0·19
Intendedness				
Intended	0	6 (1·6%)	1 (0·1%)	7 (0·4%)
Ambivalent	4 (4·7%)	7 (5·0%)	14 (2·4%)	21 (2·9%)
Unintended	96 (12·3%)	3 (4·1%)	6 (3·5%)	9 (3·7%)
p*	0·031	0·054	<0·0001	<0·0001

Percentages are the proportion of women in that age, gestational stage, or intendedness group who used emergency contraception. Information is not available for all participants. * χ^2 test for trend.

Table 4: **Trends in maternal age, gestational stage, and pregnancy intendedness among women using emergency contraception**

Discussion

In Edinburgh in 2005, only two-thirds of pregnancies destined to end in childbirth were clearly intended, one in ten was unintended, and around a quarter of women were somewhat ambivalent about their intention to become pregnant. When Barrett and colleagues (2004) developed their instrument, they took care to describe the differences in the meaning of the words "planned and unplanned", "intended and unintended", and "wanted and unwanted" [105]. In 1989, Fleissig used the word "unplanned" and the questionnaire was completed by women six months postpartum [4]. Despite the difference in timing of the survey, and differences in wording of the questionnaires, the proportion of pregnancies that are "planned" or "intended" has not changed since Fleissig's study. This finding is perhaps surprising given the demographic changes (falling birth rates, later age of first childbirth) [173], changes in sexual behaviour [174], and the increase in contraceptive choice in the past 25 years [6].

The relation between age and intendedness among women continuing their pregnancies is unsurprising. The findings that over 90% of pregnancies ending in abortion were unintended and that only 10% of women requesting abortion were ambivalent about their pregnancies is consistent with the findings of a smaller study of 300 women in Edinburgh undergoing abortion, in which a similar proportion claimed to have used emergency contraception [70]. In that study a modified version of the intendedness measure was used in a face-to-face interview rather than in a self-administered questionnaire. Despite the methodological differences, both studies show that most women who requested abortion had no intention to conceive.

One in ten women undergoing abortion and a quarter of women continuing with their pregnancies seemed to have some ambivalence about pregnancy intention. Since the number of women who were ineligible for the study or deemed to be too distressed to be given the questionnaire was small we do not believe that inclusion of these women would have altered the findings.

The observation of a relation between intendedness and emergency contraception use adds weight to the validity of Barrett and colleagues' scoring system. The results also suggest that, women who are ambivalent about their pregnancies are more likely to continue than to have them terminated. The association between use of emergency contraception and earlier gestational stage at presentation among women requesting abortion is probably related to heightened awareness of the risk of pregnancy since they recognised that they were at risk of pregnancy before missing a menstrual period. Use of emergency contraception was related to age only for women who were continuing with their pregnancy, perhaps because younger women are more ambivalent about avoiding pregnancy than older women.

The availability and use of emergency contraception varies around the world. Although use of a combination of oral contraceptive pills as a substitute for emergency contraception has always been possible, in many countries a dedicated product has only recently become available. Generally, use does not start to increase until such a product becomes available (eg, in Nigeria and the USA in 1998, in France in 1999, and in India in 2002). In much of sub-Saharan Africa, the former Soviet Union, and the Middle East, a dedicated product is not available. In a 2001 survey of 880 female undergraduates in Nigeria, of whom 34% had had an abortion in the past, 58% knew about emergency contraception, but only 2% had ever used it [156]. In a group of 623 women who sought contraception or abortion in India—where an estimated 5–6 million abortions occur each year, most of them illegal—only 6% knew about emergency contraception and none had ever used it [157]. In more developed countries, knowledge of emergency contraception is greater and more women use it. Among women undergoing abortion, the proportion who said they had used emergency contraception to try to prevent the pregnancy was 1·3% in the USA in 2000 [158], 2·9% in Sweden in 2000 [159], and 9·2% in France in 2002 [161]. Use of emergency contraception has increased in the UK since it was first licensed in 1984. In a questionnaire study of women presenting for abortion in Dundee in 1984, 1% of women had tried to prevent the pregnancy with emergency contraception

[162]. When the study was repeated in 1996, 7% of women had used emergency contraception [163]. Nevertheless, emergency contraception is unlikely to prevent many pregnancies if, as in our survey of women who became pregnant, only one in ten women who definitely did not want to become pregnant use it in cycles when they have put themselves at risk of pregnancy, and not much more than half of those use it with every act of unprotected intercourse. The availability of emergency contraception over the counter in UK pharmacies does not seem to have resulted in increased use [175].

Other studies have shown that failure to recognise the need to use emergency contraception is common. In a questionnaire study of 1365 women who had induced abortions in France, 90% had heard of emergency contraception but only a third had ever used it and only 9% had used it in the cycle in which they became pregnant [161]. 38% of the women were aware that they had put themselves at risk of pregnancy in the cycle in which they conceived—most of these were either not using contraception or were relying on condoms or withdrawal. Nine out of ten of them knew about emergency contraception but only one in four of them used it. More than half the women did not realise that they were at risk of pregnancy, and only 2·8% used emergency contraception. Lack of knowledge of how and when to use emergency contraception, difficulties with getting hold of it, and reservations about using it are all commonly cited barriers to its use [176]. However, without these barriers, most women who have become pregnant and could have used emergency contraception to prevent an unwanted pregnancy failed to do so. Several studies, from various settings, in which women were given a supply of emergency contraception in advance of need have shown that three out of four women who put themselves at risk of pregnancy, even when they had a supply at home, did not use emergency contraception because they did not recognise—or did not acknowledge—the risk [169,170,171].

Although 98% of women who wish to avoid pregnancy use contraception in the UK [177], abortion rates continue to rise [3,99]. Unintended pregnancies that end in childbirth, unless they occur in teenagers, are of less concern to policymakers than those

that end in abortion, but they do affect the lives of the women involved. Understanding of sexual behaviour and patterns of contraceptive use is crucial for development of interventions to reduce unintended pregnancy. This survey needs to be repeated in other settings, and if the findings are similar elsewhere, a strategy will need to be developed to improve contraceptive use. We need to find ways to raise awareness of the real risks of pregnancy associated with lack of use of contraception or with incorrect or inconsistent use. Emergency contraception is unlikely to make a substantial difference to pregnancy rates. Condoms and oral contraceptive pills are the most commonly used reversible methods of contraception in the UK and both rely on consistent use for their effectiveness. Condom use is commonly inconsistent, and compliance with oral contraception is not easy. In one US study, 47% of women reported missing one or more pills per cycle and 22% reported missing two or more [178]. In a study that used electronic diaries to record compliance, 63% of women missed one or more pills in the first cycle of use, and 74% did so in the second cycle [179]. We need to encourage women who clearly want to avoid pregnancy and are taking risks to use long-acting contraceptive methods (implants and intrauterine devices) that do not depend on compliance for their effectiveness [180].

Chapter 3: Determining acceptability of long-acting reversible methods of contraception – The case of Implanon®

Background

Long-acting reversible methods of contraception

These are methods of contraception which have a long duration of action and do not require any active adherence once initiated. They are sometimes, aptly, termed 'forgettable contraception' [181]. They are characterised by low failure rates [74] earning them a position at the top tier of contraceptive methods, side by side with sterilisation[2] [181]. A recent guideline from the National Institute of Health and Clinical Excellence (NICE) in the UK suggested that increased uptake of long-acting reversible methods of contraception (LARC) would reduce unintended pregnancy [180].

The current etonogestrel implant (Implanon®) is one of an array of LARC.

Implanon®

Implanon® is a long-acting reversible contraceptive method providing contraceptive protection for three years [182]. Implanon® was first marketed in Indonesia in 1998 [183]. It was licensed in the UK in 1999 and became available in the United States in 2006 [183,184]. It is currently available in more than 30 countries [183]. A new version of the etonogestrel implant, with an improved inserter and radio-opaque device for easier detection is currently undergoing assessment [185].

[2] Although the LARC, Levonogestrel releasing Intrauterine system (IUS) is actually more effective than female sterilisation.

Pharmacology and metabolism

Implanon® consists of a single rod that is 4cm long and 2mm in diameter. It contains 68mg of etonogestrel (a progestogen that is derived from 19-nortestosterone and is the biologically active metabolite of desogestrel) (Figure 1) in an ethinyl vinyl acetate (EVA) copolymer core, surrounded by an EVA membrane. The rod is inserted subdermally and releases etonogestrel at a rate of 67 micrograms/day initially, gradually decreasing, reaching 30 micrograms/day by the end of the three years [186]. The dose of 30 micrograms/day is sufficient to inhibit ovulation throughout almost the entire three years of use [187,188,189,190].

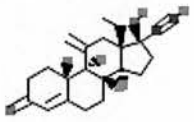


Figure 1: Chemical compound information of implanon; MW: 324.45g/mol, MF: $C_{22}H_{28}O_2$

Mode of action

Concentrations of etonogestrel are sufficient to inhibit ovulation within eight hours of insertion of Implanon® and maximum concentrations are attained within four days of inserting the implant, thus ensuring rapid and sustained ovulation inhibition [189]. Additionally, etonogestrel changes the quality of the cervical mucus and thus impairs or suppresses the access of fertile spermatozoa to the site of fertilisation [187]. Etonogestrel also suppresses endometrial development [187]. However, these additional mechanisms are (for the vast majority of users) superfluous as there is no egg to fertilise or implant. Implanon® has the advantage of being independent of compliance for its efficacy [191].

Safety

The safety profile of Implanon® has been shown to be acceptable and not essentially different from other low dose progestogen only methods [182,183]. Implanon® is assigned a category '1' classification for healthy women from menarche to before the menopause (18 to >40) (World Health Organisation Medical Eligibility Criteria (WHO MEC)) and there are very few contraindications in contrast to combined hormonal contraception [192,193] (appendix 1). Etonogestrel does not accumulate in the body [194] and ovulation is resumed within three weeks of removal in more than 90% of users [190,195].

Cancer risk

Toxicology studies have addressed both the progestogens and the polymers that make up the implant. Carcinogenicity studies reviewing the polyethylene-co-vinyl acetate (the polymer used in Implanon®) indicated that this material is safe for use [196]. Much of the data on etonogestrel is derived from its prodrug, desogestrel. The data from toxicology, genotoxicity, carcinogenicity and fetal development studies have demonstrated overall safety [197]. In a 24 month carcinogenicity study in rats with subdermal implants releasing 10 and 20 micrograms etonogestrel per day (equal to about 1.8-3.6 times the systemic steady state exposure of women using Implanon®), no drug related carcinogenic potential was observed [186]. An integrated safety analysis including 11 international studies concerning Implanon® of which 10 had a duration of at least two years found no link to cancer risk [198].

Cardiovascular disease

Epidemiological studies on the cardiovascular risk of progestagen-only contraceptives are rare. A review of the safety of implantable contraceptives by Curtis (2002) in 2002 concluded that cardiovascular events among Norplant users were rare, although there was no evidence to assess whether rates differed from those of non-hormonal controls [199]. Whilst this review was of the implant, Norplant both implant types are low-dose

progestogen-only implants and hence it seems reasonable to extrapolate from Norplant to Implanon®. Since 2002 I am aware of only two studies on this topic, One small study of 36 healthy, non-smoking women has explored the effect of Implanon® on cardiovascular risk factors, including markers of inflammation and found there to be no increase in risk in healthy young women [200]. A second non-comparative study (n=60) in Spain reported no clinically significant changes in blood pressures, blood cholesterol or glucose concentrations among Implanon® users at one year [201]. These data are reassuring.

Bone mineral density

The evidence available on this topic is limited. The American College of Obstetricians and Gynecologists states in its 2011 practice bulletin that the evidence, whilst limited, is reassuring that implants do not have a major effect on bone mineral density (BMD), a surrogate marker for fracture risk [202]. When extrapolating from pharmacokinetic studies of the etonogestrel implant and data on BMD assessment in women aged 18 years and older, they state that the implant should not affect ovarian estrodiol production or BMD in adolescents [202]. To my knowledge there are only three studies to date exploring the relationship between Implanon® and BMD [203,204,205,206]. The study by Beerhuizen et al [204] found no effect of Implanon® on BMD whereas the other two studies found a loss of BMD at the ultra-distal radius in one [203,205] and the distal radius and ulna [206] in the other. However, whilst these findings could indicate that BMD changes through the time of use evaluations at forearm sites are less predictive and accurate than those at the hip and vertebra. Further limitations of the studies include small study numbers; of short duration (two year and three year follow up of one study) and a cross-sectional survey in the case of the other, and hence the results must be treated with caution. No studies have evaluated fracture risk in current or past implant users, change in BMD after discontinuation of the implant, or the BMD in women younger than 18 years [202]. In summary more research with larger sample sizes is needed but based on the available evidence an update on hormonal contraception and bone density by Islay and Kaunitz (2011) states that skeletal health concerns should not

restrict the use of hormonal contraception, including DMPA® and in this case, Implanon® [207]

Administration

Contraceptive implants are inserted subdermally in the upper arm by a trained health professional. This requires attendance to a health facility for both insertion and removal as the implant is non-biodegradable. This has a cost-benefit implication. The benefit is that it allows for appropriate counselling and for an alternative method to be initiated if and when Implanon® is removed. It also guarantees compliance for the duration of use. The cost incurred is in the training of health professionals and upfront resource utilisation in terms of appointment time used in counselling, insertion and removal and will depend on the duration of time the implant is retained for.

Efficacy

Recent data have shown that Implanon® is one of the most effective reversible contraceptives with a 'perfect use failure rate' of 0.01 per 100 implants fitted [208] and a 'typical use' failure rate of 0.049 per 100 implants fitted [208]. This has led to some health insurance schemes fully reimbursing the cost of implant provision in a number of high resource countries and other governments subsidising or providing it free at health care facilities [209].

Side effects

Menstrual irregularity

The WHO has defined bleeding pattern indices. These indicate the extent of deviation from a normal pattern as observed in a normal menstrual cycle [210]. Bleeding patterns include amenorrhoea, infrequent bleeding, frequent bleeding, prolonged bleeding and normal bleeding [211] (Appendix 2).

This is the most common side effect of Implanon®. An integrated analysis of data from 11 international trials comprising 942 women and 24,679 cycles found Implanon use to be associated with an altered bleeding pattern [212]. These altered patterns comprised amenorrhoea (22.2%); infrequent bleeding (33.6%); frequent bleeding (6.7%) and prolonged bleeding (17.7%). In 75% of reference periods (RP) bleeding/spotting days were fewer than or comparable to those observed during the natural cycle, but they occurred at unpredictable intervals. On average, the number of days of bleeding per 90 day RP was 17.5. A positive finding of this analysis was that the group of women with favourable bleeding patterns (amenorrhoea or infrequent bleeding) during the first three months tended to continue with this pattern throughout the first two years of use, whereas the group with unfavourable initial patterns (frequent or prolonged bleeding) had at least a 50% chance that the pattern would improve [212]. However, this finding must be treated with some degree of caution because as the authors themselves surmised the prognosis of bleeding patterns based on the first RP is hampered by the fact that those discontinuing early did not contribute any further RP data to the analysis, which then leads to a more favourable pattern with time [212].

The NICE guidance published in 2005 recommends that women should be informed that bleeding patterns are likely to change while using Implanon® and that whilst 20% of women will experience amenorrhoea 50% will have infrequent, frequent or prolonged bleeding [180].

Weight gain

Weight gain is a major concern for contraceptive users. Although commonly reported in contraceptive studies it is difficult to ascertain whether weight gain is caused by the contraceptive itself because most studies lack a control group [202]. Weight gain is reported by 12% of Implanon® users in contraceptive studies but few (2.3%) discontinue because of this weight change [183]. It is unlikely to be associated with Implanon® given the low dose of Progestogen in Implanon®.

Dermatological reactions

Acne is a commonly reported adverse effect of progesterone-only contraceptives. Overall, most women (86-90%) using the implant have either no change or an improvement in reports of acne and 10-14% of users experience a worsening of symptoms [202].

Provider-dependence for insertion and removal

Both insertion and removal of Implanon® require attendance at a health facility. It also requires adequately trained providers as well as aseptic techniques. Furthermore, since initiation and discontinuation of use is provider-dependent and not controlled by the user, there may be a risk of coercion of use on the one hand or, on the other hand, difficulty in access to initiating use, if trained providers are not readily available [213].

Benefits

Implanon® is a safe, efficacious long-acting reversible 'forgettable' contraceptive. It is free from daily user compliance and thus its typical and perfect use failure rates are closely aligned.

Oestrogen-free

Similarly to other methods of contraception discussed in this thesis, Implanon® contains no oestrogen. This makes it ideal for women who are either intolerant of or who have contraindications to oestrogen-containing methods – such as focal migraine.

Return to fertility

After removal of Implanon®, serum concentrations of etonogestrel decline to below the limit of detection of the assay (20pg/ml) within one week [189] and ovulation is resumed within three weeks of removal in more than 90% of users [187,190,195,214].

Pain syndromes

Current studies, though limited in number, are encouraging that Implanon® may provide another option for pain syndromes such as dysmenorrhoea and endometriosis [181]. Examples of such studies include: A study by Funk et al (2005) of 315 patients found that rates of dysmenorrhoea decreased from 59% at baseline to 21% with Implanon® treatment [215]. Of the 187 patients with dysmenorrhoea at baseline, 81% reported improvement and only 10% reported an increase in symptoms; Mansour et al (2008) undertook an integrated analysis of 11 clinical trials and found that 77% of women who had baseline dysmenorrhoea experienced complete resolution of symptoms [212]. A pilot study by Walch et al (2009) compared Implanon® with Depot medroxyprogesterone acetate (DMPA) in women with laparoscopically proven endometriosis. It reported that both methods were associated with decreased pain scores. In the etonogestrel implant group, pain intensity scores decreased by 68% compared

with 53% in the DMPA group [216]; A case series, by Yisa et al (2005), of five women with severe endometriosis reported that four of the five (80%) had excellent relief of symptoms with the implant [217]; and a recent randomised trial, by Shokeir et al (2009), of 23 women with pelvic pain and pelvic congestion syndrome compared Implanon® treatment with no treatment. Women with Implanon® had significantly lower pain scores at one year, decreased severity of dysmenorrhoea, less monthly blood loss, and fewer days of pain than untreated controls [218]. This was a small pilot study however and larger randomised controlled trials are necessary to confirm these findings.

Amenorrhoea

Studies have shown that many women would prefer a contraceptive option that is associated with less frequent or no bleeding. The integrated analyses of 11 clinical trials (942 women) reported that 22.2% (209/942) of women experienced amenorrhoea [212]. It also revealed that only 0.7% (7/942) discontinued for this reason. From these observations it would appear that users of Implanon® in all the cultures that were studied found amenorrhoea and infrequent bleeding to be highly acceptable [212]

Economics

Implant costs include not only the price of the device, but the equipment and supplies necessary for insertion and removal, training costs, time spent with clients for counselling and procedures, follow-up visits for management of side-effects and unintended pregnancies, and the time, personnel and supplies for infection prevention [191]. Cost data in 2005 found that first year cost of Implanon® was £175 and total three year cost was £230 (table 1) [180].

Table 1: Cost data for Implanon® [180]

Implanon method cost	Baseline value	Cost component and basic assumptions	
		Component	Cost
		Ingredient cost (Implanon®)	£90.00 per device [219]
First year cost	£175	Initial GP consultation, 20 min	£44.80
Total 3 year cost	£230	Consultation for insertion, 16 min	£35.84
		Sterile pack for insertion	£4.40
		Consultation for removal, 22 min	£49.28
		Sterile pack for removal	£5.50
		Resource use and cost of sterile pack based on GDG consensus; GP unit cost = £2.24 per surgery/clinic minute [220]	

It has been suggested that this high upfront cost of Implanon® limits its availability in parts of the UK [180] and perhaps in other countries.

To date three cost-effective analyses of Implanon® have been undertaken in the United Kingdom (UK) [180,221,222].

Phillips (2000) examined Implanon® compared with Norplant, Levonorgestrel releasing Intrauterine system (IUS), Depo-Provera (DMPA) and the combined oral contraceptive pill (COC) [221]. Data for Implanon® was taken from clinical trials and data for other methods was from reports in the literature. They found that reversible long-term approaches to contraception provided an effective and efficient use of healthcare return on public investment. Phillips (2000) concluded that Implanon® produces better rates of

return than both Norplant and IUS, and is more cost-effective in terms of cost per pregnancy avoided and cost per protected year than Norplant, IUS, DMPA and COC. A limitation of this study was that the analysis was based partly on data obtained from clinical trials and which may not reflect everyday clinical practice, especially in relation to continuation rates [221].

A second modelling study by Varney and Guest (2004), performed from the perspective of the UK, NHS using data on contraceptive service use from a General Practice Research database, found Implanon® to be more cost-effective than DMPA but less cost-effective than the IUS [222]. One significant limitation of this study undertaken between 1997 and 2002 was that data on Implanon® use was limited (n=277) as compared to that for IUS (n=6080) and DMPA (n=10,478). The most likely reason for this is that Implanon® only became available in the UK in late 1999 and initially the majority of implants would have been inserted and removed in the family planning clinic environment rather than in general practice due to health professionals having to be trained in insertion and removal techniques.

More recently NICE (2005) developed an economic model to examine the cost-effectiveness of LARC methods based on the clinical effectiveness data they presented in their guideline on the effective and appropriate use of LARC [180]. Data on continuation rates were from real life studies in the UK and Europe, including data from the study presented below. The guideline development group found that all LARC methods are more cost-effective – even if only used for one year – than either the COC or condoms and that among the four LARC methods DMPA is less cost-effective than the IUD, IUS and implant, with the latter becoming more cost-effective with longer duration of use [180].

This suggests that:

- Cost-effectiveness of all methods of contraception depends on their continuation rates, but this is particularly true for the very long-acting methods (implants and intrauterine devices) and
- High initiation costs should not be a barrier to use of LARC methods [180].

Use worldwide

The etonogestrel implant (Implanon®) is a relatively new method of contraception. It's use is relatively low though it is becoming an increasingly popular choice of contraception and approximately six million women use this method worldwide [209]. In 2007, 3.7% of married women were using injectables/implants as their chosen method of contraception, worldwide [223]. This can be separated into use in more developed regions (1.3%) and less developed regions (4.2%). Admittedly the majority of this figure comprises injectable users and that component which is implants includes Jadelle and Norplant as well as Implanon®.

In the UK in 2008/9 the Office of National Statistics estimated current Implanon use amongst women aged 16-49 to be 1%. This was a third of that for Depo-Provera (3%) [224].

Reasons for discounting/discontinuing Implanon®

Discontinuation of Implanon® particularly occurs during the first six months of use and then levels off [198]. This is possibly due to one of two factors: either because side-effects such as menstrual irregularity improve over time or because those women that experience side-effects will discontinue within the first six months.
Menstrual irregularity is the most frequently cited reason for discontinuation. Mansour et al (2008) found in an integrated analysis of 11 clinical trials that 11.3% of women discontinued Implanon® prematurely due to bleeding abnormalities [212]. Blumenthal et al (2008) undertook an integrated safety analysis of Implanon® and found that 10.4% (98/942) of women discontinued Implanon® use due to bleeding irregularities [198]. Other reasons for discontinuation other than planning a pregnancy (4.1%) identified in the study by Blumenthal et al (2008) included emotional lability (2.3%), weight increase (2.3%), acne (1.3%), headache (1.6%) and depression (1.0%) [198].

Determining acceptability by investigating continuation rates

Although no single contraceptive method is perfect or appealing to all, contraceptive implants are safe and fulfil a very important need among fertility regulation methods thus increasing choice of method. To increase use of any method it must appeal and be considered acceptable to the user. There is evidence to demonstrate that the acceptability of a contraceptive method (and continuation rate) is increased when users are well informed about the side effects and risks [225,226]. Acceptability of the chosen method is likely to be fundamental to correct and consistent use and to continuation.

Thus, continuation rates are one objective measure of acceptability. This is not to say continuation rates equate to level of acceptability. That would be simplistic. There are

many factors which determine acceptability and continuation of a method may only reflect that it is the most acceptable of those methods available.

Continuation in international clinical trials of Implanon® has been shown to be high, between 90 and 95% at 6 months and 80 and 88% at 12 months [182]. However, participants in prospective clinical trials often have relatively high continuation rates because the inclusion criteria for trials are rigid and tend to bias toward a willingness/likelihood to continue the method. Regular study related follow-up serves as a positive reinforcement and in countries where women have to pay for contraception and healthcare is expensive, provision of free supplies and services through clinical trials ensures good compliance [227].

"Real life" data are much harder to come by and loss to follow up is common. Moreover much of the data for Implanon® comes from developing countries where the availability of and access to other methods of contraception is usually limited and where continuation rates are often high.

In a review of evidence from European settings the NICE guideline (2005) concluded that 20-25% of women will discontinue Implanon® within one year of insertion, and up to 44% will stop using the method within three years [180].

To date, three small studies have investigated continuation rates in the clinic setting in the UK.

The first study by Smith et al (2002) set out to assess continuation rates and also factors associated with early removal in three community services [228]. An audit was carried out by use of a retrospective review of service users who had undergone an Implanon® procedure between the date when Implanon® was introduced into each service and 31st December 2000 inclusive. Client records were reviewed after June 2001 ensuring a minimum of six months' data for each user. Kaplan Meier survival analysis techniques

were utilised to enable as much information as possible to be used from previous follow-up appointments from those subjects where it was not known whether or not they had continued with Implanon®. A total of 190 subjects were identified between the three sites. At six months, 97 had definitely continued with their implant, 22 had had it removed and 71 were lost to follow-up. It was assumed that no-one would have had their implant removed elsewhere and continuation rate was calculated at 88% or by survival analysis 84%. By the end of the study, 95 subjects had had their implant fitted at least 12 months previously. Similar analysis revealed 78% continuation and by survival analysis 67%. Whilst this study did utilise real life data, and perhaps because of this, there were a number of weaknesses; there was a high default rate to follow-up and hence estimates of continuation would be considered unreliable. Also, it was assumed that no-one had had their implant removed elsewhere. The authors themselves felt that repeat analysis of the cohort after three years when all 190 subjects should have had their implants removed would give more accurate continuation rates and hopefully the number of defaulters would have been lower as most women would hopefully have attended one of the three sites for the removal of their implant[228].

Similarly the second study, by Rai et al (2004), a retrospective case note review which followed up 147 women who had had Implanon® inserted three years previously found there was a high loss to follow up (24%). The study found that the probability that Implanon® was still being used at three years was 75% (58-85%) by Kaplan Meier survival analysis and 53% (28-73%) by confirmed analysis [229].

The third study, by Agrawal and Robinson (2005), assessed the first three years' use of Implanon® in a family planning and reproductive health care centre in Luton. Case notes were reviewed for the first 106 women who had Implanon® inserted since introduction of the implant in September 1999. Again, loss to follow up was high (19%). Continuation rates were 69.8% at one year, 44.1% at two years and 30.2% at three years [230].

The above studies would indicate that continuation rates in "real life" situations are not as high as in trial situations. However, consideration must be given to the fact that loss to follow up was high in these studies. For this reason the results must be viewed with a degree of caution.

Objective for survey:

- Explore continuation rates of a long-acting gestogen implant (Implanon®)

At the time of undertaking this research there was a need for reliable data on 'real life' continuation rates. With so many contraceptive methods on the market any new method, especially one that has a high upfront cost such as Implanon®, has to prove itself as an effective and efficient use of healthcare resources. Cost-effectiveness of all methods of contraception depends on their continuation rates, but this is particularly relevant as detailed earlier for the very long-acting methods (implants and intrauterine devices).

To determine continuation rates of Implanon® in clinical practice in Scotland, a retrospective case note review combined with a questionnaire survey three years after insertion of Implanon® in a cohort of 326 new users attending a large family planning clinic in Edinburgh, Scotland was undertaken [231].

Materials and methods

Case notes for all women attending the clinic for Implanon® insertion between January 1 and December 31, 2001, were reviewed at the end of 2004. If the implant had been removed in the clinic, data were obtained from the case records. Women who had not returned to the clinic for Implanon® removal (and who had consented to being contacted when first attending the clinic) were contacted to remind them about the need for removal of their implant before 3 years of use was completed. These women were also asked to complete a brief questionnaire to determine whether Implanon® had been

removed and, if so, the date and reason for removal of the implant, where the procedure was done, and the method of contraception used for the first 3 months after discontinuation of Implanon®. Three attempts at contact were made: twice by mail and once by telephone. An interval of 2 weeks was maintained between each attempt at contact.

Data were analyzed using Excel 2003 (Microsoft Corporation) and SPSS version 12 (SPSS, Inc., Chicago, IL, USA). Ages of women continuing for more or less than 2 years were compared using a two-sample *t* test. Cumulative discontinuation rates were computed using descriptive statistics. Many women returned to the clinic slightly before 3 years after Implanon® insertion to ensure that replacement contraception was available for use. Women returning for implant removal more than 2 years and 9 months after insertion were deemed to have used the implant for its full duration.

Results

Three hundred twenty-four women had Implanon® inserted in 2001. Median age at insertion was 26 years (range, 15– 49). Information about implant removal was available for a total of 277 women (85%). Two hundred thirty-six of these women had their implant removed in the clinic, and data were collected from their case records. Letters were sent to 87 women who had not returned to the clinic for implant removal; 1 woman chose not to disclose her home address. Forty-one women responded and returned the questionnaire. All 46 women who failed to respond to the request for information were no longer residing at the address recorded in their case records.

For the 277 women in whom information was available, continuation rates of Implanon® were 89% (CI 84–91) at 6 months, 75% (CI 69–79) at 1 year, 59% (CI 52– 63) at 2 years and 47% (CI 40 –52) at 2 years and 9 months. Thirty-three women did not return for implant removal until at least 1 month after 3 years of

use; the longest duration of use was 4 years and 8 days. Continuation rates were independent of age.

Sixty-eight women discontinued Implanon® within 1 year; 62 of them (91%) did so because of unwanted side effects. The most common documented reason for implant removal was frequent and/or unpredictable bleeding (n = 42, 62%). Weight gain accounted for 21% of removals (n = 14) and mood change accounted for 16% (n = 11). Of the six women who discontinued within 1 year for reasons other than side effects, five did so because they wished to become pregnant and one because she was no longer in a relationship.

Data on the contraceptive chosen for use following Implanon® removal were collected (table 2). Twenty-three women (8%) were actively trying to conceive. Thirty-nine percent of women had a second implant inserted, while another 14% chose an alternative long-acting method or sterilization. More than 47% of the women elected to use a less effective method of contraception.

Table 2: Contraceptive method used following removal of Implanon®

Method	**N (%)**
No method (pregnancy desired)	28 (8)
Implanon®	99 (39)
IUD/IUS	12 (5)
Depo-Provera®	8 (3)
Sterilisation	16 (6)
Combined pill	45 (18)
Progestogen-only pill	17 (7)
Condom	55 (22)
Withdrawal	2 (<1)

Discussion

Continuation rates of all methods of contraception are generally disappointing. In an international review of discontinuation rates after 1 year of use of hormonal contraception, rates varied from 19% (for Norplant) to 62% (the combined pill) [232]. Discontinuation rates are higher for methods that do not require removal by a health professional [233]. In this study of women in Edinburgh, continuation rates among women in whom data could be obtained might best be described as reasonable with just under 60% continuing for 2 years and just under half continuing for 3 years. The fact that nearly 40% continued to use Implanon® for contraception after 3 years is encouraging.

The present study involved a cohort of women attending a clinic that offers all currently licensed methods of contraception free of charge. Routine follow-up consisted of at most one visit to the clinic 6–12 weeks after insertion of the implant. Women were free to attend the clinic for review at any time, and Implanon® was removed on request at no cost. Follow-up rates in our study were quite good at 85%. In a postal survey of UK women followed up after Implanon® insertion in the East Midlands, the response rate was only 44% [234]. Although clinical trials have more frequent data collection and higher rates of follow-up, continuation rates in this cohort of Scottish women should be generally representative of UK women attending a community family planning clinic.

We have no idea as to how long those women lost to follow-up continued to use their Implanon®, and it does not seem worthwhile to guess. Only a dozen general practices (family doctors) in the area offer Implanon® removal and only to women who are registered with them. It is likely that most of these women continued to use Implanon® until they moved away from Edinburgh.

Robust data on patterns of contraceptive use in the UK are lacking. However, in the

United States, 40% of married women and 61% of unmarried women using a reversible method of contraception change it over the course of 2 years [235]. Many — especially those with less years of education — change from a more-effective method to a less-effective method. This study similarly demonstrates that almost half of the women, despite wishing to avoid pregnancy, chose a less-effective method of contraception when they stopped using Implanon®. This is unfortunate since data from the United States and the UK suggest that using condoms or oral contraceptives is much more likely to risk unintended pregnancy [70,158]. In contrast, there are data to show that contraceptive implants are associated with lower rates of unintended pregnancy than either condoms or contraceptive pills [236].

Using preliminary data from this study, the NICE guideline on LARC concluded that Implanon® is a cost- effective method of contraception and more cost-effective — even if used only for 1 year — than either the combined oral contraceptive pill or condoms [180]. Concerns that the high up-front costs of this method are unjustified because discontinuation rates are high are unfounded as this study has shown that continuation rates for Implanon® in real life in the UK are not unreasonable when compared with those of clinical trials and with data for other contraceptives. This should reassure health care providers and commissioners that increasing the uptake of this long-acting method as recommended in the NICE guideline [180] is worth the effort and the cost. The fact that more than one third of women chose to continue to use the implant when their first one had expired reflects a strong level of satisfaction on the part of the users.

Appendix 1: World Health Organisation Medical eligibility criteria (WHO-MEC) [193]

1= Yes: Use the method in any circumstance

2= Yes: Generally use the method

3= No: Use of the method is not usually recommended unless other more appropriate methods are not available or acceptable

4= No: Method NOT to be used

Categories 1 and 4 are clearly defined recommendations. For categories 2 or 3, greater clinical judgement will be needed and careful follow-up may be required. If clinical judgement is limited, categories 1 and 2 both mean the method can be used, and categories 3 and 4 both mean the method should not be used.

Appendix 2: WHO defined 'clinically important' bleeding patterns [211]

1. Amenorrhoea: no bleeding during the reference period
2. Infrequent bleeding: fewer than 2 bleeding episodes per 90 day period
3. Frequent bleeding: more than 4 episodes in a 90 day period
4. Irregular bleeding: between three and 5 episodes with a range of lengths of bleeding-free intervals exceeding 17 days
5. Prolonged bleeding: 1 or more bleeding episodes lasting 10 days or more
6. None of the above: a 'normal' bleeding pattern

Chapter 4: Adapting currently existing methods of contraception - The case of Depo-Provera®

Background

Depo-Provera®

Depo-Provera® (depot medroxyprogesterone acetate or DMPA®) is a long-acting, progestogen-only, injectable contraceptive licensed for use in over 106 countries [237].

DMPA was licensed as a contraceptive in the UK in the early 1960's. However, a number of countries (including the United States of America) were reluctant to approve it for many years. This reluctance revolved around the results of testing done on beagle dogs. The dogs were given large doses of the progestogen over a long period of time and developed tumours [238]. It was not until 1992, after the World Health Organisation (WHO) [239] published the results of a nine year study showing that users of this injectable method of contraception had no appreciable increase in the risk of breast and gynaecologic malignancies, that DMPA was finally approved for contraceptive use in the USA [239,240,241,242].

Pharmacology and metabolism

DMPA comprises one millilitre of sterile acqueous microcrystalline suspension containing 150mg of medroxyprogesterone acetate. Medroxyprogesterone is a synthetic progestin which is more potent than progesterone.

The long duration of action of DMPA (11-13 weeks) results from its slow absorption from the injection site. Immediately after injection of 150mg/ml, plasma levels have been found to be 1.7 +/- 0.3 nmol/l. Two weeks after injection, concentrations are 6.8 +/- 0.8 nmol/l but by 12 weeks concentrations have fallen to initial levels again. At lower doses, plasma levels of medroxyprogesterone acetate (MPA) appear to be directly related to the dose administered. Serum accumulation over time has not been demonstrated.

Medroxyprogesterone acetate (MPA) is eliminated via urinary and faecal excretion. At least 11 metabolites have been reported and all have been found to be excreted in the urine and some but not all are conjugated [243].

Mode of action

DMPA prevents pregnancy by two methods. Progestins diffuse freely into target cells in the female reproductive tract, mammary gland, and the pituitary and bind to the progesterone receptor. The primary mechanism of action is to prevent ovulation by acting at the level of the pituitary and hypothalamus. Once bound to the progesterone receptor, progestins decrease the pulse frequency of gonadatropin releasing hormone (GnRH) release from the hypothalamus. This decreases the release of follicle stimulating hormone (FSH) and luteinising hormone (LH) by the anterior pituitary. Decreased levels of FSH inhibit follicular development, preventing an increase in oestradiol levels. Progestogen negative feedback and the lack of oestrogen positive feedback on LH

release prevent an LH surge. The inhibition of follicular development and the absence of an LH surge suppress ovulation [244].

As with other progestin-only methods, DMPA also has an effect on the cervical mucus. The mucus becomes scanty and thick which makes it unfavourable for sperm penetration [245].

Soon after the first injection of DMPA, the endometrium starts to become thin and atrophic. Under these circumstances, DMPA could, theoretically, inhibit implantation. However, DMPA is highly effective in preventing ovulation and sperm penetration, as described above, and thus the role for inhibiting implantation is negligible. There are no data available to support prevention of implantation as a contraceptive action of DMPA [246].

Safety

The use of DMPA has been dogged by controversy about its safety from its start.

Cancer risks

Initial concerns were regarding gynaecologic malignancies. Evidence has shown concerns over ovarian, cervical and liver cancer to be unfounded as discussed previously and a large body of data exists to support that DMPA does not increase the overall risk of breast cancer either [247,248].

Bone mineral density

Skeletal impact in adult premenopausal women

More recently discussion has centred on the effect of DMPA on bone mineral density.
In the early 1990's Cundy et al (1991) reported that bone mineral density (BMD) was lower in current users of DMPA than in pre-menopausal non-users [249]. Since then,

many reports, most based on cross-sectional data, have assessed BMD with DMPA use. They have similarly observed reduced BMD in DMPA users [250,251,252,253]. This has led to concerns that this hormone-induced bone loss might translate into long-term increased fracture risk [207]. The Food and drugs administration (FDA) were sufficiently concerned as to add a black box label warning to DMPA packaging, in 2004, warning of the risk of significant bone loss and cautioning against long term use (>2yrs) [207].

Studies, of duration longer than one year, investigated the relationship between bone loss with time. Tang et al (2000) found that there was a suggestion that there was a retardation in bone loss with time [250] and Berenson et al (2004) found that in their two year study the bone loss was linear [253]. No study, to my knowledge, has found an acceleration in bone loss with time which is reassuring.

In 1994, Cundy et al (1994) went on to assess BMD during and after use of DMPA, and noted that declines in BMD with current use of DMPA were reversible on discontinuation [254]. Other studies have confirmed Cundy's 1994 observations [255,256,257,258,259] and a systematic review of ten observational studies conducted between 1996 and 2006 which examined BMD trends during and after use of DMPA concluded that declines in BMD associated with current use of DMPA are reversible [260].

Skeletal impact on adolescents

This is of particular interest as adolescence is the time of accumulation of peak bone mass [207].

A 2005 cohort study by Scholes et al (2005) following 170 adolescents (including 80 who used DMPA at baseline) found that recovery of BMD was complete within 12 months following DMPA discontinuation [261]. It was noted that the adjusted mean BMD values for discontinuers were at least as high as those of non-users for all anatomic

sites at 12 months and at all subsequent follow-up intervals. Of special note, BMD was ultimately observed to be higher in former than in never users of DMPA. Duration of DMPA use was not observed to impact the speed of BMD recovery following DMPA discontinuation.

A more recent study by Harel et al (2010) enrolled and prospectively followed 89 adolescent DMPA users aged 12-18 years. Subjects provided data for up to 240 weeks whilst receiving DMPA and for up to 300 weeks after DMPA cessation [262]. Bone mineral density was assessed at three sites using duel-energy X-ray absorptiometry (DEXA): lumber spine (LS), total hip (TH) and femoral neck (FN). At the time of their final DMPA injection (240 weeks) study participants had BMD declines from baseline of 2.7% (LS), 4.1% (TH) and 3.9% (FN) ($p<0.01$ at all three sites). Within 60 weeks of discontinuation LS BMD had returned to baseline, and 240 weeks after DMPA discontinuation, the mean LS BMD was 4.7% above baseline. Mean TH and FN BMD values recovered more slowly:240 weeks and 180 weeks, respectively, after the final DMPA injection [262]. This study substantiated the results of Scholes et al (2005) [261] that BMD loss in female adolescents is substantially or fully reversible in most girls after discontinuing DMPA.

In summary the evidence, to date, indicates that the decrease in BMD associated with current use of DMPA is reversible after discontinuation of DMPA with recovery to baseline BMD levels within one to two years [207,261,262]. This is reassuring given that peak bone mass is attained during adolescence and early adulthood and there has been much debate regarding use of DMPA in young women [261].

Skeletal impact on postmenopausal women

Concerns have been raised that long-term DMPA use may be a risk factor for postmenopausal osteoporosis and fractures. A second related concern has been that use up to menopause, without any time to let oestrogen levels to normalise may increase this risk [207]. A number of studies have been undertaken to explore this and findings to date

suggest that older reproductive age women do not appear to suffer a negative impact on their skeletal health [207,250,263,264,265,266,267].

Impact on risk of fractures

The data is very limited. A few studies have addressed fracture risk associated with DMPA use [207]. Case reports [268,269] have suggested an association between DMPA use and fractures in adult women, as have four published observational studies.

The first, by Lappe et al (2001) studied the impact of lifestyle factors on stress fractures in female army recruits [270]. Bone density was measured at baseline using quantitative ultrasound of the heel and reported as speed of sound (SOS) through bone. Women were followed for the eight weeks of basic training for occurrence of stress fractures. DMPA use at baseline was associated with higher risk of stress fracture during follow-up only among non-Hispanic Caucasian women (relative risk (RR) 1.71 95% CI 1.01-2.90). However the investigators reported that this association became non-significant when adjustment was made for SOS at baseline. They did not give the RR and 95% CI.

The second observational study, by Watson et al (2006), investigated the association between fracture incidence and DMPA use in women with developmental disabilities (DD) using a cross-sectional population based observational study [271]. They found a significant association. A considerable limitation to this study is that the investigators were not able to control for severity of disability. This is very likely to have been a confounder because women with more severe disabilities and therefore more risk factors for low bone mineral density, and hence increased risk of fracture, are also more likely to be given DMPA for menstrual suppression and/or contraception.

Vestergaard et al (2008) conducted a large national case-control study in Denmark (64,548 fracture cases: 193,641 controls) [272]. They assessed both use of DMPA and IUD. The authors found an increased risk of fractures in women who had ever used

DMPA (adjusted OR 1.44 95% CI 1.01-2.06) while IUD use appeared to be associated with a reduced fracture risk (OR 0.75 95% CI 0.64-0.87). However, the authors pointed out a number of limitations. Firstly, use of DMPA is very rare in Denmark so the numbers were very small (n=163) and hence the study will have been underpowered. Secondly, there was a lack of baseline data on BMI and smoking thus adequate control for confounding factors was not possible and hence causal inferences were not possible with respect to DMPA use. Similarly with regard to the finding that IUDs were protective against fracture. There was insufficient data on lifestyle factors and the results are more likely to have been due to the effect of residual confounding from lifestyle factors which had not been included in the study than to any real protective effect.

The most recent published observational study, another large population based British case-control study, by Meier et al (2010) (17,527 fracture cases: 70,130 controls and 11% vs 8% use of DMPA respectively) found current use to be associated with increased risk of fracture and that the risk increased with increasing DMPA exposure (OR 1.36, 95% CI 1.15-1.60 with 3-9 prescriptions; OR 1.54, 95% CI 1.33 – 1.78 with ≥10 prescriptions) [273]. Past DMPA use was also associated with significantly increased risk, again increasing with increased DMPA exposure (OR ranged from 1.17 to 1.30).

However, Isley and Kaunitz (2011) [207] have commented on their unpublished data using a retrospective cohort design to assess use of DMPA and risk of fracture in the same UK database. The results were presented at the Association of Reproductive Health Professionals annual meeting in Atlanta in 2010. Using the retrospective study design the investigators were able to take into consideration the timing of DMPA use and the timing of the fracture to compare fracture rates between DMPA users and users of other hormonal contraceptives (predominantly COCs). An increased risk of fracture was noted in DMPA users, even after just one DMPA injection. This led the authors to compare risk between women who were destined to become DMPA users to those who went on to use COC. They found that future DMPA users had an incident rate ratio of 1.28 (95% CI 1.07-1.53) for any type of fracture. They also compared fracture type and found that

DMPA users were more likely to have trauma-related appendicular fractures(OR 1.38, 95% CI 1.31 – 1.46) and less likely to have an axial fracture (OR 0.95 95% CI 0.74-1.23). The study also found that risk did not increase with increasing number of injections. These results, as Islay and Kaunitz (2011) [273] pointed out, are consistent with those of Vestergaard's Danish study [272], suggesting that DMPA users appear behaviourally different from those who use other methods of contraception. This unpublished study provides important evidence to support that the observed increase in fracture rate observed in DMPA users in some studies does not appear to be due to DMPA use and, accordingly, is not related to low BMD [273].

In summary, current understanding is that short-term skeletal changes observed during use of injections do not predict long-term impact on skeletal health [274]. The WHO recommends that women in the age range of 18 through 45 years can use DMPA without restriction (WHO MEC category 1). For women who are less than 18 or more than 45, the benefits of using DMPA generally outweigh the known or theoretical risks (WHO MEC category 2) [275,276] (Appendix 1 chapter 3).

Risk of Sexually transmitted infection and HIV-1 transmission

A key question for clinicians is whether an aetiological association exists between contraceptive methods and HIV [277]. Increased risk related to hormonal contraceptive users would be of importance to global public health because of the large numbers of women using such methods [278]. The WHO has called for high quality studies to assess the potential role of hormonal contraception in increased HIV-1 risk [279,280].

Morrison et al (2009) conducted a systematic review (studies published 1966 to 2008). They focussed on COC, DMPA and IUD. For DMPA users the data demonstrated no increase in HIV risk among women in the general public and was equivocal for women in high risk groups (e.g. sex workers) [277].

Earlier this year Heffron et al (2011) presented findings of their prospective cohort study of 3,790 heterosexual HIV-1 sero-discordant couples participating in two longitudinal studies of HIV-1 incidence in seven African countries at the meeting of the International AIDS society [278]. They found that use of hormonal contraception might increase the risk of acquiring HIV infection two-fold. Additionally they reported that HIV-infected women were two times more likely to transmit infection to their uninfected partner compared with those who did not use hormonal contraception. The contraceptive methods used by women in this analysis were primarily DMPA, with a smaller number using COC [278]. Whilst this study adds to the existing body of evidence the findings, similarly to previous studies are from observational studies, which may be biased by self-selection. Users of hormonal contraception may differ in important ways from non-users (for example, with regards to sexual behaviour and condom use). Additionally information on contraceptive use was collected by self-report, and overall, few women actually reported using hormonal contraception during the study period (at enrolment 10.8% of uninfected women and 13.5% of infected women were using DMPA). Another limitation of the study was that few new HIV infections were reported amongst contraceptive users [281].

As a result of the Heffron study findings and the public health concerns it raises, WHO is convening a Technical Consultation on 31st January -1st February to re-examine the totality of evidence on potential effects of hormonal contraception on HIV acquisition, disease progression, and infectivity/transmission to sexual partners [281]. In the meantime the consensus from systematic reviews is that the weight of evidence does not indicate that use of hormonal contraception increases the risk of HIV acquisition, transmission or disease progression among the general public, but may have an impact on women at high risk of HIV-infection, such as sex workers.

Abscess formation

As with any intramuscular injection, especially if not administered correctly there is a risk of abscess formation at the site of injection, which may require medical and/or surgical intervention [282].

Administration

The suspension must be given by deep intramuscular injection every 11 to 13 weeks [283]. This dosing schedule requires routine attendance at a health facility four times each year.

Efficacy

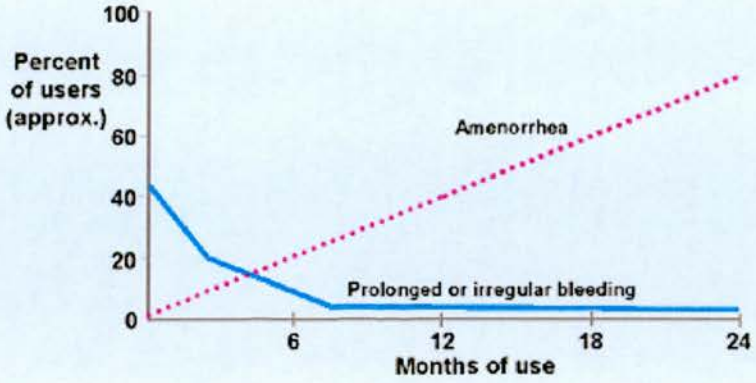
DMPA requires less user participation and as a consequence is highly efficacious [284]. Its failure rate is similar to that of the levenorgestrel releasing intra-uterine system (LNG-IUS, Mirena®) when used perfectly and it is the most effective method of reversible hormonal contraception that does not require an invasive procedure. Even with typical use it has a low rate of failure (0.3-3%) as compared to the oral contraceptive pill (0.3-8%) [74].

Side-effects

Menstrual irregularity

Most women who use DMPA experience menstrual changes. Some have prolonged or heavy bleeding, others have irregular bleeding and spotting throughout the cycle, but most become amenorrhoiec.

After the first DMPA injection over 40% of users experience irregular bleeding, spotting and prolonged episodes of bleeding (>10days) (figure 1) [285,286,287]. Following subsequent injections these bleeding changes become less common and after 12 months (4 injections) are relatively uncommon.



In contrast amenorrhoea and infrequent bleeding become more common with time (figure 1). By 12 months 50% of users are amenorrhoeic and by 24 months 80% are amenorrhoeic [285,286,287]. Opinions have been changing over time and whilst irregular bleeding, spotting and heavy bleeding are considered a side-effect amenorrhoea is more and more being considered a benefit [93].

Weight gain

As mentioned in the previous chapter weight gain is a major concern for contraceptive users. Concern can keep women from using contraception or can cause them to discontinue methods prematurely, which can lead to unintended pregnancy.

Weight fluctuation in women of reproductive age is common, regardless of hormonal contraception use and hence it is difficult to ascertain if the cause of weight gain is the contraceptive itself. Studies on weight gain during DMPA use report conflicting results [180].

Observational studies have reported variable effects of DMPA on body weight. Among adolescents and older women who used DMPA for up to one year, several studies reported non-significant changes in weight [288,289,290,291] while other studies, such as that by Clark et al (2005) [292], have found significant gains of 3-6kg

[292,293,294,295,296,297]. The study by Clark et al (2005) was the first large prospective study with a control comparison group evaluating long-term (30 months) weight change in women using DMPA. It compared longitudinal changes in weight, body fat, and ratio of central to peripheral fat mass among first-time DMPA users (n=178) to women using no hormonal contraception (n=145) [292]. The study demonstrated a change in body composition towards greater fatness and toward a central distribution of fat among DMPA users as compared to controls. It also demonstrated that higher levels of physical activity offered some protection against gains in fat mass.
The NICE guideline team when developing their guideline on LARC reported two studies on the effect of weight gain and DMPA [180]. Both reported weight gain in DMPA users. One multinational randomised controlled trial (1983) reported a mean weight gain of approximately 3kg in both DMPA (n=1587) and Norethisterone-enanthate (n=789) users at two years [298]. The second study by Mangan et al (2002) reported a significantly greater weight gain among overweight DMPA users (~6.2kg) compared with both 'normal' weight DMPA users (3.1kg) and overweight COC users (3.4kg) at one year [299].

A Cochrane database systematic review (2011) evaluating the potential association between progestin-only contraceptive use and changes in body weight has been published this year (2011). It identified 15 studies (those meeting their inclusion criteria) examining the effect of progestin-only contraceptives (POC) on weight [300]. Eleven of the studies examined DMPA. The review found that only two of the eleven studies showed differences in weight or body composition change for DMPA compared to no hormonal method. One small study (n=33) by Bonny et al (2009) found that adolescents using DMPA had a greater increase in body fat (%) versus a group using no hormonal method (mean difference 11.00; 95%CI 2.65-19.36). The DMPA group also had a greater decrease in lean body mass (%) (mean difference -4.00; 95%CI -6.93 to -1.07) [301]. In the second study by Pantoja et al (2010) (n=758) [302,303] comparing DMPA to copper intrauterine device (IUD), there was a small but significant difference in mean weight change (kg) for all three years of the study (mean difference 2.28, 2.71, 3.17

respectively). The remaining nine studies identified showed no significant weight change with use of DMPA. Unfortunately the authors were unable to conduct a meta-analysis due to the range of contraceptive methods examined and different reporting for weight change.

The data, therefore, remains inconclusive. It suggests that responses are individualised, and while weight gain is observed in a portion of patients, patients may also lose or maintain weight.

Several factors are thought to influence the differences in weight change observed in studies of hormonal contraceptives. These include demographics of the treatment population (e.g. certain racial or ethnic groups such as African Americans are more susceptible to weight gain), baseline BMI, metabolic differences between patients and the normal gains associated with aging [304].

Considering the conflicting data, the current recommendation, by NICE, is to inform women that DMPA may be associated with an increase of 2-3kg in weight over one year and counsel appropriately [180].

Delayed return to fertility

Re-establishment of ovulation and menstruation after injection of DMPA is delayed and difficult to predict. The delay is due to persistence of MPA in the circulation, because microcrystals in the injected depot may sometimes dissolve very slowly. In a large US study of women who discontinued DMPA use to try for pregnancy (response rate 61%), 68% of women who became pregnant conceived within twelve months, 83% within 15 months and 93% within 18 months of the last injection [282]. This final pregnancy rate at 18 months is within the normal reference range. The median time to conception for those who do conceive is ten months after the last injection [282]. There is no evidence that DMPA causes permanent impairment of fertility [283].

Benefits

Depo-Provera ® is an efficacious, safe and convenient method of contraception. Additionally a number of non-contraceptive health benefits have been observed among women who use DMPA.

Depo-Provera ® has demonstrated an 80% risk reduction for endometrial cancer, and this may extend to beyond its duration of use [305].

A multi-centre hospital based case control study of 3619 women (910 cases and 2709 controls) found that DMPA use has a strong duration dependent protective effect against uterine leiomyomas [306] and reduced the need for hysterectomy in women with uterine leiomyomas [307]. Cases were all newly diagnosed patients with pathologically proven diagnoses of uterine leiomyomas, admitted to one of eight hospitals in three regions of Thailand from January 1991 to June 1993. Each case was matched to three controls by sex, age within five years and date of admission within three months [306].

The decreased frequency of menstrual cycles may in itself be protective against some gynaecological malignancies such as ovarian cancer where the evidence base suggests that frequent, repetitive ovulatory cycles may be associated with an increased risk [95,308,309,310].

Case reports have described a reduction or total absence in painful crises in patients with Sickle cell anaemia during treatment with DMPA [311,312] and also a reduction in seizures in epileptic patients using DMPA [313]. Data exists to indicate that DMPA has intrinsic anticonvulsant activity [307,314,315].

Women with endometriosis have found DMPA to effectively relieve pelvic pain [316], as have women post hysterectomy who have suffered pelvic pain and dyspareunia of ovarian origin [307].

The amenorrhea experienced by the majority of users is often seen as a benefit because it improves conditions such as menorrhagia, dysmenorrhea and iron deficiency anaemia [308,317,318,319]. This particular effect is particularly beneficial to those with menstrual cycle related disorders, such as premenstrual syndrome or migraine headaches [320,321]. Amenorrheoa is also advantageous for mentally and physically handicapped women who have difficulties with menstrual hygiene [322].

Additionally, progestogen-only contraceptives such as DMPA have been recommended as appropriate contraceptive choices for women in whom oestrogen is contra-indicated [307,323].

Economics

The actual cost of the injection itself is low (£5 per dose in the UK). On top of this however is the cost of running the service that the woman attends to receive her injection. The dosing schedule as mentioned requires attendance quarterly at a health facility. In contrast, the WHO recommends that women using oral contraception need to be seen by a health professional only once each year [324]. Cost data in 2005 found that first year cost of DMPA® was £144 and subsequent years were £99/year (table 1). Thus, although an inexpensive method of contraception per se, Depo-Provera® involves increased costs to health services when compared with all other available methods [222,325]. It also creates greater cost and inconvenience for the user.

Table 1: Cost data for Depo-Provera® [180]

Injectable method cost	Baseline value	Cost component and basic assumptions	
Annual method cost		Component	Cost
First year	£144	Ingredient cost (DMPA®)	£5.01 per dose [219]
Following years	£99	Initial GP consultation (first year), 20 mins	£44.80
3 year cost	£342	Consultation for injection every 12 weeks, 8 min	£17.92
5 year cost	£540		
8 year cost	£837	Resource use based on GDG consensus; GP unit cost = £2.24 per surgery/clinic minute [220]	

Use worldwide

With time Depo Provera® has become an increasingly popular option for hormonal contraception among women. In 2007, worldwide, 3.7% of married women surveyed were using injectables[3]/implant as their chosen method of contraception [223]. This is projected to reach nearly 40 million by 2015 – more than double that in 1995 [237,326].

The use of DMPA® varies globally. In developing countries the prevalence of injectable/implant use in 2007 was 4.2% [223]. Data from Demographic and Health Surveys undertaken in 46 developing countries were reviewed, in 2009, by Family Health International. Levels of contraceptive use varied substantially among countries with the vast majority documenting relatively low levels of injectable use among married women of reproductive age (MWRA) [327]. 24 countries reported prevalence rates of injectable use among MWRA to be less than 5%. Despite these low usage rates, in ten of these countries injectable use accounts for between 23% and 46% of modern method use. Three of the 46 countries, Indonesia, Namibia and South Africa supported much higher use with more than one in five MWRA using injectable contraception in each country (28%, 22% and 28% respectively). In these countries, injectable use accounted for between 41 and 49% of modern method use. In the remaining 19 countries, the prevalence of injectable use ranged from 18% in Malawi to 5% in Ghana [327]. In Sub-saharan Africa, injectables are the most popular contraceptive method chosen by more than one out of every three women using modern contraception [328].

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[3] Recent data often refers to injectables. Injectables comprise DMPA, Norethisterone enanthat (NET-EN) and Lunelle. The first two are progestin-only contraceptives and the latter is a combined oestrogen/progesterone contraceptive. DMPA is the most widely used injectable. Thus where data refers to injectables it includes use of all three methods. Where data is available for DMPA alone I have noted that. Additionally data tends to combine results for injectable use and implant use hence the use of injectable/implant..

In contrast, DMPA is used by only a small minority of women in more developed countries (1.3%) [223]. According to the National Survey of Family Life and Growth (2010), only 3% of women in the United States in 2006-8 were using DMPA [329]. Even in the United Kingdom where use is the highest among the more developed countries [223], whilst 9% of 25-29 year olds were using DMPA in 2008/9, for women aged 16-49 the figures were identical to the US with only 3% using DMPA [224].

Reasons for discounting/discontinuing DMPA

The percentage of women who have discontinued injectable use by the end of the first year has been estimated by Trussell et al (1999) to be 30% [330]. While consideration of long-term safety may be a part of the reason why DMPA is not as popular with women as other methods, women are often more concerned about the relatively immediate effects of contraceptives such as potential changes in menstrual cycle, body weight and mood. These concerns can lead to a reluctance to start treatment or to discontinue it prematurely [331,332] and may partly explain why uptake is low in some countries of the world.

Menstrual irregularity

Menstrual disturbances are the major reason for discontinuation of DMPA [331,333,334,335,336,337]. A telephone survey by Skeggs et al (1997), of 1,864 New Zealand women, that included 252 DMPA users, found that menstrual disturbances were cited by 20% as the reason for discontinuation. These changes were equally distributed between irregular bleeding (6.8%), heavy bleeding (6.8%) and amenorrhea (6.8%) [336]. In a one year American study of 402, new DMPA users, by Davidson et al (1997), those who continued and those who discontinued reported similar menstrual changes [337].

However, perception of what menstrual changes constitute a side effect are changing and a number of more recent surveys have found that many women would prefer a contraceptive option associated with less frequent or no menses [338,339,340].

Weight gain

Another frequently given reason for discontinuation is weight gain. The telephone survey of 252 DMPA users, mentioned above, found that 12% (n=37) of women cited weight gain as their reason for discontinuation [336].

Women of all ages are concerned with weight gain. This is with good reason. Thirty years ago international nutritionists were focussed on childhood malnutrition. Today the WHO finds itself needing to deal with the new pandemic of obesity and its accompanying non-communicable diseases (NCDs) while the challenge of childhood malnutrition has far from disappeared [341]. Thus it is not surprising that weight gain is often cited by women as the reason for discontinuing hormonal contraception. However, as discussed earlier, the evidence is conflicting as to whether DMPA causes weight gain when compared to non-hormonal contraceptives.

Mood changes

Another common concern reported by many women is the effect on mood. The majority of published reports indicate that DMPA does not cause depressive symptoms [342,343,344,345,346]. Only one population based study, Civic et al (2000), of women enrolled in a health maintenance organisation (HMO) reported an association between DMPA and depressive symptoms but could not establish a causal relationship [347].

Needle phobia

A potential deterrent specific to DMPA is that it is administered via an injection. Needle phobia affects at least 10% of the population [348]. While no research exists on uptake of DMPA by needle phobics, research has shown that a fear of injections can interfere

with uptake of travel vaccines and also initiation of insulin therapy in diabetics [349,350,351]. Parallel conclusions could therefore be drawn for DMPA.

Improving choice and use of DMPA

Depo-Provera ® is an efficacious, safe and convenient method of contraception. The reasons for its appeal are multiple as described above. However, as stated, the use of DMPA has been dogged by controversy about its safety from its start.

Originally the concerns were about cancer and then effects on bone mineral density which led to black box labelling by the FDA in 2004 in the States [274]. Research into these areas has been reassuring with regard to its safety [247,248,274,276].

More recently the reasons for, not choosing to use or, discontinuing DMPA have been concerns surrounding weight gain and menstrual disturbance. The concerns surrounding weight gain appear to be unfounded [300] though meta-analysis of studies is still outstanding and menstrual irregularity, though troublesome is often temporary and can often be improved by short courses of oestrogen or shorter injection intervals [352] or tolerated in the short term if appropriately counselled [79].

Currently concerns surround the potential effects of hormonal contraception on HIV-1 acquisition, progression and transmission, and the WHO are convening a Technical Consultation in January 2012 to re-examine the evidence [281].

To improve use of DMPA, as discussed previously in chapter 1, three strategies have been identified: improve counselling; develop methods which require a low level of compliant behaviour by the user and improve the quality of life of users by minimising the negative side-effects and maximising non contraceptive benefits [79].

Counselling

As surmised by Bigrigg et al (1999), perhaps the most important issue surrounding the use of DMPA is that of patient information [352]. The method has had a particularly bad image, which naturally makes potential users anxious and subject to misinformation from poorly informed or biased sources [352,353]. A study by Glasier et al (2007), in the UK, exploring the changes in prescribing by health professionals in response to the black box labelling of DMPA, revealed that there is a need to provide clear, balanced information on new findings about adverse effects of contraceptives as otherwise it risks increasing rates of unintended pregnancy [353]. Additionally it has been found that users are more tolerant of side-effects and specifically menstrual irregularity if counselled appropriately prior to commencing DMPA [354].

Develop methods which require low user compliance

DMPA is instantly effective and only requires to be administered every three months. It is considered a long-term reversible contraceptive and as such fits the criteria for being a method requiring low compliance. If it were possible to attend less frequently for administration then this would likely further increase continuation rates.

Minimise side-effects and maximise benefits

The need to visit a health professional four times a year is considered by some as an inconvenience and hence a side-effect. It has also been found that decreasing the need to attend a health professional is linked to reducing unintended pregnancy [355]. A study by Foster et al (2011) explored how the number of packages of oral contraceptive pills dispensed related to subsequent pregnancies. They found that women who received a one-year supply of pills were less likely to have an unintended pregnancy (1.2% vs 3.3% of women receiving only three packets of OCP and 2.9% of women receiving only one packet of OCP). They also found a 46% reduction in the odds of an abortion (95% CI 0.32 – 0.93), controlling for age, race, and previous pill use. They concluded that making OCP more accessible may reduce the incidence of unintended pregnancy [355]. Thus in

keeping with this if it were possible for DMPA users to attend health services less frequently then it is likely that DMPA would potentially be more attractive to prospective users as well as current users.

In response to this a new delivery system with a new formulation of DMPA has been developed. Subcutaneous DMPA has the potential for self-administration and thus the potential for reducing visits to a health professional to annually.

Subcutaneous Depo-Provera® (DMPA-SC)

Formulation and administration

With hormonal contraception, the goal is to provide high contraceptive efficacy while using the lowest possible steroidal dose. This has resulted in progressive lowering of the standard content of oral contraceptives since they became available. A new micronised preparation of DMPA has recently been developed [356] and is now licensed for use in the UK. It is pharmacologically unique. A dose of 104 mg in 0.65ml of aqueous microcrystalline suspension (16% weight/volume) [357] is administered subcutaneously into the anterior thigh or abdomen every twelve weeks. The slower rate of absorption observed with DMPA-SC relative to the IM formulation allows for a lower peak serum medroxyprogesterone (MPA) concentration and a long duration of effect [357]

Efficacy and safety

DMPA-SC provides efficacy, safety and immediacy of onset equivalent to Depo-Provera ® intramuscular (IM) injection. Two large, open-label, Phase 3 studies assessed the one year contraceptive efficacy and safety with DMPA-SC administered every three months [356]. No pregnancies were reported in either study, which included a total of 16,023

woman-cycles of exposure to DMPA-SC and substantial numbers of overweight or obese women. The safety profile was found to be similar to that reported with Depo-Provera [356]. Because the 104 mg dose was chosen on the basis of studies in Caucasian women [357], there was concern that this dose may have variable efficacy in women of other racial groups, particularly Asian women (WHO). A single-centre, single-dose open-label study was performed, by Toh et al (2004), in Singapore in Asian women aged between 16 and 40 years. DMPA-SC was found to provide effective suppression of ovulation for at least 91 days in Asian women providing reassurance that ethnicity has no effect on the medroxyprogesterone acetate profile [358].

Side-effects

Two large phase 3 open label non-comparator multi-centre contraceptive trials have detailed that the majority of women experienced a change in menstrual pattern with DMPA-SC such as irregular spotting or bleeding, prolonged spotting or bleeding, and heavy bleeding during the first month following the first injection [356]. As women continued to use DMPA-SC, fewer experienced irregular bleeding and more experienced amenorrhea. By month six 38% of women were amenorrhoeic and this increased to 55% by month 12 [356]. Analysis of data from three studies by Arias et al (2006) found rates of amenorrhoea to be (52-64% across studies) at month 12 and 71% at 24 months [359]. These rates are comparable to those originally reported for DMPA-IM. Changes in bleeding pattern showed no consistent difference according to age or BMI but did indicate that incidence of amenorrhoea increases over time [359,360].

Similar to DMPA intramuscular the median time to ovulation was thirty weeks after the last injection and the cumulative rate of return to ovulation at 12 months was 97.4% [361].

A side effect encountered with the new preparation was that of injection site reactions. In five clinical studies carried out by Pfizer Inc. involving 2,325 women (282 treated for up to six months, 1,780 treated for up to one year and 263 treated for up to two years), 5% of women reported injection site reactions, and 1% had persistent skin changes, described as small areas of induration or atrophy [357].

The remainder of the side-effect profile is identical to that of the intramuscular formulation [356,362].

Additional benefits

DMPA-SC is licensed for management of endometriosis-associated pain. A multi-centre, evaluator-blinded, comparator-controlled trial, by Crosignani et al (2006), has demonstrated a reduction in endometriosis-associated pain in women with signs and symptoms of endometriosis [363]. The study randomised 300 women with laparoscopically diagnosed endometriosis to one of three treatment options; three monthly DMPA-SC, monthly leupromide (3.75mg) or three monthly leupromide (11.25mg). Each study assessed reduction in endometriosis-associated pain over six months of treatment and recurrence of symptoms for 12 months post-treatment. It also assessed changes in bone mineral density and productivity at six and 18 months. DMPA-SC was found to reduce endometriosis-associated pain as effectively as leupromide and improved productivity while causing significantly less decline in BMD [363,364].

Another benefit of DMPA-SC is that it can be self-administered, thus enabling DMPA-SC to be more competitively aligned with Implanon® and Mirena® in terms of cost-effectiveness. Self-administration also allows for greater convenience to the user.

Economics

As mentioned just above subcutaneous depo-provera can be self-administered.
The cost of the injection is low. If women are happy to self-administer then once trained at delivering her own injection a woman need only attend a health professional once a year rather than the four episodes of contact required with DMPA-IM. This then allows for DMPA-SC to be more cost-effective than DMPA-IM users and to be more competitively aligned with Implanon® and Mirena® in terms of cost-effectiveness.

Objective

- Assess whether adapting the injectable contraceptive, Depo-Provera® to a form where self-administration is feasible would increase acceptability and improve uptake

In anticipation of DMPA-SC becoming available in the UK, we undertook two questionnaire surveys (Appendices 1 and 2) to determine whether women currently using Depo-Provera® would want to self-administer, and whether the opportunity to do so would make DMPA a more attractive method to women who are not currently using it.

The surveys also allowed us to investigate reasons for not choosing to use DMPA or for discontinuing DMPA. It also gave an opportunity to ask discontinuers of the intramuscular method whether they would be interested in the subcutaneous formulation and to then research this further.

Subjects and methods

During December 2003, a self-administered questionnaire (Appendix A/Questionnaire 1) was offered to women attending a large family planning clinic in Edinburgh for repeat DMPA injection. The questionnaire was attached to every packet of DMPA, thus

ensuring that it was offered to everyone. Women were asked if, after appropriate instruction, they would be interested in giving themselves the 3 monthly injections. If they responded positively, they were asked whether, and how, they would prefer to be reminded of the date when their injection was due. Women who were not interested in self-administering Depo-Provera® were asked to choose one or more reason, from a list of possible reasons, why they would not want to self-administer.

For 2 weeks during the spring of 2004, a second self-administered questionnaire was offered to every woman attending the same Edinburgh family planning clinic (Appendix B/Questionnaire 2). Women were asked about current and ever use of all available methods of contraception. Those who had used but discontinued DMPA in the past were asked to choose from a list their reasons for discontinuation. Women who had never used it, but had considered it, were also asked to choose from a similar list what had deterred them from trying it.

All women were asked whether the opportunity to self-administer Depo-Provera®, and thereby to attend clinics only once a year, would make them reconsider using the method.

Data from the two questionnaires were entered into a SPSS dataset and descriptive analysis was carried out.

Results

One-hundred seventy-six women currently using Depo-Provera® completed Questionnaire 1. The response rate was 100%. Sixty-seven percent of women (118) said they would like to self-administer DMPA. Of these, 89% felt that they would need reminding of the date of injection. Thirty-three percent of women felt that being told the dates for the next three injections at the time of their annual review would suffice; 30% of them preferred to receive a reminder letter, and 25% a text message, just prior to the date of the next injection.

Of the 58 women (33%) who did not wish to self-administer DMPA, the most common reason was a dislike or fear of needles (62%), 43% were concerned that they would administer the contraceptive incorrectly, while 33% felt it was important to see a doctor every 3 months. Twelve percent of women were disinclined to self-administer because they were worried that they might forget.

Three-hundred seventy copies of Questionnaire 2 were distributed and 323 were returned completed (87%). The main method of contraception being used by the women who completed Questionnaire 2 is shown in Table 2. Ten women were using Depo-Provera® and seven of them (70%) expressed an interest in self-administration. Two hundred sixty-five women had never used Depo-Provera®; their reasons for not choosing the method are shown in Table 3. One hundred sixty-five of them answered the question about self-administration. Sixty-one percent said they would prefer to attend the clinic less often for supplies of contraception. Twenty-six percent (43 women) said they would seriously consider using Depo-Provera® if self-administration was possible.

Table 2: Primary contraceptive method used by women at time of completing Questionnaire 2

Primary method of contraception	**Number of respondents (n=323)**	**% Respondents**
Combined oral contraceptive pill	142[a]	44
Barrier methods	63	20
Intrauterine system/ intrauterine device	34	11
Progesterone-only pill	24[b]	7
Contraceptive implant	20	6
Depo-Provera® (DMPA)	10	3
No method	8	2
Withdrawal	7[c]	2
Natural family planning	5[d]	1
Vasectomy/sterilisation	3	1
Other	4	1
No response	3	1

a. 20 of the 142 women were using barrier protection as well
b. 2 of the 24 women were using barrier protection as well
c. 3 of the 7 women were using barrier protection as well intermittently
d. 2 of the 5 women were using barrier protection as well intermittently

Note: 27 women in total used barrier protection as well as another method of contraception.

Table 3: Reasons for discontinuing or not choosing Depo-Provera® among women completing Questionnaire 2

Reason	Discontinuing (48 women) % women	Not choosing (70 women) % women
Change in bleeding pattern:		
Irregular	40	26
Amenorrhoea	19	30
Weight gain	35	41
Mood swings	27	QNA*
Headaches	10	QNA*
12 weekly visits	10	11
Painful injection/ fear of needles	6	20
Wish for return of fertility/ not wanting a delay in resumption of fertility	6	20
Not being able to stop the contraception immediately	QNA*	23
Adverse opinions from friends/ family	QNA*	19

* QNA = question not asked

Forty-eight women (15%) had used DMPA in the past but had discontinued the method for reasons shown in Table 2. Thirty-four of 44 women (77%) who answered were interested in a method which allowed them to reduce the number of times they attended a clinic for contraception. Eighteen of 45 women (40%) who answered the relevant question said they would be interested in using DMPA again if it were available for self-administration.4.

Discussion

Depo-Provera® is an important method of contraception. Anecdote suggests that in the United Kingdom it is increasingly popular among young women and among women of all ages who like the convenience of amenorrhea. In the United States, the fall in teenage pregnancy rates has been attributed to increased use of Depo-Provera® [100]. The need to attend a health professional every 3 months simply for a single injection is a clear disadvantage of the method.

In our survey, two out of three women currently using Depo-Provera® expressed a theoretical interest in self-administration. It is, of course, very likely that a number of them, when offered the *real* option of self-administration would not take up the offer, and that more would find that they were unable or unwilling to give themselves an injection when the time came for it. Self-injected medication is the norm for diabetics and for some people with migraine or arthritis or undergoing infertility treatment. In most cases, the injections are self-administered daily or at least twice weekly. It is possible that women would never gain sufficient practice to become confident in self-administering an injection only four times each year. In a trial of self-administration of the once/monthly combined injectable contraceptive undertaken in Brazil, 14% refused to participate at the onset and another 31% declined after being trained to self-administer using oranges [365]. At the end of the study (three injections in total), more than 80% of women had been successful in giving their own injections and only 50% expressed a preference to continue self-administration. Just how many women, who in our survey said they would like to self-administer Depo-Provera®, would do so in reality is the subject of a future study.

It is highly likely that many health care professionals would be skeptical about women's ability to self-administer Depo-Provera® and, particularly, to do so at the correct time. Presently, if an injection is more than 2 weeks late, it is recommended that the next injection is withheld until pregnancy can be reasonably excluded [324]. If women were

given their own injections, it is possible that there would be a greater chance that an injection would be given late and possibly given when conception had already occurred. In our clinic, we do not currently send reminders to women using DMPA but simply inform them when they attend for their injection, when the next one is due. All are clearly informed when they start to use the method, that the injection interval is 12 weeks. Since we do not presently remind them to attend the clinic for their injection, only the desire to avoid late injection when pregnancy had already occurred should stimulate us to have a system in place to remind them to *self-inject*. In a randomized trial designed to test whether an intensive reminder system would improve compliance among 250 women using Depo-Provera® in the United States [366], intensive reminders did not improve continuation rates. Women receiving either mailed or telephone reminders were no less likely to return to the clinic on time than women given an appointment at the time of the first injection. One could argue that the need to make arrangements (including travel, and possibly taking time off work) to attend a clinic for injections would, in fact, be more likely to lead to mistimed injections compared with the ease of having the drug at home.

We were somewhat surprised that the ability to self-administer the injection, and thereby enable women to reduce the number of clinic visits, might increase the acceptability of Depo-Provera®, both to women who have never used it and to those who have tried but discontinued the method. It is likely, however, that this is an overestimate since the reasons for discontinuation of DMPA generally related to the side effects (Table 3) and not to the need to attend a health professional four times each year.

In conclusion, the advent of a form of Depo-Provera® which would allow women to self-administer is likely to be a benefit to perhaps as many as half of the women choosing this method. Our survey also suggests that use of injectable contraception may increase were self-administration possible. We have discussed the concept in our clinic and suggest that women who express a wish to self-administer Depo-Provera® should be given the appropriate training allowing an annual clinic visit with three injections

supplied for home use. The success of such a scheme, and the health economics involved, will need to be tested scientifically.

Appendix A. Questionnaire 1

The jag at home!!!

Imagine — no more visits to the doctors every 11 weeks for your jag! Just once a year!!

Interested? Keep on reading.

Soon the Depo-Provera injection will be available for you to give to yourself.

It is safe, easy and less painful.

Unlike today when it has to go deep into your muscle, the new way will be to inject it just under the skin (similarly to what diabetics do 3 times every day). We would supervise you the first time and if you were happy, you could take 3 injections home with you. Your next routine appointment would be a year later— just like women on the pill!!

Please answer the following 2 questions.

Your answers are strictly confidential and anonymous.

1. Would you want to give yourself your injection?

Yes___ (please go to question 2)

No ___ (please go to question 3)

2. How would you want to be reminded that it was time for your next injection? (Please pick ONE only)

Do not want any reminders __ Letter __

Told the next 3 dates in advance when I attended __ Phonecall __

Text message __ Email __

3. Why not?

Worried about giving it correctly __

Prefer to see the doctor every 11 weeks __

Would forget __

Just do not want to give it to myself __

Other reason — please tell us _____

Thank you for completing the questionnaire. Please hand it back to reception.

Appendix B. Questionnaire 2

Depo-provera contraception
- your views on a new version that can be administered at home

Depo-provera ('the jag') is a form of hormonal contraception that presently is administered 3-monthly by intra-muscular injection. This has to be given by a doctor or nurse, and so requires a visit to the health centre or family planning clinic.

It is hoped that in the near future, a new form of depo-provera injection will be available that involves an injection just under the skin (similar to the injection that diabetics give themselves) instead of the current form which is injected into the thigh muscle. The first injection would be supervised, and if you were happy about it you would take the next 3 injections home with you.

We are carrying out this survey to find out what women **who are not currently using depo-provera** think of this development. Your answers are strictly confidential and it would be appreciated if you would answer the questions below by ticking the boxes beside the most appropriate answers. Many thanks.

Q1. What method of contraception are you currently using?

- ☐ *combined oral contraceptive pill*
- ☐ *condoms(male/female)*
- ☐ *IUD / IUS (coil)*
- ☐ *diaphragm / cap*
- ☐ *other (please specify)* .....
- ☐ *progesterone only pill*
- ☐ *contraceptive implant (e.g Implanon)*
- ☐ *natural family planning / rhythmn method*
- ☐ *withdrawal*

Q2. Have you ever used the 3-monthly depot injections?

yes ☐ ☐ *no*

please go to Q3 ↙ ↘ *please go to Q4*

Q3. What was/were you reason/s for stopping it?

- ☐ *painful injection*
- ☐ *having to visit doctor/clinic every 3 months*
- ☐ *weight gain*
- ☐ *mood swings*
- ☐ *headaches*
- ☐ *wishing to return to fertility/ get pregnant*
- ☐ *irregular bleeding*
- ☐ *absent periods*
- ☐ *other (please specify)* .....
  .....

Q4. Have you ever considered using depot as a contraceptive method?

yes ☐ ☐ *no* **(please go to Q6 and Q7)**

please go to Q5 ↓

Q5. What was/were you reason/s for not using it?

- ☐ *fear of needles/ injections*
- ☐ *fear of weight gain*
- ☐ *having to attend the doctor/clinic every 3 months for the injection*
- ☐ *fear of irregular bleeding*
- ☐ *prefer to have a period every month*
- ☐ *delay in return to fertility*
- ☐ *not being able to stop the contraception straight away (it is with you for 3 months)*
- ☐ *friends/relatives have put me off the idea*
- ☐ *other (please specify*.....
  .....

Q6. Would you regard it an advantage if you could reduce the number of visits to your doctor/ clinic for contraception?

yes ☐ ☐ *no*

Q7. Would you consider depo-provera if you were able to give the injection to yourself?

yes ☐ ☐ *no*

PLEASE SEAL YOUR COMPLETED QUESTIONNAIRE IN THE ENVELOPE PROVIDED AND HAND IT IN AT RECEPTION. *THANK YOU.*

Chapter 5: Development of novel methods of contraception – The case of mifepristone

Background

Investment in longer term work is needed to discover and develop new methods of contraception that do not cause systemic side-effects, can be used on demand, and do not require partner participation or knowledge [7,81]. Fifty years have passed since the first clinical trials in Puerto Rico demonstrated that a daily pill containing ethinyl estradiol and norethynodrel was a highly effective contraceptive [367]. Since the introduction of the combined oral contraceptive pill advances in hormonal contraception to date have been limited to variations on the theme of oestrogen in combination with progestogen or progestogen alone [6,368]. Alterations to pill formulations, new progestogens and new delivery systems have increased choice, and some of these have been explored in earlier chapters [231,369]. However, as demonstrated, whilst these advances have improved acceptability, side-effects and risks have remained essentially unchanged and women continue to dislike or discontinue methods, often moving to less effective methods or no method [231,353,369,370,371,372,373]. Oestrogen free methods have the advantage of fewer serious risks such as breast cancer or venous thromboembolism but are associated with unpredictable vaginal bleeding. Unpredictable vaginal bleeding is often cited as the commonest reason for discontinuation [214,373,374,375,376,377,378]. Even the regular pattern of bleeding characteristic of the combined pill is considered undesirable by some

women [95]. When oral contraceptives were first marketed it was assumed that women would prefer to have a monthly cycle because it would be perceived as more natural [379]. More recently, it has been shown that many women, regardless of age, prefer to have either predictable bleeding less often than once a month or not to bleed at all [93,94,95]. Hence a novel oestrogen-free pill that does not cause irregular vaginal bleeding and reproducibly induces amenorrhoea in a high proportion of women should prove popular. Antiprogesterones offer the possibility of such a new method of contraception.

Definition of anti-progesterones

Anti-progesterones are compounds that bind with high affinity to progesterone receptors (PR) but which antagonise the action of endogenous progesterone [380].

Basis for exploration

Since progesterone is essential for the establishment and maintenance of pregnancy, it follows that compounds which block its synthesis or action will have anti-fertility properties.

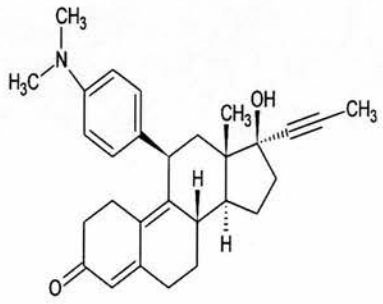
Mifepristone

Mifepristone (RU486) is a synthetic C-19 steroid which is a potent antagonist of both progesterone and glucocorticoid hormones [381]. It was first demonstrated to show both contraceptive and abortifacient properties in the early 1980's [380,382,383,384,385]. Despite this, for many years there was little progress in developing its contraceptive potential. This was not due to scientific reasons but related to the political and religious controversy surrounding RU486, or as it has more commonly become known, 'the abortion pill'. In the last ten years, however there has been a significant amount of research undertaken into selective progesterone-receptor modulators (SPRM) and specifically into their use for emergency contraception.

Structure of mifepristone

Mifepristone is a 19-norsteroid that lacks the C19-methyl group of natural progesterone and glucocorticoids. Its chemical structure has two distinctive differences from the steroidal skeleton, a 4-dimethylamino-phenyl group substituted at the 11beta position, and a 1-propynyl-chain substitution at the 17alpha position (figure 1). The 17alpha substitution is responsible for promoting higher binding affinity to the progesterone receptor and the substitution at the 11beta position is likely to be responsible for mifepristone's antagonistic action, by inducing or stabilising a biologically inert receptor conformation [386]. The chemical structures of other anti-progestins are similar to that of mifepristone.

Figure 1: Structure of mifepristone



Mode of action

Previously it was thought that the mode of action for all anti-progestogens was similar and that they were purely antagonists of progesterone [380]. However, we now know that neither of these presuppositions is true. The antagonist receptor complex is not inert and each antagonist induces a unique set of transcriptional events [387]. Also, many have both agonist and antagonist properties. The effect produced is dependent on the hormonal environment and the relative proportion of progesterone receptor alpha (PRa) and progesterone receptor beta (PRß) [387].

Pharmacology and metabolism

Absorption is rapid (70%) through the gut, and peak levels (micromolar concentrations) appear in the bloodstream within 1-2 hours post oral administration. First-pass metabolism however, reduces bioavailability to 40% of the oral dose in humans [382].

The pharmacokinetics follow two distinctive patterns which are dependent on dose. With doses lower than 50mg there is an open two-compartment model of pharmacokinetics with a peak plasma concentration of approximately 1.9 micromol/l at 1-2h and a half-life of over 27h. Serum drug concentrations increase progressively as dosage increases from 50mg to 100mg, but no further proportional increases occur after 100mg. At these higher doses (>100mg) there is an initial redistribution phase of up to 10h followed by zero-order kinetics up to >24 h [388]. There is no cumulative effect on plasma concentration after repeated doses.

Metabolism of mifepristone is extensively by demethylation and hydroxylation leading to three active metabolites, which are themselves biologically active. These can be measured within an hour of oral intake and their plasma levels increase in a dose-dependent manner [389]. They have a high relative binding affinity to progesterone receptors (PR) and their long circulating half-life suggests a possible contribution to the

overall antiprogesterone action of mifepristone [390,391]. The demethylated metabolites are then further hydroxylated or acetylated [392].

Un-metabolised drug can be detected for up to 10 days after oral intake, and the higher doses are associated with plasma levels in the micromolar range for over 2 days [393]. This relatively long half-life (20-25h) and the unusual metabolic pattern of mifepristone in humans appear to be due to plasma orosomucoid binding sites at doses above 100mg [390]. Plasma clearance rate of mifepristone in humans is 0.02 l/h/kg body weight [394]. The major excretory path is faecal, with less than 10% being renally excreted [392,395].

Safety of mifepristone

Roussel Uclaf conducted a comprehensive toxicology program in the mid-1980's demonstrating the safety of the molecule and allowing its use in humans [396].

Short-term administration of mifepristone appears to be safe. A single dose is eliminated within 6-7 days of administration [397]. Administering 200mg daily to healthy male volunteers leads to a hormonally antiglucocorticoid state and reversible cortisol overproduction, with preservation of pituitary and adrenocortical reserves, but no clinical symptoms of peripheral cortisol deprivation [398]. A different study has shown that this disinhibition of the pituitary-adrenal axis is only observed during the morning hours of the circadian rhythm thus changing the time of administration may minimise any side-effects [399]. One study reported that mifepristone at high doses (10mg/kg) taken daily for a week induced a diffuse maculopapular erythematous cutaneous eruption within two

weeks of discontinuation of the drug in the majority (73%) of the sample of healthy male volunteers [400]. This resolved spontaneously and none of the volunteers had a raised eosinophil count, thus not supporting the possibility of a drug hypersensitivity or glucocorticoid deficiency. Additionally studies where even higher doses have been administered for even longer periods have not reported similar complications [401].

Most novel indications (e.g. contraception) require though long term administration. Mifepristone's antiglucocorticoid action has raised the issue of safety. Prolonged administration of daily low dose is not associated with a cumulative increase in plasma concentration of the drug [402] but at doses, above 200mg/day clearance reaches a limiting value and this may produce elevated plasma levels over a long period of time [397]. Chronic exposure to 200mg daily has been associated with symptoms of cortisol deprivation that were severe enough to be treated with concomitant prednisolone [403]. It was suggested that this may be due to mifepristone activating and resetting the hypothalamo-pituitary-adrenal axis at a higher level [404]. However, this has not been replicated in other studies with similar doses taken for long durations [405]. At high dose, mifepristone has been linked to biochemical hypothyroidism and it has been advised to monitor thyroid function at high doses [406]. However, with very low doses of 2-5 mg/day, it is very unlikely that changes in thyroid function would be observed.

In contrast to pills containing oestrogens and/or progestogens, there is no theoretical risk that such a pill would increase the risk of breast cancer or cardiovascular disease. Rather, experimental data show that antigestogens are antimitotic in breast cancer cell lines and animal models and hence might actually reduce the risk of malignancy

[407,408,409].

The main concern with prolonged intake is that of exposure to unopposed oestrogen and the possibility of the endometrium undergoing hyperplastic or malignant changes [410]. However, studies in monkeys with mifepristone and other antogestogens have shown evidence of endometrial atrophy rather than hyperplasia [411,412,413,414]. It has been demonstrated that much of the increase in endometrial thickness is associated with cystic dilation of the endometrial glands and the cavity itself [415]. A recent study by Eisenger et al reported the results of a study in which 40 women with fibromyoma were given 5 or 10mg mifepristone/day for up to 1 year [416]. Simple hyperplasia of the endometrium without atypia was seen after six months only in a minority of women (28%) who took 10 mg but none in 5mg [416]. One of the secondary outcomes of the multicentre double-blind randomised controlled trial we undertook [417] was to investigate the effect of long term administration of daily mifepristone (5mg) on endometrial thickness and histology.

Biological effects of mifepristone

As mentioned above, antiprogestogens bind with high affinity to PR and antagonise the action of endogenous progesterone. Progesterone receptors are distributed widely throughout the body. Their principal function however, is in the reproductive tract (uterus and ovary) and also the hypothalamus and anterior pituitary. Thus mifepristone

has multiple effects on the hypothalamic-pituitary-ovarian axis and on the ovarian cycle, depending on the time of the cycle and the dose used.

Administration in the follicular phase

Progesterone is secreted in small amounts by the pre-ovulatory follicle and together with oestrodiol, is responsible for initiating the luteinising hormone (LH) surge which is characteristic of an ovulatory cycle [418]. Progesterone is contradictory, in that, in small amounts it enhances the ability of oestrogen to provoke an LH surge, whilst in large quantities it inhibits the positive feedback of oestrogen [419]. Acting locally within the follicle progesterone plays an important role in follicle rupture. Antigestogens have the potential to block all of these events induced by progesterone and hence interfere with ovulation.

During the early follicular phase, mifepristone (at a dose of 3mg/kg) causes a minimal decrease in oestrodiol level, but otherwise has no effect on the menstrual cycle [420]. Given later in the follicular phase, mifepristone decreases oestrodiol and LH levels causing arrest of folliculogenesis and inhibition or delay of ovulation [420,421,422]. This reduction in LH pulse amplitude suggests a pituitary site of action [422]. The effect of a one-off dose is to delay the LH surge and so post mifepristone dosage follicle recruitment restarts and ovulation occurs after approximately 2 weeks [423,424].

If however, mifepristone is administered daily at a dose of 2 or 5mg, it does not affect the pulsatile pattern of LH secretion. Therefore the impairment of the oestrogen induced surge is not due to an effect of mifepristone at the hypothalamic level, nor is it due to decreased responsiveness of the pituitary to exogenous gonadatrophin releasing hormone (GnRH). The conclusion is that at this dose (2 or 5mg) mifepristone interferes with the mid-cycle LH surge by a complex mechanism, possibly by directly reducing the sensitivity of pituitary gonadatrophin to the positive feedback effects of oestrogen [425].

It is important to note that if mifepristone is given post LH surge is initiated, it does not appear to arrest ovulation [116,423,426,427]. Its effect is then as described below.

Administration in the luteal phase

Antigestogens also exert powerful effects on the uterus. In the luteal phase, under the influence of progesterone the endometrium transforms from a proliferative to a secretory state. When the corpus luteum collapses, progesterone withdraws and this leads to menstrual bleeding.

Early luteal phase administration of either a single dose or multiple doses of mifepristone disrupts endometrial maturation without affecting vaginal bleeding patterns [428,429]. There is inhibition of progesterone induced down-regulation of PR and oestrogen receptor (ER) [428,430,431], plus antagonism of the action of progesterone on

endometrial markers such as prostaglandin dehydrogenase (PGDH), which are known to be progesterone dependent [432,433]. In addition, if 200mg mifepristone is administered immediately after ovulation, uterine prostaglandin (PGF2alpha) release [434], the luminal expression of COX-2 [435] and the glandular expression of leukaemia inhibitory factor (LIF) (a cytokine involved in the implantation process) are significantly reduced, while the immunoreactivity of Ki67 (a nuclear marker of cellular proliferation) is increased [431]. The reduction in the endometrial metabolism of oestrodiol [436] and the reduction in the markers of endometrial maturation [428] suggest that antiprogestogens given immediately after ovulation will delay or prevent formation of a secretory endometrium, in which case it remains proliferative and hostile to implantation [429].

If given later in the luteal phase of the cycle, mifepristone at a single dose of >50mg will induce breakdown of the endometrium and menstrual bleeding [424,437,438]. The effect of mifepristone on the endometrium (at a time when the levels of the endometrial progesterone receptor are relatively low) are poorly understood and it may be that mifepristone antagonises the action of progesterone, which at the time appears to involve the suppression of release of prostaglandins [439]. There is evidence available for a similar action of mifepristone on local prostaglandin in early pregnancy, when it has been reported to reduce the PG-metabolising enzyme PGDH [440].

Administration throughout the menstrual cycle

If given as a daily pill, the threshold dose of mifepristone required to disrupt endometrial development is 1mg/day [441,442] and when given as a once weekly pill then 5mg is sufficient to inhibit normal secretory development of the endometrium [443,444]. The endometrial histology is variable. It ranges from atrophic changes to cysts lined by a single layer of atrophic epithelium with very few mitotic cells [445]. These changes are probably due to the antiproliferative effect of mifepristone and suggestive of a low risk of atypical hyperplasia in the long-term. As a result of the absence of ovulation and the inactive endometrium most women experience amenorrhoea.

Contraceptive potential of mifepristone

Research has concentrated on four areas [446]; as a daily contraceptive pill; as a weekly contraceptive pill; as a once-a-month contraceptive pill and as post-coital emergency contraception.

Inhibition of ovulation – potential as a daily contraceptive pill

Brown et al (2002) [447,448] undertook a double blind randomised control trial of 98 women (58 Edinburgh, 40 Shanghai) who took mifepristone 2 or 5mg daily for 120 days [415,449]. Ninety women completed the study (50 Edinburgh, 40 Shanghai). Follicular activity (and therefore oestrogen secretion) continued during treatment with both doses although ovulation was suppressed in the majority of the cycles (90% of 2mg cycles and 95% of 5mg cycles). The majority of women experienced amenorrhoea (65% of women taking 2mg and 88% of those taking 5mg). Endometrial thickness increased significantly in women in Edinburgh and decreased in those in Shanghai; histology showed either atrophic or cystic changes with no evidence of hyperplasia. There were no pregnancies reported in the 200 months of exposure in 50 sexually active women who had used no other method of contraception [449]. There has only been one other study assessing the contraceptive efficacy of mifepristone [450]. In that study 32 women used 0.5mg mifepristone daily. Sixteen completed six months of use. In 141 cycles, there were five pregnancies [450].

The findings from these preliminary studies do suggest that mifepristone in low daily dose has the potential to be developed as a novel oestrogen-free daily oral contraceptive pill. The findings have to be further confirmed in larger studies and that is one of the remits of the multicentre double-blind randomised controlled trial we undertook [417]. This study and its findings are detailed below.

Disruption of follicular development – potential as a once-a-week pill

High doses of 50mg, 25mg and 10mg are associated with variable inhibition of ovulation [386,451] while small doses of 5mg and 2.5mg do not affect ovulation, the hormonal profile or the menstrual cycle [444]. All doses have resulted in delayed endometrial maturation and impaired secretory activity of the endometrium. The efficacy of the once-weekly regime has been tested in two studies. The first study by Marions et al involved 18 women taking 5mg mifepristone weekly [452]. In over 63 cycles, three pregnancies occurred. In the second study, by Godfrey et al (2004), 17 women took 10mg mifepristone weekly [453]. In 56 cycles, three pregnancies occurred. Considering these were trials the failure rate can be considered as close to that of perfect use. With the combined oral contraceptive pill failure rate for perfect use is 1/12,000 cycles [74] and in these studies the rate is 4.7/100 cycles and 5.3/100 cycles. In view of these data, weekly administration has been abandoned as a potential contraceptive method.

Inhibition of implantation – potential as a once-a-month pill

The effect of giving 200 mg mifepristone in the early luteal phase of the cycle 2 days after the mid-cycle LH surge has been tested in two small pilot studies [454,455] and one evaluation [456]. All three have found contraceptive efficacy to be high. Post pilot studies the main concerns with this once-a-month approach were regarding determining the correct timing of administration as if given at the incorrect time then there is an unpredictable disturbance in the pattern of menstrual bleeding [429]. This then makes it very difficult for the women to time the taking of mifepristone in subsequent cycles. The

feasibility of such an approach has been tested [454]. Women were required to use a daily fertility monitor to predict and identify the day of onset of the LH surge. On average women failed to perform 24% of tests in the 162 cycles analysed during the period of high fertility. Study volunteers are generally considered a highly motivated group and hence it is unlikely that women in the general population would have a greater compliance rate. Consequently, at the time of initiating our research into the efficacy of daily mifepristone, the once-a-month approach was not considered practicable until a simpler method of determining time of administration could be developed.

However, Brown et al (2003) tried a different approach and found that mifepristone could be given, pre-ovulation, as a once-a-month contraceptive pill without causing significant disruption in the menstrual cycle in the majority of women for a four day period from just prior to ovulation until LH plus 3 [457]. Subsequently, Agarwal and colleagues (2009) have undertaken a prospective case-control study where 80 women took 200mg mifepristone orally once a month on day 16 of a regular menstrual cycle of 28-30 days. They found that mifepristone can act as an effective monthly contraceptive pill, given on a fixed day in the mid-cycle without detecting LH surge in women having regular menstrual cycles [456].

Disruption of implantation or 'menstrual induction' – potential as a once-a-month pill

Administration of mifepristone in the late luteal phase of the cycle even in doses as low as 10mg will induce menstrual bleeding [458]. This will occur even in the presence of

exogenous human chorionic gonadatrophin [439]. This would suggest that administration of mifepristone in the late luteal phase would disrupt implantation and prevent pregnancy. However, although bleeding is almost always provoked in very early pregnancy by antigestogens, studies have reported an unacceptably high pregnancy rate for this regimen to be considered feasible [459,460,461]. It is unclear as to why the failure rate at this stage of pregnancy is higher than that observed when abortion is induced within 10 days of a missed period [462].

Potential for use as postcoital emergency contraception

Mifepristone has all the pharmacological properties to make it a highly effective post-coital contraception [463]. Multicentre trials have confirmed mifepristone to be an effective emergency contraception (EC) method even in doses as low as 10mg [34,35,36,37][464]. Studies have proven mifepristone to be as efficacious as levonorgestrel for emergency contraception [465,466]. Side-effects have been found to be independent of dose but menstrual delay is significantly less likely with the 10mg dose [467]. The mode of action of mifepristone varies with when in the cycle it is taken. If given before ovulation it prevents it, if given after ovulation the effect on the endometrium is highly suggestive of impaired implantation [383,468]. Currently Mifepristone has been licensed and is available for use as emergency contraception in two countries – China (Bi Yum 10mg dose) and Vietnam (Ciel 25mg dose) [469].

In summary: Mifepristone is an effective method of emergency contraception and has the potential to be developed as a once daily pill for regular contraceptive use as well as a once-a-month pill if administration at the correct time can be guaranteed.

Potential additional benefits offered

Amenorrhoea

Previous studies have demonstrated that mifepristone at low dose disrupts the menstrual cycle. One study using 1mg per day for duration of 150 days demonstrated that more than half the women showed irregular bleeding or amenorrhoea [470]. Brown et al (2002), as described earlier, studied the effects of 2mg and 5mg doses on menstrual bleeding pattern. The study was undertaken in two centres: Edinburgh and Shanghai. In Edinburgh, 17/26 women (65%) of those receiving 2mg daily were amenorrhoeic, as were 21/24 (88%) of those receiving 5mg. The mean days of bleeding were 4.4 days and 0.6 days for the 2 and 5mg groups respectively. In Shanghai, 18/20 women (90%) in both dose groups were amenorrhoiec during treatment, with mean days of bleeding of 0.4 and 0.7 respectively [415,447].

In the first study presented below, therefore, the efficacy, pattern of bleeding and other side-effects of mifepristone at a dose of 5mg/day taken for 24 weeks were directly compared with the progestogen-only-pill (POP), levonorgestrel [417]. We hypothesized that women given mifepristone would have a much higher incidence of amenorrhoea and fewer days of menstrual bleeding.

Protection against sexually transmitted infections and HIV

The HIV epidemic continues to be a major global public health challenge [471]. Heterosexual intercourse is now the main route of transmission of HIV. While barrier methods such as condoms reduce the risk of transmission, there is a pressing need for additional and complementary methods of protection [6].

The vagina is thought to be a key portal of entry for HIV and other sexually transmitted infections (STI) in women [472]. The underlying mechanisms are poorly understood. Experiments on hysterectomized rhesus monkeys suggest that it is the vagina, rather than the cervix or the uterus, that is the main portal of viral entry [473,474]. Epithelial thickness and integrity modulates the ease of access of virus to immune cells and subepithelial vasculature [475]. Oestrogen-induced surface keratinisation and hyperplasia protect rhesus monkeys against SIV inoculation [476,477], whereas oestrogen-deficient women such as those who are post-menopausal [478,479] or on long acting gestagens are at increased risk of HIV, presumably as a result of vaginal thinning. Other biological variables such as the vaginal microflora [480,481,482], immune cell populations [483,484] and natural antimicrobials also play an important role in the innate defences of the reproductive tract [485,486,487].

Mifepristone, a potent antagonist of progesterone, has the potential to be developed for contraception and other gynaecological uses [448,488,489]. Daily low-dose mifepristone inhibits ovulation but maintains follicular development, thus exposing reproductive tissues to unopposed oestrogen. Since gestagen treatment, in macaques, increases and oestrogen treatment decreases HIV/SIV transmission the aim of the second study detailed below was to investigate whether, the antigestagen, mifepristone modulates underlying mechanisms involved in transmission of HIV [490].

Study 1

Objective

A pill which contains no oestrogen and which reproducibly induces amenorrhoea in a high proportion of women should prove popular. In further exploring this the efficacy, pattern of bleeding and other side-effects of mifepristone at a dose of 5mg/day taken for 24 weeks were directly compared with the progestogen-only-pill (POP), levonorgestrel [417]. We hypothesized that women given mifepristone would have a much higher incidence of amenorrhoea and fewer days of menstrual bleeding.

Materials and Methods

This was a multicentre, double-blind, randomized controlled phase II trial comparing two daily contraceptive pills. The primary outcome was the percentage of women who had amenorrhoea throughout the study. Secondary outcomes included number of days of bleeding, endometrial thickness and histology, and number of pregnancies. Before starting, the trial was approved by the steering committee of the Contraceptive Development Network (MRC Grant No. G9523250). It was performed in accordance with Good Clinical Practice including regular monitoring of centres.

Between June 2003 and January 2004, a total of 97 healthy volunteers with regular menstrual cycles (21 – 42 days) aged 18 – 40 years were recruited from four sites (34 in Sagamu, Nigeria; 18 in Cape Town, South Africa; 10 in Hong Kong, People's Republic of China and 35 in Edinburgh, Scotland). The study was approved by local ethical committees at all centres. All women gave written informed consent before enrolment and were screened before entering the study by routine physical and gynaecological examination and measurement of height, weight, blood pressure and pulse rate. Blood samples were collected for measurement of progesterone, clinical chemistry and haematology. The size of the uterine cavity and ovarian follicles were measured by transvaginal ultrasound scan. Urinary hCG was also measured to

exclude pregnancy before entering the trial. Women who had used any form of hormonal contraception within the last 3 months of the start of the study were excluded except in Edinburgh where nine women with regular cycles while already using a POP were included without having to undergo a washout period.

Subjects were studied for one pretreatment cycle, six treatment cycles (24 weeks) and for one post-treatment cycle. Subjects were randomly allocated to receive either mifepristone 5 mg/day (one half a 10 mg tablet) (from Laboratoire Exelgyn, 6 rue Christophe Colomb, 75 008-Paris for women in Edinburgh; and from Hualian Pharmaceuticals Ltd, Shanghai, China for women in Hong Kong, Cape Town and Sagamu) or levonorgestrel 0.03 mg/day (POP) (Norgeston; Schering Health Care Limited, The Brow, Burgess Hill, West Sussex, RH15 9NE) starting on Day 1 or 2 of the cycle. Randomization was achieved by blocked computer-generated randomization performed individually for each centre to ensure good balance of numbers in the different treatment groups. Each centre was given numerical sequence of coded treatment bottles which were identical generated by statistician in Edinburgh office. Participants were enrolled in each centre by the local investigator. The numerical sequence was determined by the clinical trials manager in Edinburgh. To maximize, the number of cycles observed on mifepristone without greatly reducing the power of the randomized comparison, three times as many subjects were randomized to mifepristone as to levonorgestrel. In Cape Town, Hong Kong and Edinburgh, daily doses were issued at eight weekly intervals in pre-packed identical bottles containing mifepristone (a half tablet) with placebo, or levonorgestrel with one half placebo tablet. In Nigeria, daily doses were issued at weekly intervals. The placebo and active tablets were both white but of slightly different size. Unused medication was returned and new medication dispensed at this time. All subjects were sexually active and intending to use the study drug as their sole method of contraception. However, in Cape Town and Hong Kong some women also used condoms for protection against sexually transmitted infection. Cycles in which dual protection was used were omitted when calculating the months of exposure to the risk of pregnancy.

The women were asked to record every day the amount of vaginal bleeding and adverse events on a diary card. If bleeding occurred it was recorded as spotting (one or less pad/tampon required) or bleeding (more than one pad/tampon required).

Subjects attended the clinic for review at eight weekly intervals during the treatment phase and once more on Day 5 – 11 of the first menstrual cycle after discontinuation of the study medication. At the end of the treatment phase a vaginal examination was performed on all subjects and an endometrial biopsy performed in a subgroup of volunteers. In order to identify those women who might develop hyperplasia of the endometrium a biopsy was performed at any time during the study on all subjects if the measurement of the uterine cavity was observed to be >12 mm ('safety biopsy'). Biopsies were performed as an outpatient procedure with a Pipelle suction curette (Pipelle de Cornier, Laboratoire C.C.D, 60 rue Pierre Charron, 75 008-Paris-France, Ref. 1103000). Endometrial samples were then fixed in 10% neutral buffered formalin prior to embedding in paraffin wax. Histological examination of the endometrial sections was conducted blind by an independent pathologist and classified into five categories [proliferative, secretory, inactive, insufficient and cystic glandular dilatation (CGD) as previously described (Baird *et al.*, 2003)]. (i) Proliferative includes 'active' in which glands are tubular, with columnar epithelial cells showing nuclear stratification and frequent mitoses (>=5 per 20 gland profiles); and 'weakly'— simple tubular glands with columnar epithelial cells showing mild nuclear stratification (2 – 4 mitoses per 20 gland profiles). (ii) Inactive—cuboidal to columnar cells showing focal or diffuse nuclear stratification, in glands with simple tubular or undulating profiles without significant dilatation. Resembles endometrium of basalis in normal cycling endometrium. Glands are not atrophic, and show evidence of limited recent growth or function, but lack the findings seen in normal cycling proliferative endometrium. Mitoses are absent or uncommon (no more than one mitotic figure per 20 gland profiles). (iii) Inactive with CGD—inactive glandular epithelium as described above, with dilatation of gland lumina. Dilatation is defined as the gland having an open lumen that forms a space, i.e. greater than four times

the epithelial thickness. (iv) Secretory—glands are variably tortuous with cytoplasmic vacuolation that varies according to phase of cycle. Cells are columnar, with non-stratified nuclei showing an absence of mitoses (except for infrequent mitoses in the early secretory phase). Stromal decidual change starts around spiral vessels, becoming confluent in the late secretory phase. Some samples contained inadequate amounts of tissue for accurate histological evaluation.

Ovarian function was assessed by the measurement of progesterone in venous blood collected at screening and at 8, 16 and 24 weeks after starting treatment. The woman was classified as having ovulated at least once if the concentration was > 15 nMol/l in any of the three samples. Following centrifugation the serum was stored at -20°C in labeled sample tubes. Analysis by radioimmunoassay was carried out at the end of the study locally by each centre. Transvaginal ultra-sonography was used to assess number and size of follicles or cysts at screening and after 8, 16 and 24 weeks.

Statistical methods

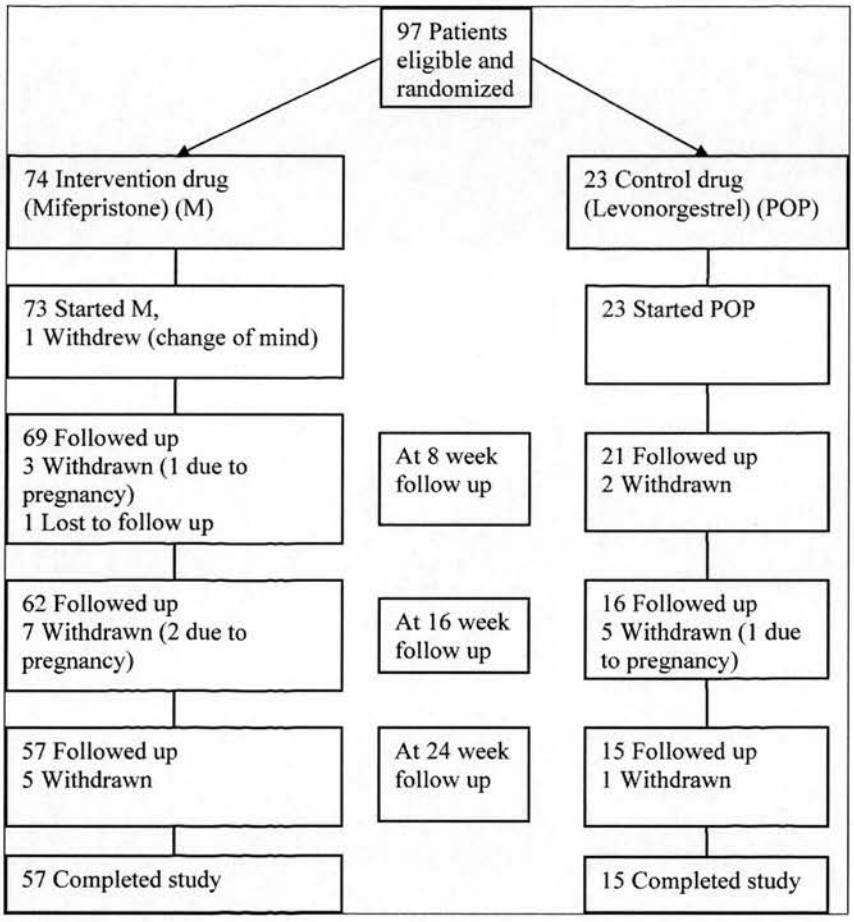
The number of subjects on mifepristone was chosen to give an upper confidence limit of <5% for the risk of pregnancy over the 6 months of treatment if no pregnancies occurred in that group. To maximize, the number of cycles observed on mifepristone without greatly reducing the power of the randomized comparison, three times as many subjects were randomized to mifepristone as to levenorgestrel. These numbers were also sufficient to give over 99% power to show a significant difference in the rate of amenorrhoea over 6 months if the rate was 50% on mifepristone when compared with the 5% anticipated in the levenorgestrel group [491]. Data analysis was carried out using SPSS version 12 (SPSS, Inc., Chicago, IL, USA) and Excel 2003 (Microsoft Corporation). The two randomized groups were compared by chi-squared test with Yates' correction and Mann – Whitney or Student's t-tests as appropriate tests for binary and continuous outcomes, respectively. Analysis of covariance adjusting for pretreatment values was used to compare the endometrial thickness in the two groups at follow-up. Confidence limits were calculated for the

incidence efficacy outcomes in the mifepristone group using the Poisson distribution based on person time at risk.

Results

A total of 97 women were recruited (Fig. 2) and randomized to treatment [35 in Edinburgh (26M, 9POP); 34 in Nigeria (26M, 8POP); 18 in Cape Town (14M, 4POP); 10 in Hong Kong (8M, 2POP)]. One subject randomized to mifepristone withdrew for personal reasons and discarded the study drugs without taking any. Therefore, the results of the 96 women who started treatment [73 received mifepristone (M), 23 received levonorgestrel (POP)] were included for analysis.

Figure 2: Flow chart of patients through the study



Of the 87 women who started the study and did not use hormonal contraception in the previous months, 66 were randomized to mifepristone and 21 to POP. An additional nine women in Edinburgh who had regular menstrual cycles while taking POP for contraception were transferred directly to study medication. In this subgroup, seven were randomized to mifepristone and two to POP.

When all centres were combined (Table 1) there were no statistically significant differences in age, weight, height, BMI or parity between the two treatment groups (two-sample Student's *t*-tests). However, there were some differences in the characteristics of the women from different centres. The women in Nigeria were significantly older (years 33.9 ± 4.2 SD, $P = 0.001$) than the women in both Cape Town (26.2 ±7,7) and Edinburgh (28.7 ± 6.0). The BMI of women in Hong Kong (19.8 ± 1.7 kg/m2) was significantly lower than that of women in Edinburgh (22.9 ±1.9, $P < 0.001$), Nigeria (24.3 ±3.9, $P = 0.001$) and Cape Town (23.1 ± 3.6, $P = 0.014$).

Table 1: Mean (SD) age, weight, height and BMI according to study drug

	M	POP
Age (years)	30.3 (6.3)	30.4 (6.9)
Weight (kg)	60.7 (9.1)	58.4 (6.2)
Height (cm)	161.5 (5.9)	161.3 (6.3)
BMI (kg/m^2)	23.3 (3.6)	22.4 (1.8)

Twenty-four women (16M, 8POP) did not complete the full 24 weeks of treatment. Eight women withdrew for personal reasons unrelated to the study medication (e.g. moved from the area, relationship ended); six were discontinued by the investigators because of persistent protocol violations (e.g. poor compliance, unavailable for follow-up). Two women withdrew because of anxiety about potential adverse affects of the pills. Four women in the POP group discontinued because of persistent irregular bleeding, no women in the mifepristone group discontinued for this reason ($P < 0.01$). Four women withdrew because of pregnancy: three in the mifepristone group and one while using levonorgestrel.

Seventy-two women completed the study (57M, 15POP) and took the medication for at least 24 weeks (168 – 192 days).

Menstrual bleeding pattern

There were significant differences in the pattern of menstrual bleeding between the groups (Fig. 3). Of the 73 women who started mifepristone, 36 (49%) were amenorrhoeic for the duration of drug treatment and only three women bled or spotted on average for five or more days per month. In contrast, none of the women in the POP group were amenorrhoeic. Although the number of days of bleeding was within the range of a normal menses in the majority (61%), nine women in this group bled or spotted for five or more days per month and four women withdrew from the study prematurely because of continuous irregular bleeding.

Of the 57 women who took mifepristone for the full 6 months, 25 (44%) were amenorrhoeic and a further 22 (39%) had bleeding or spotting for a total of 10 days or less (Table 2). In comparison, of the 15 women who took POP, none (0%) were amenorrhoeic (compared with mifepristone $x^2 = 8.01$, $P = 0.005$).

The pattern of bleeding in those women who continued to menstruate was irregular and unpredictable in the majority in both groups. Although episodes of bleeding in the women who took mifepristone were infrequent and slight, they were mostly unpredictable, except in Nigeria where 4 of the 15 women who continued to bleed had regular monthly periods. Nigerian women were more likely to experience bleeding over the 6 month study duration (13/21; 62%) than those from other centres [Edinburgh 4/22 (18%); Cape Town 3/9 (33%); Hong Kong 1/6 (17%)]. There was a significant difference in bleeding frequency between Nigeria and Edinburgh ($x^2 = 6.86$; $P = 0.009$).

Figure 3: Percentage of women who bled for a given number of days on average per month in 96 women randomized to daily mifepristone or levonorgestrel (POP).
0, amenorrhoea; > 0 < 2, any bleeding < 2 days per month; 2 < 5, 2 to < 5 days bleeding per month; 5+, 5 or more days bleeding per month

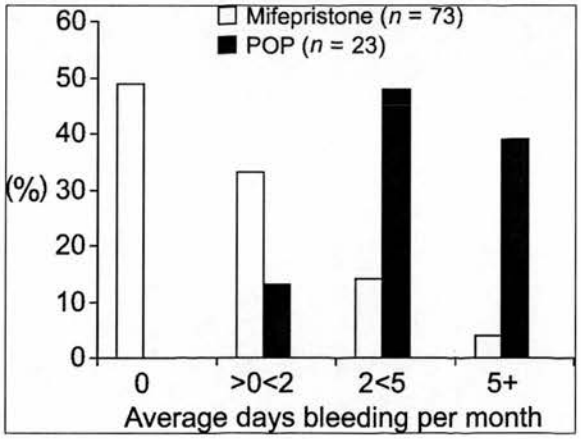


Table 2: Distribution of mean (SD) numbers of days bleeding and/or spotting while on drug according to treatment group, in the 72 women who took the treatment for at least 6 months (%)

Number of days	Bleeding/spotting		Bleeding only		Spotting only	
	M	POP	M	POP	M	POP
0	25 (44)	0 (0)	36 (63)	0 (0)	39 (68)	6 (40)
1	4 (7)	0 (0)	0 (0)	0 (0)	6 (10)	2 (13)
2.5	8 (14)	2 (13)	5 (9)	2 (13)	8 (14)	1 (7)
6 – 10	10 (18)	1 (7)	7 (12)	1 (7)	3 (5)	1 (7)
11 – 20	5 (9)	3 (20)	5 (9)	5 (33)	1 (2)	2 (13)
21+	6 (11)	9 (60)	5 (9)	7 (47)	1 (2)	3 (20)

Ovarian function

Ovarian function was assessed at baseline, 8, 16 and 24 weeks after starting treatment by measurement of progesterone in blood. In 33 of the 97 pretreatment samples, the concentration of progesterone was >15 nmol/l indicating that in just over a third of the women the blood was collected in the luteal phase after ovulation. At each eight-week review, there was evidence of ovulation in some women in both groups. If a woman showed evidence of ovulation at any of the three review visits she was classified as ovulatory. Ovulation was less likely to occur in women taking mifepristone (14/73; 19%) than in those in the POP group although there was no statistically significant difference (7/23, 30%; $x^2 = 0.72$; $P = 0.40$). However, there were significant differences between centres in the incidence of ovulation and amenorrhoea. For example, while taking mifepristone only one of the 26 women in Edinburgh (4%) ovulated when compared with 11/26 women in Nigeria (42%: $x^2 = 8.78$; $P = 0.003$). The proportion of women who ovulated while taking the POP was identical in the two centres (37%).

Ultrasound examination revealed the presence of numerous small and medium-sized follicles in the ovaries of women in both groups throughout treatment. Follicular cysts (diameter >30 mm) were detected on eight occasions (six among women using mifepristone and two in the POP group). The cysts were asymptomatic and resolved spontaneously by the next examination without treatment.

Endometrial thickness

In the mifepristone group, the width of the cavity and the thickness of the endometrium increased with time relative to the baseline (Table 3). In contrast, there was no statistically significant change in the POP group, and by 16 weeks the difference in the size of the uterine cavity between the groups was statistically significant (P = 0.043). There were differences between the centres in the extent of the increase in size with the thickest endometrium at 24 weeks occurring in Edinburgh(5.2 ± 2.1 mm before starting, increasing to 13.8 ± 7.1 at 24 weeks)(Table 4).

Table 3: Mean (SD) endometrial thickness at different times according to treatment group

Weeks	Mifepristone	POP	*P*-value
0	6.1 (2.1) [73]	5.8 (2.4) [22]	NS
8	6.6 (3.4) [68]	5.3 (2.7) [19]	0.11
16	8.0 (5.2) [60]	5.1 (2.8) [15]	0.043
24	10.3 (6.8) [58]	4.0 (2.0) [15]	<0.001

The *P*-value is from an analysis of covariance adjusted for the pretreatment value. Numbers of subjects are shown in square brackets.

Table 4: Mean (SD) endometrial thickness at different times according to centre – for mifepristone only

Weeks after starting	Edinburgh	Cape Town	Hong Kong	Nigeria
0	5.2 (2.1)	6.7 (1.7)	6.6 (2.6)	6.4 (1.8)
8	4.9 (3.4)	7.6 (3.3)	6.4 (4.4)	8.0 (3.2)
16	9.2 (5.7)	8.0 (5.0)	8.9 (7.4)	6.5 (4.4)
24	13.8 (7.1)	11.5 (8.7)	8.3 (3.6)	6.8 (4.4)

Endometrial biopsies

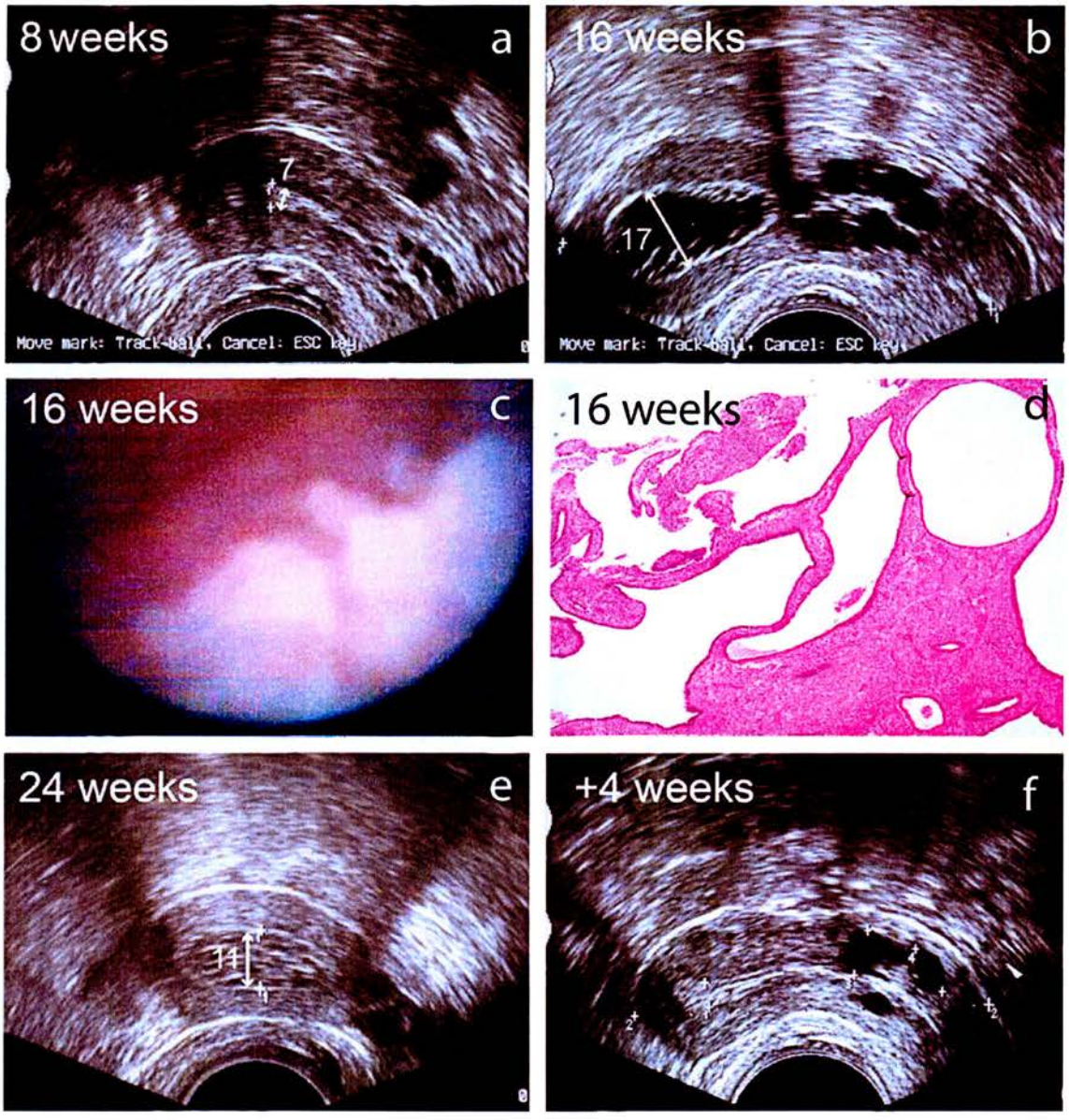
A total of 39 women had at least one endometrial biopsy. Six women in the mifepristone group had two biopsies and one had three biopsies, making a total of 47 biopsies available for examination. Twenty women (19 in the mifepristone group and 1 in the POP group) had a 'safety biopsy' because the endometrial cavity on ultrasound was >12 mm. A total of 31 biopsies were available from women who had completed 24 weeks of treatment (27 mifepristone and 4 POP).

CGD was found in the endometrium of 13 of the 27 women who had a biopsy at 24 weeks on completion of the study. The remainder were proliferative (4), inactive (3), or insufficient for histological examination (7). Of the 19 women on mifepristone who had a 'safety' biopsy because the uterine cavity was dilated above 12 mm, 11 showed CGD on at least one occasion. CGD also occurred in four of nine women with normal-sized cavity who volunteered to have an endometrial biopsy at 24 weeks. The endometrium in the sole woman in the POP group who had a safety biopsy (cavity 13 mm) was too scanty for histological examination. None of the women in either group showed evidence of hyperplasia or atypia.

Hysteroscopy was performed as an outpatient procedure on two women (both in Edinburgh) with an enlarged uterine cavity (29 and 16 mm) detected on routine ultrasound at 16 weeks. The uterine cavity was dilated with mucous fluid and a pale oedematous looking endometrium which showed CGD on histological examination (Fig. 4). No serious pathology was detected and one woman opted to continue with the trial. The endometrial cavity returned to normal size after completion of the trial.

Figure 4: Appearance of the uterus of subject 135 who took 5 mg mifepristone per day for 24 weeks

The subject remained amenorrhoeic throughout. (a) After 8 weeks the thickness of the endometrium and cavity was normal; (b) at 16 weeks the cavity was dilated to 17 mm with fluid. Cyst like structures had become apparent in the cervix; (c) the cavity was lined by pale dilated endometrium; (d) showing CGD on histology (X10); (e) by 24 weeks the cavity was still dilated; (f) by 4 weeks after stopping the cavity had returned to normal after menses. Note the persistence of dilated cervical glands.



Contraceptive efficacy

Not all the subjects were at risk of pregnancy for the duration of the study. Some women separated from their partner, while a number of women in Cape Town and Hong Kong used barrier methods as well as the study drug for duel protection. In total, there were 356 months of exposure in women who took mifepristone as their sole method of contraception and 85 in women who took POP.

Five women became pregnant during the study: four of them in the group of women randomized to mifepristone. However, there were only three pregnancies while the subjects were taking the study drugs. In the mifepristone group, two pregnancies occurred (both in Capetown) during treatment and were recognized after 62 and 115 days of treatment, respectively. Conception occurred in both within the first 2 months of treatment. One woman underwent a vacuum aspiration and the other opted to continue with the pregnancy and delivered at term a healthy baby. Both of these women were using condoms for dual protection. Inclusion of these two women in the analysis of efficacy gives an estimate of 0.6% (95% confidence limits 0.07 to 2.0%) for the risk of pregnancy per month while exclusion results in an estimate of 0% (0 and 1.1%). Two additional pregnancies occurred before starting or several weeks after stopping mifepristone. One pregnancy occurred in a woman using levonorgestrel and was only detected because of a routine pregnancy test 8 weeks after starting treatment. She had a blighted ovum and the uterus was evacuated surgically. There was no significant difference in the pregnancy rates between the groups although the study was not powered to detect differences.

Adverse effects

There were no major adverse events in either group. Eight women (35% of those taking POP) and 19 (36% of those on mifepristone) reported a range of symptoms none of which were significantly different between the groups including abdominal discomfort (12% mifepristone versus 4% POP), irregular bleeding (7%)

mifepristone versus 9% POP), headache (8% mifepristone versus 22% POP), flushes (7% mifepristone versus 0% POP) and mood change (3% mifepristone versus 0% POP).

Discussion

This study has demonstrated striking differences in the pattern of menstrual bleeding between women taking two types of oral contraceptive pills, which contain no estrogen. The results confirm our previous study, which reported that the majority of women taking 5 mg mifepristone every day for four months were amenorrhoeic [448]. In the present study, over 80% of women who took mifepristone were either amenorrhoeic or had episodes of bleeding or spotting <2 days per month. In comparison, women on levonorgestrel had frequent irregular bleeding (>5 days per month) and none was amenorrhoeic. Four women discontinued the POP because the bleeding pattern was unacceptable.

This study has its strengths and weaknesses. The placebo was similar but not identical to either of the active drugs and hence the study was not truly double blind. It would have been possible for the persistent subject and/or researcher to identify group differences by the size of the treatment tablets. We think that this is unlikely because each woman was supplied with two identical opaque bottles each containing 8 weeks supply of either placebo or active drug prepared by a member of the research team not involved with contact with the subjects. Moreover, the difference in bleeding patterns (the primary end point) was so striking that we think it unlikely that unblinding of an individual subject would have had a significant impact on the overall results.

Nine women (all in Edinburgh) who were taking POP were recruited directly to the study without at least 3 months 'wash-out'. It could be argued that in these women there was a 'carry over' effect on the ovary and/or endometrium from the

previous treatment. We think that it is unlikely because all women had regular menstrual bleeding while on the POP. Moreover, at recruitment they had evidence of ovarian activity, i.e. luteal levels of progesterone, follicles >10 mm and/or endometrium >8 mm. Because they were randomized 2:7 POP:mifepristone, they would not bias the comparison between the groups. The seven women in this subgroup who were randomized to mifepristone had amenorrhoea for at least 3 months after starting, illustrating one of the clinical uses when the drug becomes available.

There were minor differences between centres in the response to the drug. However, in all centres the bleeding pattern was better with mifepristone and the contraceptive efficacy was high.

It is arguably in Africa where there is the greatest unmet need for contraception. The fact that we show that it is effective in a range of cultures, including Africa, should help facilitate its use worldwide particularly in those cultures where women who are menstruating are subject to social taboos.

In the women in the mifepristone group who continued to bleed there were differences between the centres in the pattern of menses. Nigerian women were more likely to experience bleeding over the 6 months study and to show biochemical evidence of ovulation than women in the other centres. For logistic reasons, our assessment of ovarian function by measurement of the concentration of progesterone every 8 weeks was imprecise. We classified a woman as being 'ovulatory' if the level of progesterone was in the luteal phase range on any one of the three occasions when it was collected. We argued that there would be about a one in three chance that the progesterone level will be raised in any single sample collected at random from normal cycling women as was found in the control cycle. Although the majority of women in all centres failed to ovulate while taking mifepristone,

there were significant differences in the incidence of anovulation between centres.

These differences between centres are intriguing. We have previously reported that ovulation and menstruation were more easily suppressed by mifepristone in Chinese women in Shanghai than in Caucasians in Edinburgh [448]. The daily dose of 5 mg mifepristone was chosen because it resulted in amenorrhoea in over 90% of women in Edinburgh. We have previously suggested that these differences in response may be due to differences in diet and/or in metabolism of steroids. Alternatively, there could be differences in compliance between centres although there was no evidence from the number of returned pills that the women in Nigeria omitted more pills than those in other centres. Moreover, a large number of tablets would have to be missed before sub-therapeutic levels of mifepristone were reached because of the long half-life of mifepristone [492].

One of the main reasons that women discontinue hormonal contraception is because of menstrual irregularity [493] and this was true of women using the POP in this study. All four women who discontinued the study specifically because of menstrual irregularity were taking POP. The amenorrhoea or scanty bleeding associated with mifepristone should be perceived as an advantage by many women [93,94].

It has been argued that prolonged amenorrhoea is unnatural and even harmful. Monthly menstruation has however been the norm only for the last 100 years. Prior to that, most women spent their short lives either pregnant or breastfeeding and amenorrhoeic. Absence of periods per se does no harm. If associated with hypoestrogenism (as in the menopause or during treatment with analogues of gonadotrophin releasing hormone) it is associated with increased risk of osteoporosis and heart disease; if associated with a high dose of progestogen (as during the use of Depo-Provera®) or prolonged exposure to COC (as in extended pill use) it may be

associated with increased risk of breast cancer and heart disease [491]. Amenorrhoea during mifepristone use is not accompanied by hypoestrogenism and, as stated earlier the risk of breast cancer may be reduced [407,408]. A recent paper reported that mifepristone prevented the development of breast cancer in transgenic mice with null mutation of BRCA1/p53 [409].

Concern has been expressed that with prolonged intake of antiprogestogens the endometrium would undergo hyperplastic or malignant changes due to continued exposure to unopposed oestrogen [410]. However, studies in monkeys with mifepristone and other antigestogens have shown evidence of endometrial atrophy rather than hyperplasia [411,412,413,414]. In keeping with our previous report, the endometrial cavity widened progressively with time in women in Edinburgh, but not in the other centres [448]. We have previously reported that much of the apparent increase in endometrial thickness is associated with cystic dilation of the endometrial glands and the cavity itself [447]. A recent paper reported the results of a study in which 40 women with fibromyoma were given 5 or 10 mg mifepristone/day for up to 1 year [416]. Simple hyperplasia of the endometrium without atypia was seen after 6 months only in a minority of women (28%) who took 10 mg but none at 5 mg.

In the present study, the commonest histological picture was of inactive CGD. This CGD was only found in those women taking mifepristone. The cause of this unusual change is unknown. It is unlikely to be due to the effects of unopposed estrogen as it has been demonstrated to occur in women who have profound suppression of ovarian follicular development and where oestrogen levels are low [447,448]. Moreover, it occurs more commonly in those women taking 10 mg mifepristone per day than in those taking 5 mg in whom the secretion of ovarian oestradiol is higher [416].

Novel observations in this study were the hysteroscopy finding of atrophic and/or

oedematous endometrium. Hysteroscopy revealed that the apparent thickness of the endometrial cavity as measured on ultrasound reflected dilation of the glands and cavity rather than true hyperplasia of the endometrium. It demonstrates the limitation of using measurements of endometrial thickness by ultrasound alone as a marker of endometrial pathology. The cause of this accumulation of fluid and its nature is unknown but similar findings have been reported in rabbits following treatment with mifepristone [414]. It may be that the mechanism, which normally allows the passage/and or re-absorption of fluid from the endometrial glands and uterus, is obstructed leading to an accumulation of fluid within the uterus.

This study has confirmed that mifepristone is potentially a highly effective contraceptive. Even in Nigeria where there was biochemical evidence of ovulation in 42% of women there were no pregnancies in the mifepristone group. In our previous study, we reported no pregnancies in 50 women who used mifepristone at a dose of 2 or 5 mg/day for 4 months [448]. The present study extends our contraceptive experience to a total of 556 women cycles. The two pregnancies in women taking mifepristone occurred in women in South Africa who were also using condoms as protection against sexually transmitted disease including, HIV/ AIDS. It is possible that these women may have omitted to take their pills every day because they thought that they were protected from the risk of pregnancy by the use of condoms.

In conclusion, the present study demonstrates that mifepristone at a daily dose of 5 mg is a safe and potentially effective contraceptive. The relatively high incidence of amenorrhoea or reduced amount of scanty bleeding is likely to be better accepted by women than the irregular unpredictable menstrual bleeding that occurs in the majority of women taking the POP. The reduction of menstrual blood loss should convey health benefits to women particularly in developing countries where the incidence of anaemia is high. Because of its antagonism of progesterone the risk of breast cancer may be reduced rather than increased as is the case with COCs containing estrogen and progestogens [494]. A large multicentre phase III trial is required

further to assess contraceptive efficacy and safety, particularly with respect to endometrial cancer.

Study 2

Objective

Protection again sexually transmitted diseases and HIV would be a significant non-contraceptive benefit for any method of contraception. We explored whether daily low-dose antigestogen, mifepristone, modulates underlying mechanisms involved in transmission of STI's including HIV by investigating its effects on vaginal morphology, steroid receptor and natural antimicrobial [secretory leukocyte protease inhibitor (SLPI), human beta defensins mRNA (HBD1, HBD2, HBD3, HBD5), granulysin and elafin] content [490]. We also report the pharmacokinetics and pharmacodynamics of 5 mg mifepristone supplied by Hualian Pharmaceuticals Co. Ltd. (Shanghai, China), which has been used in the present study as well as in previous studies [448].

Subjects and methods

We report two pilot studies using 5 mg mifepristone — the study on vaginal epithelium that was carried out in Edinburgh and the pharmacokinetic study in Helsinki.

Effects of mifepristone on vagina

A single-center, open, single-group study concerning female volunteers was undertaken. Eight healthy subjects with a mean age of 35 years (range, 27–39 years) and a mean body mass index (BMI) of 23 kg/m2 (range, 17.3–28.2 kg/m2) who had regular menstrual cycles (25–42 days) were recruited to the study. The women agreed to refrain from the use of vaginal medications during the study period or from sexual intercourse 48 h prior to vaginal biopsy. Women who had breast-fed or had taken hormonal contraception less than 3 months prior to the study and those with vaginal or pelvic infections (current or past) were excluded. The proposal was approved by the local ethics committee (institutional review board). All women gave written

informed consent before enrolment and were screened before entering the study. Screening included a full medical, gynaecological history and examination, including measurement of height, weight, blood pressure and pulse. Blood samples were collected for measurement of routine clinical chemistry and haematology (liver function tests, urea and electrolytes, glucose, full blood count). ßHCG was measured to exclude pregnant women from the study. Subjects were studied for one pre-treatment cycle, one cycle of treatment (approximately 33 days) and one post-treatment cycle. Each subject was reviewed on Day 12 of the pre-treatment menstrual cycle (Visit 1), at the end of treatment (Visit 2)
and on Day 12 of the post-treatment menstrual cycle (Visit 3). Subjects were given a menstrual record card and were asked to record all vaginal bleeding.

Assessment of ovarian function

Ovarian function was monitored by measurement of ovarian steroids in urine and plasma and by transvaginal sonography. All subjects collected twice weekly samples of early morning urine during the study period, starting in the early follicular phase (Days 1–5) of the pretreatment cycle. Aliquots were frozen and stored at -20°C until assayed for estrone glucuronide (E1G), pregnanediol glucuronide (PdG) and creatinine (Cr). PdG was measured using a direct enzyme immunoassay, while E1G was measured by direct immunoassay. Ovarian follicular activity during treatment was compared with that during the follicular phase of the pre-treatment cycle, and the activity was scored as complete suppression, partial suppression or continued follicular activity. Ovulation was deemed to have occurred if the excretion of PdG exceeded 0.5 mmol/mol Cr and was at least threefold higher than that in the preceding week. A detailed description of this methodology is given in our previous report [448]. Blood samples were collected at all study visits and assayed for oestradiol (E2) and progesterone (P) using radioimmunoassay (RIA). Assay characteristics and methodology have been described in our previous reports [448]. A transvaginal ultrasound scan was carried out at all study visits, and ovarian dimensions, follicle number and diameter and presence of ovarian cysts were recorded.

Vaginal biopsy

A full-thickness vaginal biopsy was taken from the lateral vaginal wall 4 cm proximal to the hymeneal ring on Day 12 of the pre-treatment cycle (Visit 1) and, again, after completion of treatment (Visit 2). One ampoule of Citanest with octapressin (3%; prilocaine hydrochloride, 30 mg/mL; felypressin, 0.03 U/mL; Dentsply) was injected into the lateral vaginal wall as a local anesthetic and haemostatic agent. This also elevated the target vaginal tissue sufficiently to permit easy access for a biopsy. Vaginal biopsy was performed using a long Schumacher forceps. Vaginal tissues were stored in RNAlater (Applied Ltd., Cambridgeshire, UK; RNAlater is an aqueous storage reagent to stabilize and protect RNA) and neutral-buffered formalin (NBF; for future preparation of paraffin-embedded tissue for immunohistochemistry). A second biopsy was taken if adequate sample was not obtained from the first biopsy. Vaginal

bleeding from the biopsy site was controlled using either silver nitrate or, if necessary, a Vicryl 3-0 suture (Ethicon, UK), depending on the amount of bleeding.

An endometrial biopsy was collected using Pipelle endometrial sampler (Prodimed, Neuilly-en-Thelle, France) at end of treatment (Visit 2), fixed in NBF and embedded in paraffin. Endocervical and posterior fornix swabs were collected at all study visits and cultured for pathogenic organisms, for example, gonococcus, trichomonas and streptococcus, which might influence the parameters studied in target vaginal tissues.

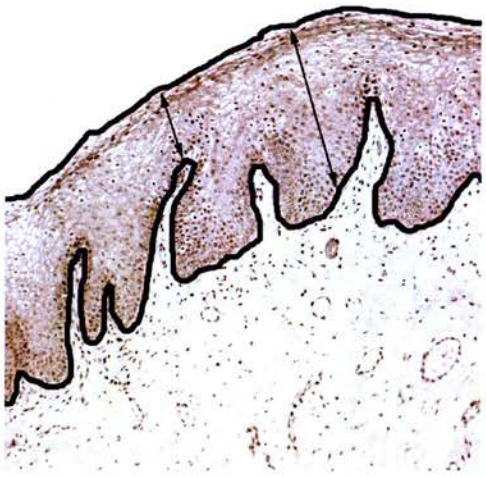
Safety parameters

At each study visit, blood pressure and pulse were measured, and blood was taken for measurement of routine clinical chemistry and hematology. In addition, each subject was asked to report any health problems or adverse events that had occurred since the last visit.

Vaginal thickness measurement

Vaginal tissue samples were embedded in paraffin, and serial 5um sections were cut at a 90° angle to the vaginal surface epithelial layers. The sections were stained with hematoxylin and eosin, and digital images were captured at x10 eyepiece magnification using a Spot microscope connected to a Windows PC computer. Image Proplus 4.5 (Media Cybernetics, Silver Spring, CO, USA) software was calibrated to match the eyepiece used to capture the image. The surface and basement membranes of vaginal epithelium were outlined using a trace tool within the software (Fig. 5). The software automatically calculated the average distance between the two traced outlines.

Figure 5: Vaginal thickness measurement. Average thickness between surface and basement membrane (trace tool, Image Proplus 4.5, Media Cybernetics).



Immunohistochemical (IHC) localization of oestrogen receptor alpha (ERa), oestrogen receptor beta (ERß), progesterone receptor (PR), androgen receptor (AR) and proliferation marker phospho-histone H3 (PH3)

Immunohistochemistry was carried out on both the vaginal and endometrial biopsies for the following proteins of interest: ERa (Novocastra, Newcastle-upon-Tyne, UK), ERß (Serotec, Oxford, UK), PR (A+B) (Novocastra), AR (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), PH3 (Upstate Biotechnology, Poole, UK) and SLPI (Hycult Biotechnology, Cambridge, UK). All antibodies used were mouse monoclonal, except for PH3, which was a rabbit polyclonal. They were tested individually at a range of dilutions and at different antigen retrieval conditions to determine the protocol that gave the least background and highest specific staining (Table 5). Positive and negative controls were included in every run. In most cases, negative controls were performed by adding a matched IgG control antibody (mouse IgG, Sigma, Poole, Dorset, UK; rabbit IgG, Vector Laboratories, Peterborough, UK) of the same species and at the same antibody concentration as the primary antibody. Protocols were carried out either on the bench or with the use of a Bond-X automated immunohistochemistry staining machine (Vision Biosystems, Newcastle, UK).

All tissue sections were initially prepared in a similar manner. Five-micron paraffin-embedded tissue sections were dewaxed in Histoclear (National Diagnostics, Hessle, UK) and rehydrated in descending grades of alcohol to distilled water (dH2O). Antigen retrieval was then carried out by heating the sections either in a microwave oven (setting: high) or in a Tefal Clipso pressure cooker (Setting 2/high, Tefal, Nottingham, UK). The buffer concentration and duration of antigen retrieval varied depending on protocol (Table 1). Sections were left to cool in both cases for 20 min. Endogenous peroxidase activity was quenched by immersion in 3% hydrogen peroxide (BDH, Poole, UK) in methanol for 30 min at room temperature. For AR only, sections were incubated in avidin for 15 min at room temperature (Vector Laboratories), followed by incubation in biotin (Vector Laboratories), also for 15 min at room temperature. Nonspecific binding of the primary antibody was blocked by incubating the sections for 20 min at room temperature in a 1:5 dilution of nonimmune serum (Autogen Bioclear, Holly Ditch Farm, Wilts, UK) in phosphate-buffered saline containing 5% bovine serum albumin. All immunostaining methods employed a detection system dependent on visualization of the reaction using a horse-radish peroxidase enzyme and the chromagen 3′ 3-diaminobenzidine (DAB). After the DAB step, the sections were counterstained in hematoxylin before dehydrating them in ascending grades of alcohol and mounting them from xylene with Pertex (Cellpath plc., Hemel Hempstead, UK). Full details of each individual protocol are given in Table 5.

Table 5: Immunohistochemistry protocol

Protein	Tissue	IHC method	Antigen retrieval	Primary antibody	Negative control	Detection system
ERa	Vagina	Bond-X machine ABC detection	Pressure cook: 0.01M sodium citrate, pH6, 5min	1:100 mouse anti-Era 3h at room temperature	MIgG1 1:1300	Biotinylated secondary and ABC detection (Vision biosystems)
	Endometrium			1:1000 mouse anti-Era 3h at room temperature	MigG1 1:13,000	
ERß	Vagina	Biotinylated secondary and ABC-streptavidin	Pressure cook: 0.05M glycine/EDTA, pH 8, 7min	1:40 mouse anti-ERß Overnight at 4°	Serum [a]	Biotinylated rabbit antimouse antibody and ABC-streptavidin (both from DAKO, Cambridgeshre, UK)
	Endometrium					
PR	Vagina	Goat antimouse envision system	Microwave: 0.01M sodium citrate, pH6, 10 min	1:80 mouse anti-PR 37°C for 60min	MIgG1 1:2000	Goat antimouse envision system (DAKO)
	Endometrium					
AR	Vagina	Biotinylated secondary and ABC-Elite	Pressure cook: 0.01M sodium citrate, pH 6, 5min	1:400 rabbit anti-AR Overnight at 4°C	RIgG 1:2000	Biotinylated goat antirabbit antibody and ABC-Elite (both from Vector Laboratories
	Endometrium					
H3	Vagina	Goat antirabbit envision system	Pressure cook: 0.01M sodium citrate, pH6, 10 min	1:1000 rabbit anti-H3a Overnight at room temperature	RIgG1 1:1000	Goat antirabbit envision system (DAKO)
	Endometrium					
SLPI	Vagina	Biotinylated secondary and ABC-Elite	Microwave: 0.01M sodium citrate, pH6, 10min	1:50 mouse anti-SLPI Overnight at 4°C	MIgG 1:500	Biotinylated horse antimouse antibody and ABC-Elite (both from Vector Laboratories)
	Endometrium					

[a] The anti-ERß antibody has been previously preabsorbed with the peptide to which it has been raised [495]

IHC analysis

We used a descriptive methodology as there is no quantitative or semiquantitative methodology established for analysis of vaginal tissue samples in our laboratory. The intensity and distribution of immunostaining for ER, ERß, PR, AR, PH3 and SLPI are described for vagina (epithelium and stroma) and endometrium (glands, stroma and surface epithelium), and differences between pre- and post-treatment samples were analyzed.

RNA extraction

Tissue was minced using a standard sterile surgical scalpel blade and immersed in 2 mL of Tris-buffered saline (Sigma-Aldrich, St. Louis, MO, USA). The mixture was homogenized for 60 s and incubated overnight at 4°C. The following day, tissue sample was warmed to room temperature and 200ug of bromochloropropane was added. The mixture was centrifuged at 14,000 rpm at 4°C for 15 min, and aqueous-phase RNA (supernatant) was transferred to a fresh tube. Five hundred microliters of isopropanol was added and incubated at 4°C for 60 min. The mixture was centrifuged for 10 min; the supernatant was discarded, and the pellet was washed with 1 mL of 70% ethanol. The mixture was centrifuged for 5 min,
and the supernatant was discarded, allowing the pellet to dry for 5 min. The pellet was resuspended in 20 uL of RNA solution. To standardize measurements between the various
biopsy specimens, we assessed the same amount of RNA in each sample. The amount of specific amplicon is related to ribosomal 18S, which is constant relative to the amount of
cDNA present and, subsequently, to an experimental internal control. The RNA was reverse transcribed (TaqMan Reverse Transcription Reagents Kit, Applied Biosystems, Foster City,
USA) and polymerase chain reaction (PCR) amplified (TaqMan Universal Master Mix, No Amp Erase UNG, Applied Biosystems) according to the manufacturer's instructions. PCR amplification of cDNA was performed on an ABI Prism 7700 Sequence Detection System (Applied Biosystems). Specific forward and reverse

primers (300 nmol/L) and probe (200 nmol/L, all synthesized by BioSource UK, Nivelles, Belgium) for the natural antibiotic were also added. Ribosomal 18S cDNA was measured using TaqMan Ribosomal RNA Control Reagents (VIC dye, Applied Biosystems) in each sample as an internal control following the manufacturer's protocol. Samples were measured in triplicate, and no template controls were included in all runs. Primers and probes for quantitative PCR were designed using the PRIMER EXPRESS program (Applied Biosystems; Table 6) [496,497,498,499,500,501], and probes were fluorescently labeled with the proprietary dyes FAM (5′) and TAMRA (3′).

Table 6: Sequences of quantitative PCR primers and probes for natural antimicrobials

	Forward primer	Reverse primer	Probe
SLPI	GCATCAAATGCCTG GATCCT	GCATCAAACATTGGC CATAAGTC	TGACACCCCAAACCCAACAA GGAGG
HBD1	TCAGCAGTGGAGGG CAATG	CCTCTGTAACAGGTG CCTTGAAT	CTCTATTCTGCCTGCCCGATC TTTACCAA
HBD2	CTGATGCCTCTTCCA GGTGTTT	CTGGATGACATATGG CTCCACTCT	AAGGCAGGTAACAGGATCG CCTATACCACCA
HBD3	CAGAGGCGGCCGGT GT	CGAGCACTTGCCGAT CTGTT	CTGTGCTCAGCTGCCTTCCA AAGGA
HBD4	GGCAGTCCCATAAC CACATATTC	TGCTGCTATTAGCCG TTTCTCTT	TGTCCAATTCAAATTCGCTTC TCACTGGA
HBD5	ACCTCAGGTTCTCAG GCAAGAG	AGAGGGACTCACGGG TAGCA	CTGCTATTGCCGAACCGGCC GT
Granul ysin	CAGGGTGTGAAAGG CATCTCA	GGAGCATGGCTGCAA GGA	CGGCTGCCCCACCATGGC
Elafin	TGGCTCCTGCCCCAT TATC	CAGTATCTTTCAAGC AGCGGTTAG	ATCCGGTGCGCCATGTTGAA TCC

Pharmacokinetic study

A pharmacokinetic study of 5 mg of mifepristone was carried out among the same cohort of women who had previously participated in another pharmacokinetic study [502]. The study was approved by the Institutional Review Board of the Helsinki Central Hospital and the Finnish National Agency for Medicines; all subjects signed an informed consent document. Subject characteristics, methodology, collection of sample, analysis of serum levels of mifepristone and calculation of pharmacokinetic parameters were as described previously [502]. In brief, six healthy women, with regular menstrual cycles (23–36 days), a mean age of 32 years (range, 21–45) and a BMI that ranges from 19 to 26 kg/m2, volunteered for the study. The 10-mg mifepristone tablets supplied by Hualian Pharmaceuticals Co. Ltd. were halved, and a dose of 5 mg, po, was ingested on Day 10 or 11 of the menstrual cycle. Blood samples were collected at 0, 1, 2, 4 and 8 h and, thereafter, daily for the next 6 days and on Day 10 following mifepristone ingestion. Mifepristone was measured in serum by RIA after extraction with n-hexane:ethyl acetate and separation by column chromatography using ChromosorbR [503]. The detection limit is 0.36 nmol/L, and the intra-assay and interassay coefficients of variation were 8.4% and between 10.3% and 13.6%, respectively.

Statistical analysis

Statistical analysis was performed using SPSS (SPSS, Inc., Chicago, IL, USA) and Excel 2002 (Microsoft Corporation, Reading, UK). Sex steroid, vaginal thickness and pharmacokinetic data are expressed as mean with either standard error of the mean or standard deviation. Menstrual cycle data are expressed as mean and range. Nonparametric tests (Friedman's test, Wilcoxon Signed Rank Test and Mann–Whitney test) were used to compare sex steroid level, menstrual data, vaginal thickness and natural antimicrobial RNA content before and after treatment.

Results

Effects of mifepristone on vagina

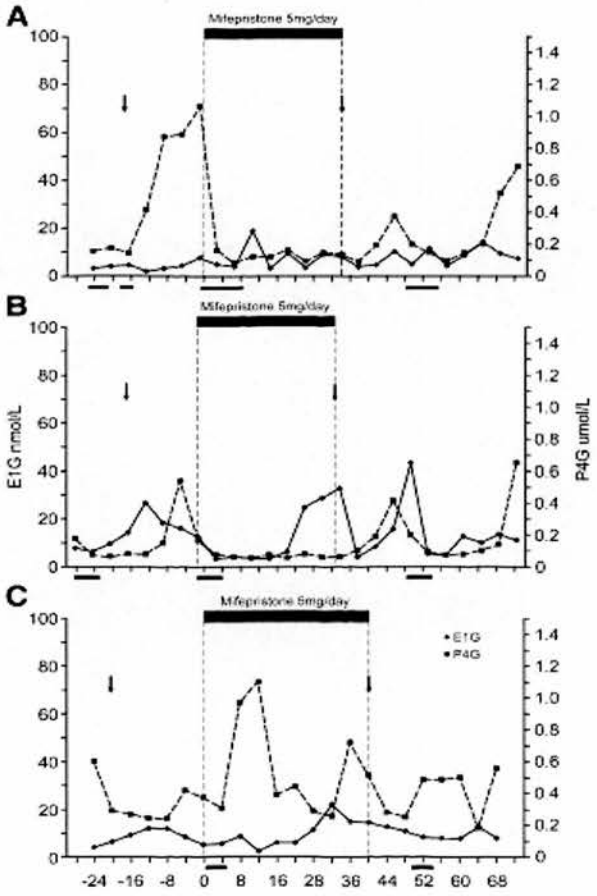
All eight subjects completed the study. The subjects took mifepristone 5 mg/day, po, for an average of 33 days (range, 28–40).

The average length of the control menstrual cycle was 27 days (range, 24–29), while that of the control menstrual period was 5.7 days (range, 4–9). All eight women reported amenorrhea during ingestion of mifepristone. The time from discontinuing the mifepristone treatment to the next bleeding episode was 17 days (range, 10–23); thus, the length of the mifepristone cycle was 50 days (range, 38–63). Average length of the bleeding episode after discontinuation of mifepristone was 5.1 days (range, 4-7).

During the treatment with mifepristone, seven of the eight subjects experienced either complete suppression of ovarian activity (3/8 women, Fig. 6A, Subject 8) or persistent follicular activity but no ovulation (4/8 subjects, Fig. 6B, Subject 4). In the remaining subject (Subject 7, Fig. 6C), there was a threefold rise in the excretion of pregnanediol in the first 10 days of treatment, suggesting the formation of a corpus luteum. However, there was no menstrual bleeding when the level of pregnanediol dropped 14 days after starting the mifepristone. In this subject, a persistent ovarian cyst with a 42-mm diameter was detected at completion of treatment (Day 40) when the level of P (14 nmol/L) was slightly raised, which was consistent with a persistent unruptured partially luteinized follicle.

Multiple follicles were detected by transvaginal ultra- sound (diameter of the largest ranged from 10 to 29 mm) in all eight subjects. The concentrations of E2 in blood samples collected at Visit 2 on the last day of mifepristone were compatible with persistent follicular activity (496±pmol/L). In seven of eight women, the concentration of P was <10 nmol/L (3±1 nmol/L), indicating lack of ovulation.

Figure 6. Excretion of metabolites of ovarian steroids in women taking 5 mg of mifepristone for 31–36 days. Estrone, E1G; pregnanediol, PdG; x-axis shows timescale; Day 0, start of treatment. Pretreatment (negative values), treatment (boxed) and follow-up cycles are shown. Black bars represent menstrual episodes; arrow denotes vaginal biopsy. (A) Complete suppression of ovarian activity. (B) Persistent follicular activity but no ovulation.(C) Single ovulatory episode and no menstruation following fall in pregnanediol levels.



Endometrial histology

Endometrial biopsies obtained at the end of mifepristone treatment displayed inactive or weakly proliferative endometrium in seven of the eight subjects. In one subject (Subject 9), tortuous glands with evidence of intraluminal secretion were seen. As expected, there was strong immunostaining of ERa and ERß as well as of PR (A+B) and AR in both glands and stroma. Histological evidence of mitosis was absent or infrequent in all samples.

Vaginal histology and vaginal thickness

Vaginal biopsy was obtained in all subjects without complications both before and after mifepristone administration. In seven of eight subjects the pretreatment vaginal biopsy was performed between Day 6 and Day 16 of the follicular phase prior to ovulation as confirmed by the hormone levels (mean±SEM: E2, 659±141 pmol/L, P, 4.3±10 nmol/L). In the remaining subject (Subject 5), ovulation had already occurred on the day of biopsy (Day 14 of menstrual cycle) as indicated by high circulating concentration of P (44 nmol/L). Post-treatment vaginal biopsy was performed on Day 33 (range, 28–40) of treatment.

The histology of the vagina showed the expected basal layer of epithelium mounted by up to 20 layers of more superficial desquamating cells (Fig. 5). Vaginal thickness was not altered during administration of mifepristone (342±40um vs. 303±69um; p =0.2, NS).

Steroid receptor expression in the vagina (ERa, ERß, AR and PR)

There was nuclear staining of ERa, ERß and AR in both vaginal stroma and in the epithelium. Immunoreactivity extended through the basal and intermediate layers but not through the superficial layer of vaginal epithelium (Fig.7). Staining was far more evident in the epithelium as compared with the stroma. There was no significant difference in sex steroid receptor immunostaining after mifepristone administration.

Apart from a few scattered nuclei in the stroma, the basal layer of vaginal epithelium immunostaining for PR was confined to stroma (Fig. 7). There was no significant difference in PR immunoreactivity after administration of mifepristone.

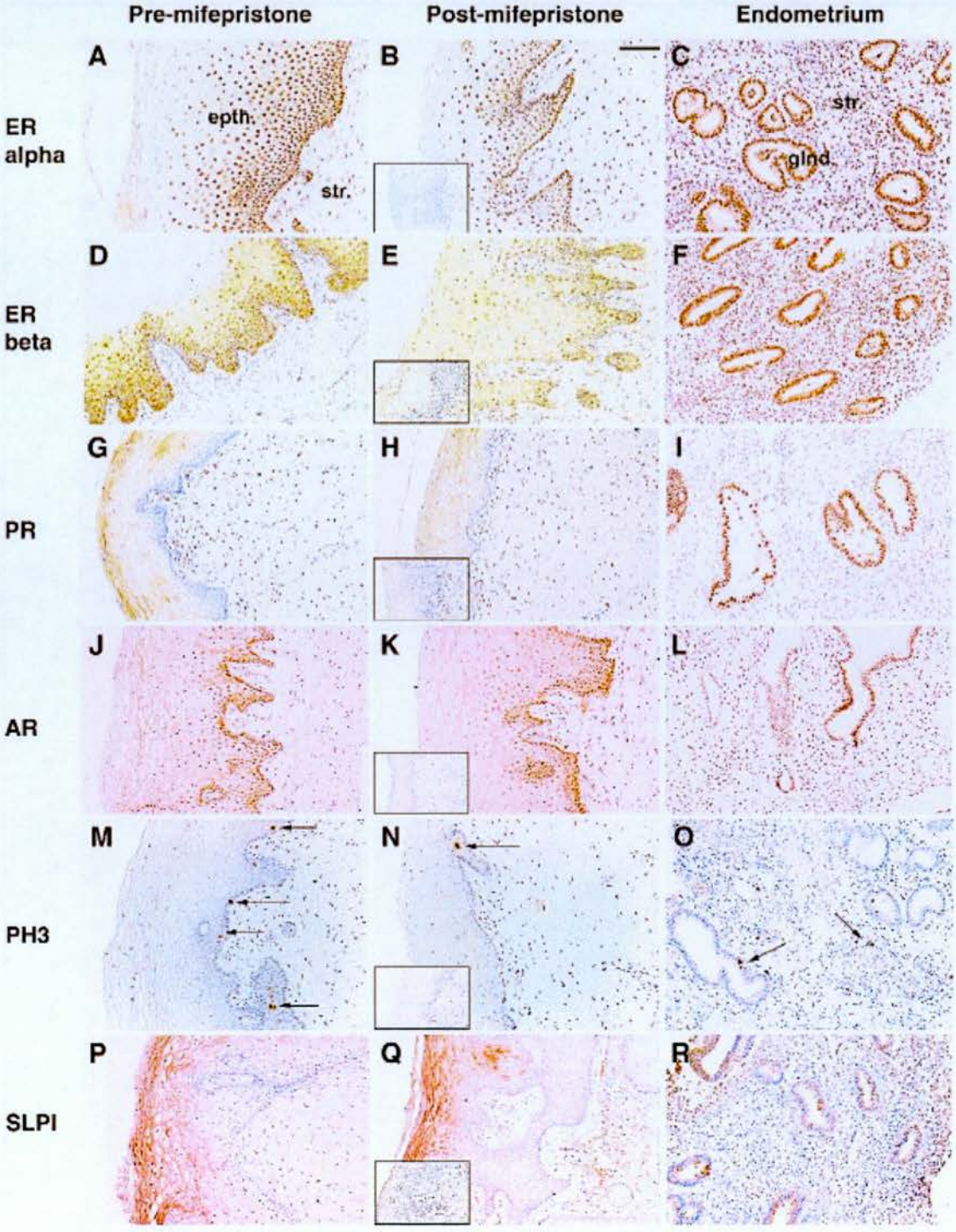
PH3 immunoreactivity

PH3 immunostaining was confined to scattered nuclei in the basal layers of the vaginal epithelium (Fig. 7). There was no significant change in immunoexpression following treatment with mifepristone.

Natural antimicrobial mRNA and protein expression

SLPI mRNA was present in vagina, and this epitope was localized to the superficial layer of the vaginal epithelium both before and after mifepristone administration (Fig. 7). SLPI immunostaining was also demonstrated in the superficial layers of luminal and glandular epithelium of the endometrium (Fig. 7). SLPI mRNA expression was unchanged following treatment with mifepristone ($p>0.05$, Wilcoxon test). HBD1, HBD2, HBD3, HBD5, granulysin and elafin mRNA were present in the vagina. The expression was unchanged following treatment with mifepristone ($p>0.05$, Wilcoxon test).

Figure 7. IHC localization of ERa, ERß, PR, AR and PH3 in the vagina [epithelium (epth) and stroma (str)] and endometrium [gland (glnd) and stroma (str)] before and after treatment with 5 mg mifepristone. Scale bar = 100 Am; inserts denote negative controls. Strong expression of ERa (A and B), ERh (D and E) and AR (J and K) in the basal and parabasal layers of epithelium and a relative lack of PR (G and H) in the epithelium (there was no observed change following treatment); PH3 expression in the basal layers (M and N; arrows) and endometrium [postmifepristone (O; arrows)]; strong expression of all steroid receptors in the posttreatment endometrium (C, F, I and L); SLPI confined to superficial layers of vaginal epithelium (P and Q) and endometrial glands (R).



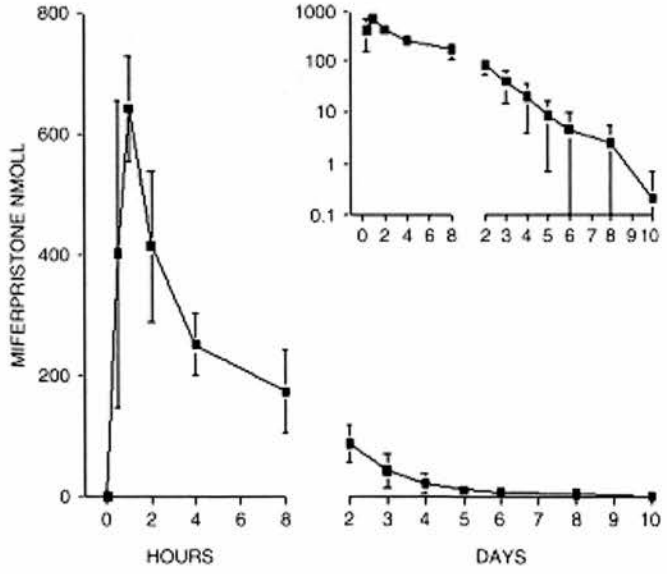
Safety parameters

There was no derangement in heart rate, blood pressure or in haematology and biochemistry parameters including liver function tests during the study. Vaginal and cervical bacteriology swab tests cultured negative for pathogenic organisms in all subjects. Vaginal biopsy was well tolerated by all women. One woman had possible low-grade endometritis following endometrial biopsy and was successfully treated with a 7-day course of antibiotics.

Pharmacokinetics of 5 mg mifepristone

Serum levels (mean±SD) of mifepristone following ingestion of 5 mg mifepristone are summarized in Fig. 8. Mean C_{max} measured at 1 h was 641.7 nmol/L (range, 502–740 nmol/L). All subjects showed a similar pattern of descending serum concentrations of mifepristone. The elimination phase half-life was 18±5.1 h (mean±SD). The mean (SD) areas-under-concentration-curves AUC_{0-8} h and AUC_{0-24} h were 2.4 (0.5) and 4.8 (1.3) Amol/L, respectively.

Figure 8. Serum levels (mean±SD) of mifepristone following ingestion of 5 mg. The data are depicted on both linear (lower) and semilogarithmic (insert) scales.



Discussion

The vagina is a key portal of entry for HIV and other STIs. In this article, we report the effect of a potential new contraceptive pill on different parameters involved in the natural defenses of the vagina to infection. Vaginal epithelial thickness, steroid receptor and natural antimicrobial content and distribution were unchanged following treatment with mifepristone for 30–40 days. Vaginal epithelial thickness is regulated by the levels of circulating oestrogen. Epithelial thickness is maximal at time of ovulation [504,505,506,507] and decreases in the luteal phase and postmenopause [504]. There is a significant reduction in circulating oestrogen levels following long-term gestagen treatment [506]. Severe vaginal atrophy has been demonstrated in primate studies, and this clearly increases the risk of SIV transmission [508]. However, the response of human vaginal epithelium to gestagen-induced hypo-oestrogenism is variable. A small but significant decrease (10%) in thickness has been demonstrated in one study [506], whereas most other studies have demonstrated no change [509,510]. Paradoxically, vaginal epithelial hyperplasia has been reported in users of depot medroxyprogesterone acetate (DMPA), oral contraceptive pill and P implants [472,509,511]. E2 levels in all three treatment groups were significantly lower compared with normal menstruating women. The steroid receptor content and distribution were similar except for PR, which was suppressed in the DMPA group.

It is difficult to reconcile all available data into a working hypothesis due to differences in study designs and methodology. The vagina has diverse embryological origins [512]; Ildgruben et al. (2005) used a cross-sectional study design and sampled lateral vaginal fornices, whereas we used a longitudinal study design and sampled lateral midvaginal wall in keeping with other reports [511].

Although vaginal epithelium clearly responds to circulating oestrogen, the underlying cellular and molecular mechanisms are poorly understood. We localized PH3, a marker for mitosis and cellular proliferation, to a few scattered nuclei in the basal layers of the vaginal epithelium. The strong nuclear expression of ER and AR in the basal and parabasal layers suggests a role in the regulation of epithelial proliferation. Oestrogen treatment induces surface keratinization and hyperplasia of primate vaginal epithelium, and we expected a similar change in mifepristone-treated vaginal samples due to unopposed oestrogen effect. The observed epithelial thickness in control samples in the present study (mean pre-treatment thickness, 342um) is comparable to other reports [472,509,510]. Thickness was unchanged following mifepristone treatment, and this agrees with similar work in cynomolgus monkeys [513]. There are several possible explanations for our findings. Firstly, mifepristone treatment did not have any effect on level of circulating steroid hormones. Secondly, PR was localized to a few scattered nuclei in the basal layers of vaginal epithelium and, hence, mifepristone, a high-affinity PR ligand, did not have any direct epithelial effects.

Consistent with previous reports, we have demonstrated a strong immunoexpression of ER and AR as well as a relative lack of PR in the vaginal epithelium compared to subepithelial stroma [514,515,516,517,518,519,520,521]. The distribution of steroid receptors has obvious implications for the development of topical and parenteral steroid treatment. Available data, including the present study, indicate that it is likely that estrogen preparations will act via epithelial ER, whereas P preparations are likely to have an endocrine and paracrine effect after binding with the subepithelial PR. The role of AR in the genital tract is unclear, but AR may play a role in regulating endometrial proliferation [522,523,524] and in modulating vaginal blood flow [514] and female genital sexual arousal [521].

The endometrium sampled at the end of mifepristone treatment showed persistent proliferative histology and strong ER, PR and AR expression, which is consistent with our previous reports [447,523] and which demonstrates that the mifepristone preparation used in this study is comparable to that we have used previously from another source (Exelgyn, Paris).

The expression, regulation and role of natural antimicrobial compounds in the female

reproductive tract are extensively reviewed elsewhere [498]. We have investigated natural antimicrobials that regulate innate protection at mucosal interfaces. SLPI has been shown to play an important role in limiting transmission of HIV [486,525,526] and other lower genital tract infection [485]. SLPI mRNA has been demonstrated in vaginal fluid previously [485,486]. In the present article, we demonstrate mRNA in vaginal tissue and immunolocalize the protein to the superficial layers of the vaginal epithelium. The expression in superficial layers of surface and glandular endometrium is in keeping with previous reports [497]. HBD1 [527] and elafin [528] mRNA have been reported in human vagina. We show that HBD2, HBD3, HBD5 and granulysin mRNA are present in vaginal tissue. HBD4 mRNA was not previously demonstrated. Natural antimicrobial expression is modulated by hormonal treatment, and up-regulation of endometrial SLPI by P is attenuated in the presence of mifepristone [501]. Reassuringly, expression and distribution of natural antimicrobials were unchanged following mifepristone treatment.

Low-dose mifepristone suppressed menstruation and ovulation in a majority of women in the present study. This adds to similar observations in our previous reports [448,529] and also demonstrates the biological activity of mifepristone supplied by Hualian Pharmaceuticals Co. Ltd. The pharma-cokinetic properties of the 5-mg dose from the above supplier follow a first-order linear kinetic pattern [502,530]. The half-life of 18 h is shorter than the 20 h previously reported for 10 mg mifepristone, whereas the AUC_{0-24} h was half that of the 10-mg dose [502]. All women received planned treatment for at least 5 days beyond the expected date of menstruation (33 days) so that any post-treatment vaginal bleeding was due to withdrawal of mifepristone rather than to a spontaneous menstruation. Fortuitously, Subject 7 was administered mifepristone for 40 days. She did not bleed, although there was evidence of ovulation and formation of corpus luteum followed by a significant decrease in pregnanediol levels. This supports our previous reported findings that mifepristone-induced amenorrhea is a result of direct endometrial effects that are possibly mediated by a rise in glandular and luminal glucocorticoid and AR [531].

In summary, we have shown that low-dose mifepristone does not influence vaginal thickness and proliferation. We have demonstrated that ER (ERa and ERß), PR, AR, SLPI and other natural antimicrobials are present in the human vagina. There is no

change in epitope expression following mifepristone treatment. The presence and distribution of vaginal steroid receptors have implications for the development of topical and systemic preparations to modulate this sex steroid responsive organ in health and disease.

Chapter 6: Discussion

Reproductive health has been identified as both a fundamental human right and also a social and economic imperative [8].

Unintended pregnancy is common worldwide. A study by Hubacher et al (2008) estimates that in sub-Saharan Africa just over one third (39%) of pregnancies are unintended [532]. Our study similarly found that one third (35%) of all pregnancies were not clearly intended (10% of pregnancies were clearly unintended and 25% were ambivalent) [46].

Contraception is a means to reduce unintended pregnancy.

In the context of this background the studies included in this thesis were devised. The overarching theme of the thesis was to improve choice and use of contraception primarily by development of new methods of contraception – in this case, mifepristone. However recognising that development of any method, let alone mifepristone which is surrounded by political and ethical controversy, takes at least ten to fifteen years as not only does the method require to be developed but often the infrastructure to support the method has to be established too, it was felt to be imperative that we continue to improve uptake and use of existing methods by trying to achieve a better understanding of current behaviours of women and couples, with respect to contraceptive use and risk taking, and also identify what adaptations to current methods and services might be acceptable. Findings could then be translated to adapt existing methods and shape services to better suit those at risk of unintended pregnancy.

Study 1: Exploring levels of unintended pregnancy and use of emergency contraception

A study was undertaken to explore levels of unintended pregnancy and use of emergency contraception (EC) [46] in an effort to better understand behaviours of women and couples with respect to contraceptive use and risk taking. Worldwide the prevalence of use of contraception has increased with time [223] and this study found that of those attending for induced abortion, 74% were using a method of contraception in the month they became pregnant (appendix 1). This finding is slightly lower than those of Schunmann et al (2006) (84%) [70] and Garg et al (2001) (98% of those undergoing repeat abortion and 83% of those undergoing first time abortion) [73]. Data suggests that true method failure accounts for only 5% of unintended pregnancies occurring when contraception is correctly and consistently being used [74,75,76]. The remaining 95% is due to non-use or less effective use of modern methods of contraception. This very high failure rate is likely to reflect the difficulty of using non-permanent methods consistently and correctly.

The study demonstrated that even when risk of unintended pregnancy has occurred only one in ten women acted to try and prevent pregnancy by using emergency contraception (EC) and only 65% of those women used EC with every act of unprotected intercourse in the month of becoming pregnant. Other studies have demonstrated that failure to recognise risk of pregnancy is common [161] and neither advance provision of EC to keep at home or availability of EC over the counter in UK pharmacies have resulted in increased use or reduced unintended pregnancies [169,170,171,175].

This study exploring intendedness of pregnancy and use of EC does require to be replicated in different settings to see if findings elsewhere are similar. However, it seemed evident from the results of the survey that there is a real need for women and couples to receive accurate information about pregnancy risk and that unless this risk

is recognised and acknowledged EC is unlikely to make a substantial difference to pregnancy rates. It also made evident that for contraception to be successful it must firstly be used but equally importantly must be used correctly and consistently. Thus firstly there is a need to make contraceptive methods, including EC, as accessible as possible and as attractive as possible. Secondly there is a need to encourage women, especially those who want to avoid pregnancy and are taking risks, either by non-use or by incorrect or inconsistent use of contraception, to take up use of methods which have long duration of action and do not require any active adherence once initiated. Such methods comprise implants, injectables and intra-uterine devices and are better known as long-acting reversible contraception (LARC).

Study 2: Assessing continuation rates of Implanon®

Consequently a study was undertaken to attempt to quantify acceptability of Implanon® by assessing continuation rates [231]. As discussed in chapter 3, continuation rates act as one objective measure of acceptability. Continuation rates of all methods of contraception are generally disappointing [233] and are lower for methods which do not require intervention by a health professional [233]. In clinical trials of Implanon® where participants are highly motivated and where there is regular follow-up they are between 90 and 95% at six months and 80 and 88% at 12 months. In clinical practice users of Implanon® are at most followed up once after three months and thereafter are only reviewed if there are any problems or when the implant is due for removal. In this 'real life' study of Implanon® use continuation rates were found to be encouraging with 75% continuing at 1 year, 59% at two years and just under half (47%) continuing for 2 years and 9 months [231]. These rates should reassure healthcare providers and commissioners that increasing uptake of this LARC as recommended in the National Institute of Health and Clinical Excellence (NICE) guideline (2005) [180] is worth the effort and the cost. The fact that more than one third of women chose to continue to use Implanon® once their first implant had expired reflects a strong level of satisfaction on the part of users.

It is widely acknowledged that at the time this study was conducted (2005) and even more recently [224] the most commonly used forms of reversible hormonal contraception are condoms and oral contraceptive pills [224]. These are notoriously difficult to use consistently and correctly [86,179]. The NICE guideline published in October 2005 recommended that healthcare providers give information on and offer a choice of all methods, including LARC [180]. They defined LARC to comprise any of three methods: intrauterine devices – both non-hormonal (IUD) and hormonal (IUS); Implanon® and Depo-provera® [180].They stated that contraceptive service providers should be aware of three things: that all currently available LARC are more cost-effective than the combined oral contraceptive pill even at one year of use; that the IUD, the IUS and Implants are more cost-effective than the injectable contraceptives; and that increasing the uptake of LARC methods will reduce the numbers of unintended pregnancies [180]. The data from this study on continuation rates added to the body of knowledge being collated by the NICE at the time in producing guidance on the effective and appropriate use of LARC [180]. It also added impetus to the argument for increased provision of Implanon® outside of the family planning setting thus making it more easily available to women.

Study 3: Exploring the acceptability of self-administration of subcutaneous Depo-Provera®

In keeping with the proposed medium term strategy to adapt existing methods to increase acceptability and taking into consideration that Depot medroxyprogesterone acetate (DMPA) when given intramuscularly was less cost-effective than other methods of LARC [180] the opportunity was taken to explore the theoretical, acceptability of self administered subcutaneous depot medroxyprogesterone (DMPA-SC) [369].

DMPA is another important method of long-acting reversible contraception. Anecdote suggests that in the UK it is increasingly popular among young women, and among women of all ages who like the convenience of amenorrhoea. In the USA the fall in teenage pregnancy rates has been attributed to increased use of Depo-Provera®

[100]. The need to attend a health professional every three months simply for a single injection is a clear disadvantage of the method, both for users and for commissioners of healthcare services due to its expense relative to other methods [180].

Subcutaneous DMPA should therefore be far more cost-effective as women would only have to attend a clinic annually. Additional costs would be incurred in setting up and maintaining a reminder system and a potential rise in telephone enquiries. Additionally the outset cost would be higher as women would require training to self-administer however if the system is adhered to then in the long-term the cost would be lower as found with other long-acting methods of contraception [180].

In the survey administered to current DMPA users a third of all DMPA users were approached and completed the survey. Two thirds (67%) of current users expressed a theoretical interest in self-administration. In the second survey 70% of current users, 40% of ex-users and 26% of never-users expressed a theoretical interest in self administration. We were somewhat surprised that the ability to self-administer the injection, and thereby the reduction of the number of clinic visits, might increase the acceptability of Depo-Provera® both to women who have never used it and to those who have tried but discontinued the method. It is likely, however, that this is an overestimate since the reasons for discontinuation, given by ex-users, generally related to the side effects and not to the need to attend a health professional four times each year. Similarly the reasons given for not using DMPA related to side-effects and not the frequency of clinic attendance.

In conclusion, the advent of a form of Depo-Provera® which would allow women to self-administer is likely to be a benefit to perhaps as many as half of the women choosing this method and the results from the surveys also suggest that use of injectable contraception may increase were self administration possible [369].

Since the surveys were undertaken subcutaneous depot medroxyprogesterone (DMPA-SC) has been licensed and made available for use. To date, it remains a method that is administered in health clinics. A study by Picardo et al (2010) explored the feasibility of administering DMPA-SC in a pharmacy setting in a pilot randomised controlled trial of 50 women [533]. They found that most women found the pharmacy

setting convenient (70%), private (100%) and were satisfied with the pharmacy as a clinical site (89%). There were no significant differences in satisfaction between the two sites (family planning clinic vs. pharmacy) and they concluded that administration by a pharmacist is a feasible option.

Having demonstrated that self-administration of DMPA is theoretically an attractive option for women, in considering the reality of self-administration, the success of such a scheme and the health economics involved need to be tested scientifically. Our findings have been taken forward by researchers at the University of Carolina [534] and also the PATH organisation [535].

Researchers at the University of North Carolina have recently completed a one year prospective observational study assessing user-satisfaction, feasibility and continuation rates of self-administration of DMPA-SC [534]. Results of this study are awaited.

The PATH organisation published a briefing summary earlier this year (July 2011) specifying that the next steps for DMPA-SC are to assess acceptability of self-administration in developing countries; assess the training, systems, policies, and infrastructure necessary to sustainably implement a home-based delivery program including self-administration and to assess storage and waste disposal requirements and options in developing countries [535].

Most importantly subcutaneous DMPA has the potential to expand the choice of available contraception and it is only by offering choice that the maximum number of women will be protected and the greatest savings to the health service be gained [222].

Studies 4 and 5: Further development of mifepristone as a potential novel contraceptive

Alongside these studies exploring how best to improve choice and use of existing methods two studies were undertaken to further develop a potential new method of contraception, mifepristone.

Mifepristone is a novel oestrogen-free contraceptive method with potential to be developed as either a monthly pill or a daily pill. It has been proven effective as a method of emergency contraception but is licensed in only two countries due to the politics and controversy that surround it.

In the first study the effects of 5mg daily mifepristone were compared to the daily progesterone-only-pill (POP), levonorgestrel, and demonstrated that mifepristone at a daily dose of 5mg is a safe and potentially effective contraceptive [417]. Striking differences in the bleeding patterns between the two comparison groups were demonstrated. Eighty percent of women randomised to mifepristone were either amenorrhoeic or had less than 2 days bleeding or spotting per month. In contrast, none of the women in the POP group were amenorrhoeic and 20% had less than 2 days bleeding or spotting per month. These results confirmed those of previous studies of mifepristone [415].

The relatively high incidence of amenorrhoea or reduced amount of scanty bleeding experienced with mifepristone is likely to be better accepted than the irregular unpredictable menstrual bleeding that occurs in the majority of women taking the POP. The reduction in blood loss should convey health benefits to women particularly in developing countries where the incidence of anaemia is high.

Concern has been expressed that with prolonged intake of antiprogestogens the endometrium would undergo hyperplastic or malignant changes due to continued exposure to unopposed oestrogen [410]. In our study, the commonest histological picture was of inactive cystic glandular dysplasia (CGD). This CGD was only found in those women taking mifepristone. The cause of this unusual change is unknown

and is unlikely to be due to the unopposed effect of oestrogen as it has been demonstrated to occur in women who have profound suppression of ovarian follicular development and where oestrogen levels were low [447,448]. Novel observations from the study were the hysteroscopy finding of atrophic and /or oedematous endometrium. Hysteroscopy revealed that the apparent thickness of the endometrial cavity as measured on ultrasound reflected dilation of the glands and cavity rather than true hyperplasia of the endometrium. The cause of this accumulation of fluid and its nature is unknown but similar findings have been reported in rabbits following treatment with mifepristone [414]. It may be that the mechanism that normally allows the passage/ and or re-absorption of fluid from the endometrial glands and uterus, is obstructed leading to an accumulation of fluid within the uterus.

This study demonstrated that mifepristone at a daily dose of 5mg is a safe and potentially effective contraceptive [417].

The next step in its development would be to undertake a large multicentre phase III trial to further assess contraceptive efficacy and safety, particularly with respect to endometrial cancer.

In the second study changes in vaginal morphology, steroid receptor and natural antimicrobial content following treatment with low-dose mifepristone were assessed. It is known that the vagina is a key portal of entry for HIV and other sexually transmitted infections (STIs). The aim was to investigate whether mifepristone modulated underlying mechanisms involved in transmission of HIV/SIV [490]. The absence of changes in vaginal thickness, steroid receptor and natural antimicrobial content and their distribution in this preliminary study suggest that in contrast to other oestrogen-free contraceptives, mifepristone is unlikely to be associated with increased risk of transmission of HIV and other STIs [490].

Progress with development of antigestogens since 2005

Mifepristone

These studies of mifepristone were completed by 2005. Since then we are no further forward in developing mifepristone's potential as a daily low-dose pill. However, a prospective case-control study undertaken by Agarwal and colleagues (2009) found that mifepristone 200mg can act as an effective monthly contraceptive pill, given on a fixed day in the mid-cycle without detecting LH surge in women having regular menstrual cycles [456]. Regardless of this development the path of mifepristone's development is faced with hurdles and political controversy. It is most commonly known for its abortifacient properties and whilst both daily and monthly mifepristone appear to be effective, marketing either of these does run the risk of enabling women to easily access medication that can be overdosed in order to induce an abortion. These issues need to be resolved before further development of either of these methods.

However, progress has been made in two areas – firstly with regard to safety concerns and secondly with respect to development of antigestogens as contraceptives.

Safety of progesterone receptor modulators (PRMs)

A potential concern about continuous daily treatment with a progesterone antagonist is that the endometrium would be chronically stimulated by oestrogen (unopposed oestrogen) leading to development of endometrial cancer. There has been much debate about the histological findings, post treatment with PRM's, of the endometrium.

The primary concerns have been centred on possible associations with endometrial hyperplasia. Although, several changes have been identified in endometrial samples from women receiving PRM treatment, descriptions of these changes have not fitted into the existing lexicon for histology or pathology, and at the time of our study no common labels had been devised. Instead, how samples were diagnosed was

dependent largely on the pathologist's experience in examining PRM-treated endometrial tissue. Therefore, in 2006 a National Institute of Child Health and Human Development workshop was held to discuss the properties of progesterone receptor modulators (PRMs), the effects of perturbed hormonal control of the endometrium and the need for further understanding of the biology of progesterone receptor action to facilitate the development of new PRMs [536]. A panel of seven expert pathologists was convened at this meeting to evaluate endometrial changes associated with a minimum of three months of chronic treatement of PRMs. Four different agents were used in the treatment regimens but the pathologists were blinded to treatment regimen or agent. Some of the slides used were from our double blind randomised controlled trial of mifepristone versus levenorgestrel where women took mifepristone for six months.

Overall a "class effect" was noted, designated as PRM associated endometrial change (PAEC). The panel agreed that the endometrial biopsies did not fit into a classification of either proliferative or secretory endometrium but exhibited an ususual architecture that could be characterised as glandular dilatation. There was no evidence of hyperplasia or cytological atypia in any of the endometrial biopsies. Expression of proliferative markers Ki67 and PH3 was increased in post-treatment endometrial glandular epithelium compared to baseline, however, baseline biopsies were taken during the secretory phase when proliferative activity is at very low levels. Consequently, the panel concluded that overall there was little evidence of mitosis and this was consistent with a proposed anti-proliferative effect of PRMs. The panel concluded that the biopsies did not reveal evidence of safety concern. They did, however, feel that more study would be needed to identify long-term outcomes of PRM treatment, rather than relying on pathologists and histologists to extrapolate information from biopsies obtained after a short period of PRM exposure [536,537].

Development as an emergency contraceptive

A selective progesterone receptor modulator (SPRM), ulipristal acetate (ellaOne®), has been developed for use as an oral emergency contraceptive tablet and was licensed for use in the UK on October 1st 2009 [469].

Ulipristal acetate (ellaOne®)

EllaOne® is now widely available throughout Europe (27 countries) and also in Singapore, Malaysia and the United States [469]. It is used to prevent unintended pregnancy for up to five days post unprotected sexual intercourse or contraceptive failure.

Mechanism of action

Administering ulipristal acetate before ovulation causes delayed follicle development and release, probably as a result of suppression of oestrodiol levels [538]. If taken during the LH peak, follicular rupture and ovum release may also be delayed [539]. During the latter part of the menstrual cycle, ulipristal acetate's effect may be attributed to its ability to decrease endometrial thickness [538].

Efficacy

The safety and efficacy of ulipristal acetate 30mg for EC has been evaluated in two pivotal phase three trials [540,541]. Fine et al. (2010) evaluated ulipristal acetate use by a total of 1,241 women. Pregnancy rate was 2.1% (95% CI 1.4-3.1%) which met the protocol-defined criteria for success. Additionally, efficacy did not decrease over time. Pregnancy rates were: 2.3% (1.4-3.8%), 2.1% (1.0-4.1%) and 1.3% (0.1-4.8%) for intervals of 48 to 72 hours, more than 72-96 hours and more than 96-120 hours respectively [541]. The second phase three trial was by Glasier et al. (2010) [540]. The study assessed the non-inferiority of ulipristal acetate to levonorgestrel. Women were randomly assigned to either ulipristal 30mg (n=1104) or levonorgestrel 1.5mg (n=1117). In the efficacy-evaluable population, administration of EC-drug within 72 hours of unprotected intercourse resulted in 37 pregnancies. Of that group, 22 women (2.6%; 95% CI 1.7-3.9) were in the levonorgestrel group (n=852) and 15 women (1.8% 95% CI 1.0-3.0) were in the ulipristal acetate group (n=844). This finding was not statistically significant. Of those women treated at 72-96 hours post unprotected intercourse (n=203), three pregnancies occurred. All three were in the levonorgestrel group. This difference was statistically significant (P=0.037). Overall the pregnancy rate (0-72hours) with ulipristal (1.4%) was significantly lower than the expected rate

(5.5%) (p=0.001). The pregnancy rate (0-72hours) with levonorgestrel was 2.6%; the expected rate had been 5.4% (p=0.001). Therefore, the authors concluded that ulipristal is non-inferior to levonorgestrel [538,540].

Safety and tolerability

Ulipristal acetate is generally well tolerated and its adverse effects and risks are considered to be no more severe than those of levonorgestrel [539,541]. It may cause a delayed onset of the next expected menstrual period, although this effect is usually transient and the menstrual cycle typically returns to normal for subsequent cycles [538].

Dosage and administration

The recommended dosage is a single 30mg oral tablet, taken after unprotected intercourse or contraceptive failure. The drug should be taken as soon as possible and within five days (120 hours) of the incident [538].

Strengths and limitations of the studies presented in this thesis

Each of the studies presented in this thesis had their various strengths and limitations.

Unintended pregnancy and use of emergency contraception study

This was a large questionnaire survey which aimed to survey every pregnant woman over seven and a half months in a variety of clinic locations. Its strengths included use of a validated tool (LMUP) to measure intendedness of pregnancy and, considering the complex logistics of clinic locations spread throughout Lothian, both the handout (78% abortion clinic; 93% antenatal clinic; 92% pregnancy support clinics) and response rates were excellent (78% abortion clinics; 86% antenatal clinics; 79% pregnancy support clinics). The main limitation of this study was selection bias. Health care professionals, either due to time pressures or due to professional judgement of the woman's emotional state, did not hand out a questionnaire to every woman. This was more prevalent in the abortion clinics (22%) than in the antenatal or pregnancy support centre clinics (7-8%) $p<0.001$. A further limitation of the study

was that in an effort to ensure completion we tried to minimise the number of questions. However it would have been valuable to have some demographic questions, especially postcode. If this study were to be repeated I would suggest that the demographic fields could be completed ahead of time by staff and this would reduce the likelihood of selection bias and allow for comparison of baseline demographic characteristics of responders to non-responders. It would, however, require a time commitment from the staff looking after these women, both of which are difficult due to the multiple pressures faced in these clinics.

The strengths and limitations of the methodology are discussed separately below.

Implanon® survey

This was a questionnaire survey which aimed to assess continuation rates of Implanon® use in a large family planning clinic setting. The main strength of this survey was that it used 'real life' data. Due to the multiple capture methodology of case note review followed by postal questionnaire followed by telephone the loss to follow up was minimised (15%). Limitations of the study included lack of ethical approval. For the study we undertook, ethical approval was not required but had we obtained it we would have been able to collect more demographic data, including postcode, and this would have allowed us to undertake some comparative analyses. For those who responded via letter or telephone they were asked to recall when they had their implant removed. For some this will have been some time previously and hence recall bias is very likely in these cases. We could have further strengthened our study by carrying out a survival analysis similar to previous studies looking at continuation rates [228,229]. The survey methodology is discussed below.

Depo-provera® survey

This was a pilot study to identify if women would be interested in self-administration of subcutaneous depo-provera® and it found that many women would in theory. Its strengths included that it managed to survey a third of the depo-provera users in the clinic. The overall response rate was excellent (100% for questionnaire 1 and 87% for questionnaire 2). This is likely to have been due to having very short questionnaires and also because both were to be completed on site.

Similarly to the previous study limitations included lack of ethical approval and hence no demographic data being collected which then limited the statistical analyses

possible. The poor layout of questionnaire two was an additional limitation. It led to questions 6 and 7 being very poorly answered. When the data was inspected we found that current users and past users had response rates of 92 and 94% respectively whilst non-DMPA users had response rates of 72% for those who had never considered DMPA and 39% for those who had considered DMPA in the past but not used it. This led us to reviewing the layout of the questionnaire and finding that having questions 6 and 7 below question 3 meant that it was very likely that non-DMPA users felt it did not apply to them. Additionally below the question directed to non-DMPA users (Q5) was a request to seal the envelope and return the questionnaire to reception so it is likely that many felt that there were no further questions to respond to.

Mifepristone versus levenorgestrel study

This was a multi-centre randomised controlled trial comparing the frequency of amenorrhoea (primary outcome), bleeding patterns, side-effects and efficacy in women taking 5mg daily mifepristone or 0.03mg levenorgestrel for a duration of 24 weeks. This study had many strengths. It was a randomised control trial and it was performed in accordance with good clinical practice guidelines. Assessment bias was reduced by double blinding. By being a multi-centre study it showed effectiveness in a range of cultures and allowed for generalisability. There was a focussed question where the population was well defined as were the intervention and the outcomes studied. A power calculation was undertaken for amenorrhoea though not for efficacy or ovulation and this would have been useful. The use of a 3:1 ratio for mifepristone:levenorgestrel was very useful for increasing the power of the study. Using block computer generated randomisation performed individually for each centre ensured a balanced 3:1 allocation.

Limitations included that the study was potentially not truly double blind. The placebo tablet was similar but not identical to either of the active drugs and hence it would have been possible for the persistent subject and/or the researcher to identify which was which. Whilst a multicentre approach has great benefits with respect to generalisability and recruitment it also has its limitations. It is possible that whilst there was a clear protocol there may have been some centre differences in research methods. We tried to minimise this by holding regular meetings between centres, having the network director (Professor Baird) and the network coordinator (Dr Morrow) visit each site annually and review both the methodologies and the case

notes during these visits. The assessment of ovarian function by eight weekly progesterone levels was imprecise and if we were to repeat the study it might be better to ask for twice weekly urine samples. In the analysis we possibly should have used an intention to treat analysis and thus included the one lady who left the trial before it started.

Mifepristone effect on the vagina study

This was a small pilot study to assess the changes in vaginal morphology, steroid receptor and natural antimicrobial content following treatment with low-dose mifepristone. The aim was to understand if mifepristone might be protective against STI's including HIV. The study had a number of strengths. It had ethical approval and was performed in accordance with good clinical practice. Vaginal biopsies were taken both before and after treatment allowing for comparison to be made. Ovarian function was measured in a much more reliable way, using urinary tracking, than in the previous study. The next step would be to carry out a larger study with a longer duration of intake of mifepristone.

Questionnaire survey methodology strengths and limitations

Three of the studies (chapters 2-4) involved structured questionnaire surveys [46,231,417]. Structured questionnaires involve the use of fixed (standardised) questions and/or scales which are presented to respondents in the same way, with no variation in question wording, and with mainly pre-coded response choices. We administered them in a variety of ways – on-site completion, postal and by telephone. Of these three routes postal completion is the least successful and our response rates clearly demonstrated this (47% response rate for postal returns as opposed to 100% and 89% response rate for questionnaires completed on-site and by telephone). The reasons for using this methodology included: (i) the ability to collect unambiguous and easy to count answers, leading to quantitative data for analysis; (ii) greater ease of data collection and analysis; (iii) the ability to include large samples of people (especially in the study of levels of unintended pregnancy and EC use) at low cost. Additionally we were able to collect routine information from questionnaires and supplement information from medical notes, where permission to access them had been obtained.

Disadvantages of this methodology included that pre-coded responses are not necessarily sufficiently comprehensive and not all answers may be easily accommodated. Some respondents may therefore have felt 'forced' to choose inappropriate pre-coded answers that may not have fully represented their views. Additionally, structured self-administered questionnaires rely on the assumption that questions can be worded and ordered in a way that will be understood by all respondents. As we found this is not always be justified. In the second self-administered DMPA-SC questionnaire offered to every woman attending an Edinburgh family planning clinic we found that unfortunately the response rates for questions regarding self-administration and clinic attendance (questions 6 and 7, questionnaire 2) were lower than expected when compared to other questions on the same questionnaire. On further investigation it was found that response to these questions by current and previous users were 92% and 94% respectively whilst response by non-DMPA users was 65% and 68% suggesting that poor design layout might have been the cause. We were however able to allow for some measure of validity and reliability of the questionnaires as both questionnaires administered for the purpose of the DMPA-SC survey indicated that 70% of users would be interested in self-administration. Two further disadvantages of questionnaire surveys are recall bias, loss to follow up (specifically due to change of addresss in the case of the Implanon® survey) and non-response bias which reduces the effective sample size and can result in loss of precision of the survey estimates. The latter is especially a problem where surveys are not completed on-site.

Relevance of this research in Scotland

The research findings presented in this thesis have been particularly relevant to recent policy developments in Scotland. Specifically, the findings have advocated for increasing awareness of risk of unintended pregnancy in women and couples; increasing ease of access to emergency contraception; encouragement of correct and consistent use of contraception and specifically LARC methods; and further development of novel methods of contraception as well as exploring the reality of self-administration of DMPA-SC.

In January 2005, as we were completing our research, the Scottish government launched the first phase of 'Respect and Responsibility: A Strategy and Action Plan for Improving Sexual Health' [542]. For the first time government policy set out a framework for improving sexual health in Scotland and access to information and services, whilst allowing flexibility for local services to respond to local needs. It recognised that sexual health is not just the absence of disease, but includes a range of ethical, moral, cultural and social issues and is firmly based on the principles of respect for self, respect for others and strong relationships.

Actions were geared towards:

- Improving the quality, range and consistency, accessibility and cohesion of sexual health services
- Supporting everyone in Scotland to acquire and maintain the knowledge, skills and values necessary for good sexual wellbeing and thus avoid sexually transmitted infections and unintended pregnancy
- Positively influencing cultural and social factors that impact on sexual health

The aim of the first phase of the strategy (2005-8) was to focus on supporting improvements in sexual health provision [542]. The NICE guidance (to which our data contributed) published later that year was absorbed into the frameworks evidence base and became a key part of the strategy [180].

One of the outcomes from this framework during the first phase, in keeping with our research findings, was that EC became available for free from pharmacies (LNG not ellaOne which is only available on prescription) [543].

The second phase of the plan was launched in 2008 and is now nearing its end [544]. In the second phase focus shifted from supporting improvements in sexual health provision to achieving cultural and behavioural change. A key focus has been encouraging awareness, access to and use of LARC, all in keeping with the findings from the research presented in this thesis. New developments within the second phase included a new Scottish government website (launched June 2009) (http://www.sexualhealthscotland.co.uk/), social marketing campaigns [545], led by the Scottish Government and NHS Health Scotland, as well as the publication of an HIV Action Plan.

The social marketing campaign was launched in July 2009 and it was specifically aimed at 'Giving you more choice' and even more importantly was called the longer-lasting contraception campaign [545]. Its aims in the short term were to increase awareness of contraceptive choices, particularly the IUD, IUS and implants; in the medium term increase use of these and the number of health professionals offering these methods and in the long term reduce the number of unintended pregnancies and repeat terminations.

The Scottish Government are currently in the process of drafting a new sexual health framework which will outline the strategic aims for sexual health for 2011-15.

The above policy and its outcomes appear to be making an impact on levels of unintended pregnancy. For the past two years there has been a fall in the number and rate of abortions with 12,826 in 2010, compared with 13,108 in 2009 and 13, 902 in 2008 (representing rates of 12.3/1000 women aged 15-44 in 2010, 12.6 in 2009 and 13.3 in 2008) [3]. This fall is a change to the overall pattern of increase since the implementation of the 1967 Abortion Act, although small dips for short periods (e.g. 1981-3) have been observed before and one cannot guarantee that this fall will continue nor can we be certain as to the cause [3].

Conclusion

In this thesis I have sought to explore a number of areas, all pertaining to trying to reduce the levels of unintended pregnancy, in an effort to increase the knowledge base in this field. It has now been some years since this research was carried out. The benefit of this is that I can see that the findings have been both relevant and useful to other researchers, practitioners and policymakers.

Appendix 1: Contraceptive use in month of becoming pregnant by those attending for termination of pregnancy

Method of contraception	**No. women using (%) N=907**
No method	238 (26.2)
Withdrawal	33 (3.6)
Breastfeeding	5 (0.6)
Fertility awareness based method (NFP)	18 (2.0)
Diaphragm/cap	6 (0.7)
Condom	502 (55.3)
COC	155 (17.1)
POP	35 (3.9)
Depo-provera	3 (0.3)
IUD/IUS	11 (1.2)
Implanon	2 (0.2)
Sterilisation	0 (0)

74% of those attending for termination were using a method of contraception in the month that they conceived.
Over one third (36%; 278/770) women who were using a method when asked admitted
"I/we were using contraception but not on every occasion".
71% of those who scored less than 3 on the LMUP i.e had a clearly unintended pregnancy were either using no method of contraception or less effective methods (condoms/withdrawal, NFP, breastfeeding or cap/diaphragm).

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Unintended pregnancy and use of emergency contraception among a large cohort of women attending for antenatal care or abortion in Scotland

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Summary

Background Unintended pregnancy is common. Although many unintended pregnancies end in induced abortion, up to a third of those proceeding to birth might be unplanned. Some of these pregnancies could be prevented by emergency contraception. We have sought to establish how many pregnancies ending in either childbirth or abortion are unintended, and what proportion of women use emergency contraception to try to prevent pregnancy.

Methods 2908 women who attended an Edinburgh hospital for antenatal care and 907 attending for abortion fully completed a self-administered questionnaire including a validated measure of pregnancy intention and questions about emergency contraceptive use.

Findings 814 (89·7%) of 907 pregnancies among women requesting abortion were unintended compared with only 250 (8·6%) among 2908 women who planned to continue pregnancy. However, only 1909 (65·6%) of continuing pregnancies were intended. The rest of the women were ambivalent about pregnancy intention. In women who continued with their pregnancies intendedness was related to age, with unintended pregnancy most probable in young women ($p<0·0001$). Emergency contraception was used by 113 (11·8%) of women who requested abortion but only 40 (1%) of those planning to continue pregnancy. In those whose pregnancy was continuing, the proportions reporting use of emergency contraception were higher in young women than in older women and in those who reported that their pregnancies were unintended than in those who meant to become pregnant (both $p<0·0001$).

Interpretation Unintended pregnancy is common, even among women planning to continue pregnancy. However, EC use is low even among women with no intention of conceiving, and is thus unlikely to reduce unintended pregnancy rates. Rather, we need to find ways to improve the use of regular contraception.

Introduction:

Unintended pregnancy is common. In the UK, almost 200 000 pregnancies are terminated every year.[1,2] Additionally, a substantial number of births result from unintended conception. In a questionnaire survey of 2000 mothers who were randomly selected from birth registrations in 1989 and interviewed 6 months after childbirth, almost a third of pregnancies (31·3%) were "unplanned".[3] A simple measure of pregnancy intention was devised and validated by Barrett and colleagues[4] in 2004 (table 1); however, there have been no estimates of pregnancy intendedness in women in the UK since 1989.

Up to a quarter of pregnancies that end in induced abortion in the UK arise from unprotected sexual intercourse; most of the rest are the result of inconsistent or incorrect use of contraceptives (eg, missed pills) or accidental damage of barrier contraceptives (eg, a burst condom).[5,6] Emergency contraception can prevent pregnancy if used within 72 hours of unprotected sex.[7,8] The only marketed product in the UK (oral levonorgestrel 1·5 mg) is thought to prevent over 80% of pregnancies depending on how soon after intercourse it is used.[7] Trussell and colleagues[9] estimated that if every woman in the USA used emergency contraception every time it was needed, 1·7 million unintended pregnancies, over half of which end in abortion, could be prevented every year. Although knowledge of emergency contraception in the UK is high, use is rather low. In 2003–04, 6% of women aged 16–49 years had used emergency contraception within the previous year although more than 94% of women knew about it.[10] One small study showed that about 11% of women presenting for abortion in the UK in 2000 had used emergency contraception to try to prevent the pregnancy,[5] but there are no data on the use of emergency contraception among women who continued with their pregnancies.

In 2005, we undertook a questionnaire survey designed to quantify pregnancy intention and use of emergency contraception among women who presented over the course of 8 months to a large teaching hospital in Edinburgh, UK, for antenatal care, or to attend the pregnancy support centre (women who have a history or high likelihood of miscarriage), or for termination of pregnancy.

Methods

From July 5, 2004, until Feb 28, 2005, all women booking for antenatal care or abortion in the New Royal Infirmary of Edinburgh, were invited to complete a self-administered questionnaire asking about pregnancy intention and use of emergency contraception. We excluded women whose fetus was shown to have died, by ultrasonography, and

those who were unable to read or write English well enough to understand or complete the questionnaire. Women who were judged by the nursing staff to be distressed about the clinical consultation were not offered the questionnaire. This judgment applied mainly to women presenting at the abortion clinic.

Before seeking ethics approval for the study, we asked 207 women to read and comment on a draft information sheet and questionnaire. The final questionnaire consisted of 15 questions about age, pregnancy gestation, contraceptive use (including emergency contraception) in the month of conception, and pregnancy intention. Intendedness was measured by using Barrett and colleagues' instrument (table 1).[4] For the intendedness score, the answer to each of the six questions was scored from 0 to 2, so the total scores ranged from 0 (least intended) to 12 (most intended). Although Barrett and colleagues emphasised that there were no obvious cutoff points on the range of scores obtained, they suggested that three score groups were identifiable: 10–12 (planned), 4–9 (ambivalent), and 0–3 (unplanned).[4] These three groups were used for the purposes of analysis and discussion. Ethics approval for the survey was obtained from the local research ethics committee.

On the basis of an estimated proportion of emergency contraceptive use of at least 10%[5] among women presenting for abortion and our estimate of 2% for women continuing their pregnancies, a sample size of 1000 women undergoing abortion and 4500 booking for antenatal care was needed to ensure that the upper confidence limit for proportion of emergency contraceptive use was only 50% higher than the lower limit. The study was powered to 90% and the confidence intervals set to 95%. Quality control checks were done on 5% of all data entries. Data were analysed by use of Excel (2003) and SPSS (version 12).

Groups were compared by χ^2 tests for binary data, Mann-Whitney tests for intendedness scores, and two-sample *t* tests for age and gestation. Ages in different intendedness groups were compared by one-way ANOVA.

Results

5686 pregnant women attended the hospital during the study period, 5630 of whom were eligible to participate (figure 1). Less than 1% of women were judged to be too distressed to be offered a questionnaire. 1285 (78%) of 1645 women attending for abortion were given the questionnaire and it was returned by 1006 (78%). 2905 (93%) of women attending the antenatal clinic and 810 (92%) of those attending pregnancy support centre were given the questionnaire; of those women, 2496 (86%) and 643 (79%), returned the questionnaire, respectively (figure 1). Women attending for abortion were younger than those planning to continue their pregnancies and women seeking abortion or attending the pregnancy support clinic attended hospital at an earlier gestation than those women attending an antenatal clinic (table 2).

Question	Answer	Score
Q1. At the time of conception	Always used contraception	0
	Inconsistent use	1
	Not using contraception	2
Q2. In terms of becoming a mother	Wrong time	0
	OK but not quite right	1
	Right time	2
Q3. Just before conception	Did not intend to become pregnant	0
	Changing intentions	1
	Intended to get pregnant	2
Q4. Just before conception	Did not want a baby	0
	Mixed feelings about having a baby	1
	Wanted a baby	2
Q5. Before conception	Had never discussed children	0
	Discussed but no firm agreement	1
	Agreed pregnancy with partner	2
Q6. Before conception	No actions	0
	Health preparations (1 action*)	1
	Health preparations (≥2 actions*)	2

Health preparations included: taking folic acid supplements; stopping or reduction of smoking; stopping or reduction of drinking alcohol; healthy eating; seeking medical advice before conception.

Table 1: **Pregnancy intendedness instrument[4]**

3815 women answered all six questions in the intendedness measure, 2908 of them continuing their pregnancy and 907 who requested abortion. 814 (89·7%) of women who requested abortion had a total score of 3 or less, which suggests that their pregnancies were unintended (table 2). Only two women who wanted an abortion scored 10 or more (intended) and 91 (10·0%) were ambivalent about the intendedness of the pregnancy

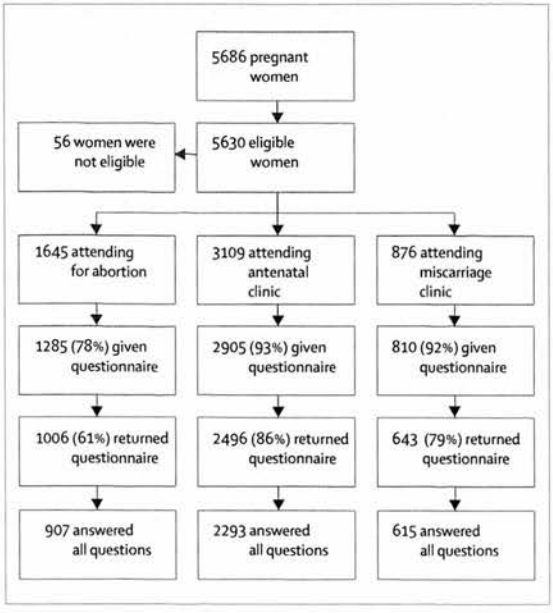

Figure 1: **Study profile**

	Seeking abortion	Continuing pregnancy			*	†
		Miscarriage clinic	Antenatal clinic	Total		
Age (years)						
Mean (SD)	25·0 (6·7)	30·3 (6·2)	29·6 (5·8)	29·7 (5·9)	<0·0001	0·002
Range	10–45	14–44	15–44	14–44		
Gestation (days)						
Mean (SD)	56 (19)	58 (15)	92 (17)	87 (21)	<0·0001	<0·0001
Range	7–181	16–129	44–270	16–270		
Total intendedness score, n (%)						
10–12 (intended)	2 (0·2%)	402 (65·4%)	1507 (65·7%)	1909 (65·6%)	<0·0001	0·42
4–9 (ambivalent)	91 (10·0%)	140 (22·8%)	609 (26·6%)	749 (25·8%)		
0–3 (unintended)	814 (89·7%)	73 (11·9%)	177 (7·7%)	250 (8·6%)		
EC used in conception cycle						
Yes	113 (11·8%)	17 (2·7%)	23 (1·0%)	40 (1·4%)	<0·0001	0·002
No	844 (88·2%)	603 (97·3%)	2308 (99·0%)	2911 (98·6%)		
EC after all episodes of unprotected sex ‡	74 (65%)	7 (41%)	13 (56%)	20 (50%)	0·12	0·42

C=emergency contraception. *Seeking abortion versus total continuing with their pregnancy. †Attending miscarriage clinic versus those attending the antenatal clinic. ‡Percentage is of those answering "Yes" to use of emergency contraception in conception cycle.

Table 2: **Characteristics of women, use of emergency contraception, and intendedness score**

(score 4–9), (figure 2). Of the women who planned to continue their pregnancies, 250 (8·6%) scored less than 3 (unintended), 1909 (65·6%) scored 10 or more (intended) and 749 (25·8%) had some ambivalence about their intention to conceive (score 4–9) (table 2, figure 2). Intendedness was associated with age in women who chose to continue with their pregnancies; women who had intended to become pregnant were significantly older than those who were ambivalent or had unintended pregnancy. There was no significant association between age and intendedness in women presenting for abortions (table 3). 113 (11·8%) women presenting for abortion used emergency contraception to try to prevent pregnancy whereas only 40 (1·4%) of those continuing with their pregnancies had done so (table 2). Of the women who used emergency contraception, 74 (65%) of those attending for abortion and 20 (50%) of those continuing with their pregnancy said they had used it after every episode of unprotected intercourse during the menstrual cycle in which they got pregnant. Women who used emergency contraception were significantly more likely to score low rather than high on the intendedness scale, both in the abortion group and in those continuing their pregnancies (table 4). In women continuing with their pregnancies, young age was significantly associated with emergency contraception use ($p<0·0001$). Age was not related to emergency contraception use in women seeking abortion, but in this group, women who presented before 39 days of gestation were significantly more likely to have used emergency contraception than those presenting later.

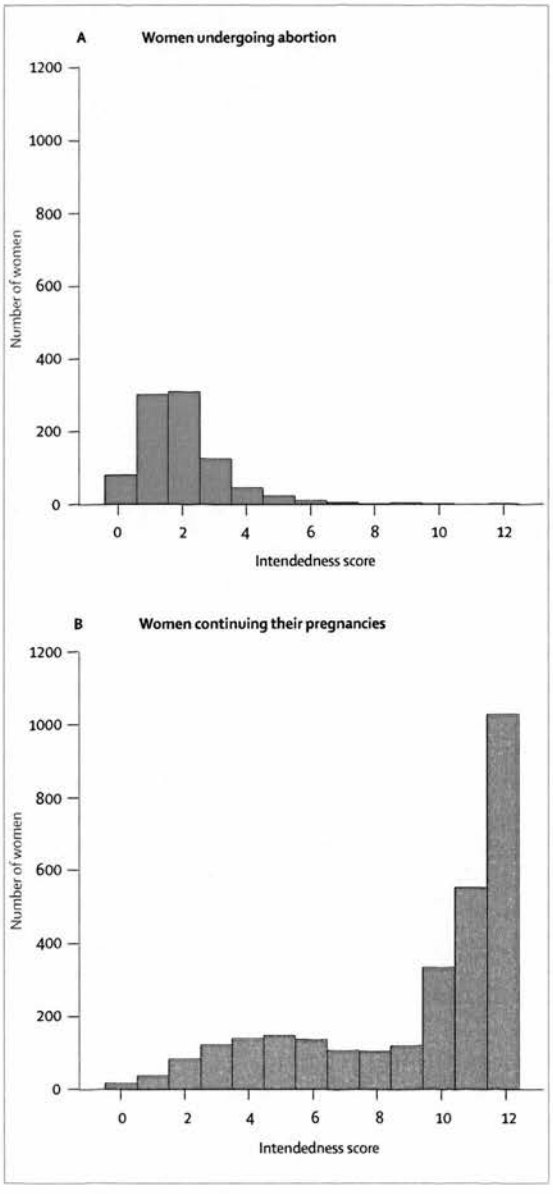

Figure 2: **Spread of intendedness scores for women undergoing induced abortion and for all women continuing their pregnancies (including those seen in the miscarriage clinic).**
0=least intended pregnancies, 12=most intended.

Discussion

In Edinburgh in 2005, only two-thirds of pregnancies destined to end in childbirth were clearly intended, one in ten was unintended, and around a quarter of women were somewhat ambivalent about their intention to become pregnant. When Barrett and colleagues developed their instrument, they took care to describe the differences in the meaning of the words "planned and unplanned", "intended and unintended", and "wanted and unwanted".[4] In 1989, Fleissig[3] used the

	Mean age (years)			
	Seeking abortion	Miscarriage clinic	Antenatal clinic	Continuing pregnancy total
Intended	31·0	31·9	31·0	31·2
Ambivalent	24·9	28·3	27·3	27·5
Unintended	24·8	25·8	25·6	25·7
p	0·28	<0·0001	<0·0001	<0·0001

p values are from one-way ANOVA.

Table 3: **Association between mean age (years) and intendedness**

	Seeking abortion	Continuing pregnancy		
		Miscarriage clinic	Antenatal clinic	Total
Age (years)				
0–19	19 (9·3%)	1 (3·7%)	6 (3·6%)	7 (3·6%)
20–24	35 (11·1%)	8 (7·8%)	7 (2·3%)	15 (3·7%)
25–29	34 (17·4%)	2 (1·6%)	5 (0·9%)	7 (1·0%)
30–34	10 (8·8%)	4 (2·1%)	2 (0·2%)	6 (0·6%)
≥35	11 (10·4%)	2 (1·2%)	3 (0·7%)	5 (0·8%)
p*	0·64	0·011	0·0001	<0·0001
Gestation (days)				
0–39	21 (22·8%)	2 (5·0%)	0	2 (5·0%)
40–79	74 (10·6%)	9 (2·5%)	2 (0·9%)	11 (1·8%)
80–119	6 (8·7%)	1 (2·5%)	17 (1·0%)	18 (1·0%)
≥120	2 (14·3%)	0	3 (3·8%)	3 (3·8%)
p*	0·015	0·48	0·13	0·19
Intendedness				
Intended	0	6 (1·6%)	1 (0·1%)	7 (0·4%)
Ambivalent	4 (4·7%)	7 (5·0%)	14 (2·4%)	21 (2·9%)
Unintended	96 (12·3%)	3 (4·1%)	6 (3·5%)	9 (3·7%)
p*	0·031	0·054	<0·0001	<0·0001

Percentages are the proportion of women in that age, gestational stage, or intendedness group who used emergency contraception. Information is not available for all participants. * χ^2 test for trend.

Table 4: **Trends in maternal age, gestational stage, and pregnancy intendedness among women using emergency contraception**

word "unplanned" and the questionnaire was completed by women 6 months post partum. Despite the difference in timing of the survey, and differences in wording of the questionnaires, the proportion of pregnancies that are "planned" or "intended" has not changed since Fleissig's study. This finding is perhaps surprising given the demographic changes (falling birth rates, later age of first childbirth)[11], changes in sexual behaviour,[12] and the increase in contraceptive choice in the past 25 years.[13]

The relation between age and intendedness among women continuing their pregnancies is unsurprising. The findings that over 90% of pregnancies ending in abortion were unintended and that only 10% of women requesting abortion were ambivalent about their pregnancies is consistent with the findings of a smaller study of 300 women in Edinburgh undergoing abortion, in which a similar proportion claimed to have used emergency contraception.[6] In that study a modified version of the intendedness measure was used in a face-to-face interview rather than in a self-administered questionnaire. Despite the methodological differences, both studies show that most women who requested abortion had no intention to conceive.

One in ten women undergoing abortion and a quarter of women continuing with their pregnancies seemed to have some ambivalence about pregnancy intention. Since the number of women who were ineligible for the study or deemed to be too distressed to be given the questionnaire was small we do not believe that inclusion of these women would have altered the findings.

The observation of a relation between intendedness and emergency contraception use adds weight to the validity of Barrett and colleagues' scoring system. The results also suggest that, women who are ambivalent about their pregnancies are more likely to continue than to have them terminated. The association between use of emergency contraception and earlier gestational stage at presentation among women requesting abortion is probably related to heightened awareness of the risk of pregnancy since they recognised that they were at risk of pregnancy before missing a menstrual period. Use of emergency contraception was related to age only for women who were continuing with their pregnancy, perhaps because younger women are more ambivalent about avoiding pregnancy than older women.

The availability and use of emergency contraception varies around the world. Although use of a combination of oral contraceptive pills as a substitute for emergency contraception has always been possible, in many countries a dedicated product has only recently become available. Generally, use does not start to increase until such a product becomes available (eg, in Nigeria and the USA in 1998, in France in 1999, and in India in 2002). In much of sub-Saharan Africa, the former Soviet Union, and the Middle East, a dedicated product is not available. In a 2001 survey of 880 female undergraduates in Nigeria, of whom 34% had had an abortion in the past, 58% knew about emergency contraception, but only 2% had ever used it.[14] In a group of 623 women who sought contraception or abortion in India—where an estimated 5–6 million abortions occur each year, most of them illegal—only 6% knew about emergency contraception and none had ever used it.[15] In more developed countries, knowledge of emergency contraception is greater and more women use it. Among women undergoing abortion, the proportion who said they had used emergency contraception to try to prevent the pregnancy was 1·3% in the USA in 2000,[16] 2·9% in Sweden in 2000,[17] and 9·2% in France in 2002.[18] Use of emergency contraception has increased in the UK since it was first licensed in 1984. In a questionnaire study of women presenting for abortion in Dundee in 1984, 1% of women had tried to prevent the pregnancy with emergency contraception.[19] When the study was repeated

in 1996, 7% of women had used emergency contraception.[20] Nevertheless, emergency contraception is unlikely to prevent many pregnancies if, as in our survey of women who became pregnant, only one in ten women who definitely did not want to become pregnant use it in cycles when they have put themselves at risk of pregnancy, and not much more than half of those use it with every act of unprotected intercourse. The availability of emergency contraception over the counter in UK pharmacies does not seem to have resulted in increased use.[21]

Other studies have shown that failure to recognise the need to use emergency contraception is common. In a questionnaire study of 1365 women who had induced abortions in France, 90% had heard of emergency contraception but only a third had ever used it and only 9% had used it in the cycle in which they became pregnant.[18] 38% of the women were aware that they had put themselves at risk of pregnancy in the cycle in which they conceived—most of these were either not using contraception or were relying on condoms or withdrawal. Nine out of ten of them knew about emergency contraception but only one in four of them used it. More than half the women did not realise that they were at risk of pregnancy, and only 2·8% used emergency contraception. Lack of knowledge of how and when to use emergency contraception, difficulties with getting hold of it, and reservations about using it are all commonly cited barriers to its use.[22] However, without these barriers, most women who have become pregnant and could have used emergency contraception to prevent an unwanted pregnancy failed to do so. Several studies, from various settings, in which women were given a supply of emergency contraception in advance of need have shown that three out of four women who put themselves at risk of pregnancy, even when they had a supply at home, did not use emergency contraception because they did not recognise—or did not acknowledge—the risk.[23–25]

Although 98% of women who wish to avoid pregnancy use contraception in the UK,[10] abortion rates continue to rise.[1,2] Unintended pregnancies that end in childbirth, unless they occur in teenagers, are of less concern to policymakers than those that end in abortion, but they do affect the lives of the women involved. Understanding of sexual behaviour and patterns of contraceptive use is crucial for development of interventions to reduce unintended pregnancy. This survey needs to be repeated in other settings, and if the findings are similar elsewhere, a strategy will need to be developed to improve contraceptive use. We need to find ways to raise awareness of the real risks of pregnancy associated with lack of use of contraception or with incorrect or inconsistent use. Emergency contraception is unlikely to make a substantial difference to pregnancy rates. Condoms and oral contraceptive pills are the most commonly used reversible methods of contraception in the UK and both rely on consistent use for their effectiveness. Condom use is commonly inconsistent, and compliance with oral contraception is not easy. In one US study, 47% of women reported missing one or more pills per cycle and 22% reported missing two or more.[26] In a study that used electronic diaries to record compliance, 63% of women missed one or more pills in the first cycle of use, and 74% did so in the second cycle.[27] We need to encourage women who clearly want to avoid pregnancy and are taking risks to use long-acting contraceptive methods (implants and intrauterine devices) that do not depend on compliance for their effectiveness.[28]

Contributors

A Glasier was the main investigator, conceived the study, and wrote the report. F Lakha assisted with the design of the questionnaire, piloted it, and coordinated the data collection, data entry, and analysis, and assisted with writing of the report.

Conflict of interest statement

We declare that we have no conflict of interest.

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Contraception

Original research article

Continuation rates of Implanon® in the UK: data from an observational study in a clinical setting

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Abstract

Background: Long-acting reversible methods of contraception can potentially reduce unintended pregnancy. There are few data on "real-life" continuation rates of the contraceptive implant Implanon®.

Materials and Methods: Three hundred twenty-four women choosing Implanon® in a community family planning clinic in Scotland were followed up by case note review ($n=236$) or postal questionnaire ($n=87$) 3 years after insertion of the implant (1 woman chose not to disclose her home address).

Results: Data were available for 85% of the women. Continuation rates were 89% (CI 84–91) at 6 months, 75% (CI 69–79) at 1 year, 59% (CI 52–63) at 2 years and 47% (CI 40–52) at 2 years and 9 months. Of the 68 women who discontinued Implanon® within 1 year, 62 (91%) did so because of unwanted side effects, the most common being frequent and/or unpredictable bleeding ($n=42$, 62%). Almost half changed to a less-effective method of contraception; however, one third ($n=99$, 39%) chose to use a second implant when the first one expired.

Conclusions: Continuation rates of Implanon® in this clinic setting in the UK make it a cost-effective method of contraception and justify its widespread provision.

Keywords: Contraception; Implants; Implanon®; Continuation

1. Introduction

Unintended pregnancy is a global problem. A recent guideline from the National Institute of Health and Clinical Excellence (NICE) in the UK suggested that increased uptake of long-acting reversible methods of contraception (LARC) would reduce unintended pregnancy [1]. Implanon® is a long-acting reversible contraceptive that lasts for 3 years and is independent of compliance for its effectiveness [2]. It has been suggested that the high up-front cost of the implant (UK £90 in 2005) limits its availability in parts of the UK [1] and perhaps in other countries. Cost-effectiveness of all methods of contraception depends on their associated continuation rates, but this is particularly relevant for the very long acting methods (implants and intrauterine contraceptives). Continued use of Implanon® has been shown to be high in international clinical trials, with more than 80% of women still using the method after 2 years [3]. However, participants in prospective trials often have relatively high continuation rates of drugs because the inclusion criteria for trials are rigid and tend to bias toward a willingness/likelihood to continue the method. Regular study-related follow-up visits serve as a positive reinforcement, and in countries where women have to pay for contraception and where healthcare is expensive, provision of free supplies and services through clinical trials ensures good compliance [4]. "Real-life" data are much harder to come by. Moreover, much of the data for Implanon® come from developing countries where the availability of and access to other methods of contraception are usually limited and where continuation rates are often high. In a review of evidence from European settings, the NICE guideline concluded that 20–25% of women will discontinue Implanon® within 1 year of insertion and that up to 44% will stop using the method within 2 years [1].

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Only two small studies have investigated continuation rates in the clinic setting in the UK. One study followed up 191 women for only 1 year [5]. In the other study, which followed up very few women for 3 years, the probability that Implanon® was still being used at 3 years was .53 [6]. To determine continuation rates of Implanon® in clinical practice, we undertook a retrospective case note review and questionnaire survey 3 years after insertion of Implanon® in a cohort of 326 new users attending a large family planning clinic in Edinburgh, Scotland.

2. Materials and methods

Case notes for all women attending the clinic for Implanon® insertion between January 1 and December 31, 2001, were reviewed at the end of 2004. If the implant had been removed in the clinic, data were obtained from the case records. Women who had not returned to the clinic for Implanon® removal (and who had consented to being contacted when first attending the clinic) were contacted to remind them about the need for removal of their implant before 3 years of use was completed. These women were also asked to complete a brief questionnaire to determine whether Implanon® had been removed and, if so, the date and reason for removal of the implant, where the procedure was done, and the method of contraception used for the first 3 months after discontinuation of Implanon®. Three attempts at contact were made: twice by mail and once by telephone. An interval of 2 weeks was maintained between each attempt at contact.

Data were analyzed using Excel 2003 (Microsoft Corporation) and SPSS version 12 (SPSS, Inc., Chicago, IL, USA). Ages of women continuing for more or less than 2 years were compared using a two-sample t test. Cumulative discontinuation rates were computed using descriptive statistics. Many women returned to the clinic slightly before 3 years after Implanon® insertion to ensure that replacement contraception was available for use. Women returning for implant removal more than 2 years and 9 months after insertion were deemed to have used the implant for its full duration.

3. Results

Three hundred twenty-four women had Implanon® inserted in 2001. Median age at insertion was 26 years (range, 15–49). Information about implant removal was available for a total of 277 women (85%). Two hundred thirty-six of these women had their implant removed in the clinic, and data were collected from their case records. Letters were sent to 87 women who had not returned to the clinic for implant removal; 1 woman chose not to disclose her home address. Forty-one women responded and returned the questionnaire. All 46 women who failed to respond to the request for information were no longer residing at the address recorded in their case records.

Table 1
Contraceptive method used following removal of Implanon®

Method	n (%)
No method (pregnancy desired)	28 (8)
Implanon®	99 (39)
IUD/IUS	12 (5)
Depo Provera®	8 (3)
Sterilization	16 (6)
Combined pill	45 (18)
Progestogen-only pill	17 (7)
Condom	55 (22)
Withdrawal	2 (<1)

For the 277 women in whom information was available, continuation rates of Implanon® were 89% (CI 84–91) at 6 months, 75% (CI 69–79) at 1 year, 59% (CI 52–63) at 2 years and 47% (CI 40–52) at 2 years and 9 months. Thirty-three women did not return for implant removal until at least 1 month after 3 years of use; the longest duration of use was 4 years and 8 days. Continuation rates were independent of age.

Sixty-eight women discontinued Implanon® within 1 year; 62 of them (91%) did so because of unwanted side effects. The most common documented reason for implant removal was frequent and/or unpredictable bleeding (n=42, 62%). Weight gain accounted for 21% of removals (n=14) and mood change accounted for 16% (n=11). Of the six women who discontinued within 1 year for reasons other than side effects, five did so because they wished to become pregnant and one because she was no longer in a relationship.

Data on the contraceptive chosen for use following Implanon® removal were collected (Table 1). Twenty-three women (8%) were actively trying to conceive. Thirty-nine percent of women had a second implant inserted, while another 14% chose an alternative long-acting method or sterilization. More than 47% of the women elected to use a less effective method of contraception.

4. Discussion

Continuation rates of all methods of contraception are generally disappointing. In an international review of discontinuation rates after 1 year of use of hormonal contraception, rates varied from 19% (for Norplant) to 62% (the combined pill) [7]. Discontinuation rates are higher for methods that do not require removal by a health professional [8]. In this study of women in Edinburgh, continuation rates among women in whom data could be obtained might best be described as reasonable with just under 60% continuing for 2 years and just under half continuing for 3 years. The fact that nearly 40% continued to use Implanon® for contraception after 3 years is encouraging.

The present study involved a cohort of women attending a clinic that offers all currently licensed methods of contraception free of charge. Routine follow-up consisted

of at most one visit to the clinic 6–12 weeks after insertion of the implant. Women were free to attend the clinic for review at any time, and Implanon® was removed on request at no cost. Follow-up rates in our study were quite good at 85%. In a postal survey of UK women followed up after Implanon® insertion in the East Midlands, the response rate was only 44% [5]. Although clinical trials have more frequent data collection and higher rates of follow-up, continuation rates in this cohort of Scottish women should be generally representative of UK women attending a community family planning clinic.

We have no idea as to how long those women lost to follow-up continued to use their Implanon®, and it does not seem worthwhile to guess. Only a dozen general practices (family doctors) in the area offer Implanon® removal and only to women who are registered with them. It is likely that most of these women continued to use Implanon® until they moved away from Edinburgh.

Robust data on patterns of contraceptive use in the UK are lacking. However, in the United States, 40% of married women and 61% of unmarried women using a reversible method of contraception change it over the course of 2 years [9]. Many — especially those with more years of education — change from a more-effective method to a less-effective method. This study similarly demonstrates that almost half of the women, despite wishing to avoid pregnancy, chose a less-effective method of contraception when they stopped using Implanon®. This is unfortunate since data from the United States and the UK suggest that using condoms or oral contraceptives is much more likely to risk unintended pregnancy [10,11]. In contrast, there are data to show that contraceptive implants are associated with lower rates of unintended pregnancy than either condoms or contraceptive pills [12].

Using preliminary data from this study, the NICE guideline on LARC concluded that Implanon® is a cost-effective method of contraception and more cost-effective — even if used only for 1 year — than either the combined oral contraceptive pill or condoms [1]. Concerns that the high up-front costs of this method are unjustified because discontinuation rates are high are unfounded as this study has shown that continuation rates for Implanon® in real life in the UK are not unreasonable when compared with those of clinical trials and with data for other contraceptives. This should reassure health care providers and commissioners that increasing the uptake of this long-acting method as recommended in the NICE guideline is worth the effort and the cost. The fact that more than one third of women chose to continue to use the implant when their first one had expired reflects a strong level of satisfaction on the part of the users.

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Contraception

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Original research article

The acceptability of self-administration of subcutaneous Depo-Provera

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Abstract

Depo-Provera (depot medroxyprogesterone acetate, or DMPA) is an important contraceptive option for women worldwide. Currently, it is only available in intramuscular form requiring regular quarterly routine attendance at a health facility. A new subcutaneous preparation has been developed. This is self-administrable and could potentially reduce need for routine attendance to an annual visit.

In a questionnaire survey of 176 women currently using DMPA, 67% would prefer to self-administer. Of the 33% who did not wish to self-administer, the most common reasons were a fear of needles (62%) and concern regarding incorrect administration (43%). In a second survey of 313 women not currently using DMPA, 64% of women said they would prefer to attend less often for contraceptive supplies. Twenty-six percent of women who had never used DMPA and 40% of ex-users would seriously consider DMPA if self-administration were possible.

Our findings would suggest that the advent of subcutaneous self-administrable Depo-Provera with appropriate training and reminder system is likely to be beneficial and popular with many women.

Keywords: Depo-Provera; Subcutaneous; Self-administration; Acceptability; Contraception

1. Introduction

Depo-Provera® (depot medroxyprogesterone acetate, or DMPA) is a long-acting, progestogen-only, injectable contraceptive currently used by around 13 million women worldwide. Depo-Provera is very commonly used in some developing countries; in South Africa, for example, more than 50% of women using contraception use DMPA [1]. In contrast, Depo-Provera is used by only a small minority of women in more developed countries. According to the National Survey of Family Life and Growth, only 3% of women in the United States in 1995 were using Depo-Provera [2]. Even in the United Kingdom, where use is perhaps the highest among the more developed countries, only 6% of women aged 25–29 years were using Depo-Provera in 2000/2001 [3].

Depo-Provera is currently formulated as an aqueous microcrystalline suspension administered by deep intramuscular injection at a dose of 150 mg every 12 weeks. This dosing schedule requires routine attendance at a health facility four times each year. In contrast, the World Health Organization recommends that women using oral contraception need be seen by a health professional only once each year [4]. Thus, although an inexpensive method of contraception per se (£5 per dose in the United Kingdom), Depo-Provera involves increased costs to health services when compared with all other available methods. It also creates greater cost and inconvenience for the user.

A new micronized preparation of DMPA has recently been developed [5]. A dose of 104 mg (in 0.65 mL) given by subcutaneous injection has an efficacy and duration of action similar to DMPA-IM. However, the subcutaneous preparation, DMPA-SC, can be self-administered. In anticipation of DMPA-SC becoming available in the United Kingdom, we undertook two questionnaire surveys to determine whether women currently using Depo-Provera would want to self-administer and whether the opportunity to do so would make DMPA a more attractive method to women who are not currently using it.

2. Subjects and methods

During December 2003, a self-administered questionnaire (Appendix A/Questionnaire 1) was offered to women

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[1] The author is a member of the International Advisory Board to Pfizer for DMPA-SC.

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attending a large family planning clinic in Edinburgh for repeat DMPA injection. The questionnaire was attached to every packet of DMPA, thus ensuring that it was offered to everyone. Women were asked if, after appropriate instruction, they would be interested in giving themselves the 3 monthly injections. If they responded positively, they were asked whether, and how, they would prefer to be reminded of the date when their injection was due. Women who were not interested in self-administering Depo-Provera were asked to choose one or more reason, from a list of possible reasons, why they would not want to self-administer.

For 2 weeks during the spring of 2004, a second self-administered questionnaire was offered to every woman attending the same Edinburgh family planning clinic (Appendix B/Questionnaire 2). Women were asked about current and ever use of all available methods of contraception. Those who had used but discontinued DMPA in the past were asked to choose from a list their reasons for discontinuation. Women who had never used it, but had considered it, were also asked to choose from a similar list what had deterred them from trying it.

All women were asked whether the opportunity to self-administer Depo-Provera, and thereby to attend clinics only once a year, would make them reconsider using the method. Both questionnaires were analyzed using SPSS.

3. Results

One-hundred seventy-six women currently using Depo-Provera completed Questionnaire 1. The response rate was 100%. Sixty-seven percent of women (118) said they would like to self-administer DMPA. Of these, 89% felt that they would need reminding of the date of injection. Thirty-three percent of women felt that being told the dates for the next three injections at the time of their annual review would suffice; 30% of them preferred to receive a reminder letter, and 25% a text message, just prior to the date of the next injection.

Of the 58 women (33%) who did not wish to self-administer DMPA, the most common reason was a dislike or 'fear' of needles (62%), 43% were concerned that they would administer the contraceptive incorrectly, while 33% felt it was important to see a doctor every 3 months. Twelve percent of women were disinclined to self-administer because they were worried that they might forget.

Table 1
Contraceptive method currently used by women completing Questionnaire 2

Main method of contraception	Number of respondents	% Respondents
Combined oral contraceptive pill	143	44
Barrier methods	64	20
Intrauterine system/intrauterine device	34	11
Progesterone-only pill	24	7
Contraceptive implant	20	6
Depo-Provera (DMPA)	10	3
No method	8	2
Withdrawal	7	2
Natural family planning	5	1
Vasectomy/sterilization	3	1
Other	2	0.6

Table 2
Reasons for discontinuing or not choosing Depo-Provera among women completing Questionnaire 2

Reason	Discontinuing (48 women) % Women	Not choosing (70 women) % Women
Change in bleeding pattern:		
Irregular	40	26
Amenorrhea	19	30
Weight gain	35	41
Mood swings	27	Qna
Headaches	10	Qna
12 Weekly visits	10	11
Painful injection/fear of needles	6	20
Wish for return of fertility/not wanting a delay in resumption of fertility	6	20
Not being able to stop the contraception immediately	Qna	23
Adverse opinions from friends/family	Qna	19

Qna=question not asked.

Three-hundred seventy copies of Questionnaire 2 were distributed and 323 were returned completed (87%). The main method of contraception being used by the women who completed Questionnaire 2 is shown in Table 1. Ten women were using Depo-Provera and seven of them (70%) expressed an interest in self-administration. Two hundred sixty-five women had never used Depo-Provera; their reasons for not choosing the method are shown in Table 2. One hundred sixty-five of them answered the question about self-administration. Sixty-one percent said they would prefer to attend the clinic less often for supplies of contraception. Twenty-six percent (43 women) said they would 'seriously consider' using Depo-Provera if self-administration was possible.

Forty-eight women (15%) had used DMPA in the past but had discontinued the method for reasons shown in Table 2. Thirty-four of 44 women (77%) who answered were interested in a method which allowed them to reduce the number of times they attended a clinic for contraception. Eighteen of 45 women (40%) who answered the relevant question said they would be interested in using DMPA again if it were available for self-administration.

4. Discussion

Depo-Provera is an important method of contraception. Anecdote suggests that in the United Kingdom it is increasingly popular among young women and among women of all ages who like the convenience of amenorrhea. In the United States, the fall in teenage pregnancy rates has been attributed to increased use of Depo-Provera [6]. The need to attend a health professional every 3 months simply for a single injection is a clear disadvantage of the method.

In our survey, two out of three women currently using Depo-Provera expressed a theoretical interest in self-administration. It is, of course, very likely that a number of them, when offered the *real* option of self-administration

would not take up the offer, and that more would find that they were unable or unwilling to give themselves an injection when the time came for it. Self-injected medication is the norm for diabetics and for some people with migraine or arthritis or undergoing infertility treatment. In most cases, the injections are self-administered daily or at least twice weekly. It is possible that women would never gain sufficient practice to become confident in self-administering an injection only four times each year. In a trial of self-administration of the once/monthly combined injectable contraceptive undertaken in Brazil, 14% refused to participate at the onset and another 31% declined after being trained to self-administer using oranges [7]. At the end of the study (three injections in total), more than 80% of women had been successful in giving their own injections and only 50% expressed a preference to continue self-administration. Just how many women, who in our survey said they would like to self-administer Depo-Provera, would do so in reality is the subject of a future study.

It is highly likely that many health care professionals would be skeptical about women's ability to self-administer Depo-Provera and, particularly, to do so at the correct time. Presently, if an injection is more than 2 weeks late, it is recommended that the next injection is withheld until pregnancy can be reasonably excluded [4]. If women were given their own injections, it is possible that there would be a greater chance that an injection would be given late and possibly given when conception had already occurred. In our clinic, we do not currently send reminders to women using DMPA but simply inform them when they attend for their injection, when the next one is due. All are clearly informed when they start to use the method, that the injection interval is 12 weeks. Since we do not presently remind them to attend the clinic for their injection, only the desire to avoid late injection when pregnancy had already occurred should stimulate us to have a system in place to remind them to *self-inject*. In a randomized trial designed to test whether an intensive reminder system would improve compliance among 250 women using Depo-Provera in the United States [8], intensive reminders did not improve continuation rates. Women receiving either mailed or telephone reminders were no less likely to return to the clinic on time than women given an appointment at the time of the first injection. One could argue that the need to make arrangements (including travel, and possibly taking time off work) to attend a clinic for injections would, in fact, be more likely to lead to mistimed injections compared with the ease of having the drug at home.

We were somewhat surprised that the ability to self-administer the injection, and thereby would enable women to reduce the number of clinic visits, might increase the acceptability of Depo-Provera, both to women who have never used it and to those who have tried but discontinued the method. It is likely, however, that this is an overestimate since the reasons for discontinuation of DMPA generally related to the side effects (Table 2) and not to the need to attend a health professional four times each year.

In conclusion, the advent of a form of Depo-Provera which would allow women to self-administer is likely to be a benefit to perhaps as many as half of the women choosing this method. Our survey also suggests that use of injectable contraception may increase where self-administration possible. We have discussed the concept in our clinic and suggest that women who express a wish to self-administer Depo-Provera should be given the appropriate training allowing an annual clinic visit with three injections supplied for home use. The success of such a scheme, and the health economics involved, will need to be tested scientifically.

Acknowledgments

The authors would like to thank the staff of the Lothian Primary Care NHS Trust Family Planning Service for their help with both questionnaire surveys and Fiona Hutchison, Clinical Effectiveness Facilitator for the Clinical Governance Support Team of Lothian Primary Care NHS Trust, for her assistance with statistical input and analysis of Questionnaire 2.

Appendix A. Questionnaire 1

The jag at home!!!

Imagine—no more visits to the doctors every 11 weeks for your jag! Just once a year!!

Interested? Keep on reading.

Soon the Depo-Provera injection will be available for you to give to yourself.

It is safe, easy and less painful.

Unlike today when it has to go deep into your muscle, the new way will be to inject it just under the skin (similarly to what diabetics do 3 times every day). We would supervise you the first time and if you were happy, you could take 3 injections home with you. Your next routine appointment would be a year later—just like women on the pill!!

Please answer the following 2 questions.

Your answers are strictly confidential and anonymous.

1. Would you want to give yourself your injection?

Yes ☐ (please go to question 2)

No ☐ (please go to question 3)

2. How would you want to be reminded that it was time for your next injection? (Please pick ONE only)

Do not want any reminders	☐	Letter	☐
Told the next 3 dates in advance when I attended	☐	Phonecall	☐
Text message	☐	Email	☐

3. Why not?

Worried about giving it correctly	☐
Prefer to see the doctor every 11 weeks	☐
Would forget	☐
Just do not want to give it to myself	☐
Other reason — please tell us _____	

Thank you for completing the questionnaire. Please hand it back to reception.

Appendix B

Depo-provera contraception
- your views on a new version that can be administered at home

Depo-provera ('the jag') is a form of hormonal contraception that presently is administered 3-monthly by intra-muscular injection. This has to be given by a doctor or nurse, and so requires a visit to the health centre or family planning clinic.

It is hoped that in the near future, a new form of depo-provera injection will be available that involves an injection just under the skin (similar to the injection that diabetics give themselves) instead of the current form which is injected into the thigh muscle. The first injection would be supervised, and if you were happy about it you would take the next 3 injections home with you.

We are carrying out this survey to find out what women **who are not currently using depo-provera** think of this development. Your answers are strictly confidential and it would be appreciated if you would answer the questions below by ticking the boxes beside the most appropriate answers. Many thanks.

Q1. What method of contraception are you currently using?

- ☐ *combined oral contraceptive pill*
- ☐ *condoms(male/female)*
- ☐ *IUD / IUS (coil)*
- ☐ *diaphragm / cap*
- ☐ *progesterone only pill*
- ☐ *contraceptive implant (e.g Implanon)*
- ☐ *natural family planning / rhythmn method*
- ☐ *withdrawal*
- ☐ *other (please specify)* .....

Q2. Have you ever used the 3-monthly depot injections?

yes ☐ ☐ *no*

please go to Q3 ↙ ↘ *please go to Q4*

Q3. What was/were you reason/s for stopping it?

- ☐ *painful injection*
- ☐ *having to visit doctor/clinic every 3 months*
- ☐ *weight gain*
- ☐ *mood swings*
- ☐ *headaches*
- ☐ *wishing to return to fertility/ get pregnant*
- ☐ *irregular bleeding*
- ☐ *absent periods*
- ☐ *other (please specify)* .....

.....

Q4. Have you ever considered using depot as a contraceptive method?

yes ☐ ☐ *no* **(please go to Q6 and Q7)**

please go to Q5 ↓

Q5. What was/were you reason/s for not using it?

- ☐ *fear of needles/ injections*
- ☐ *fear of weight gain*
- ☐ *having to attend the doctor/clinic every 3 months for the injection*
- ☐ *fear of irregular bleeding*
- ☐ *prefer to have a period every month*
- ☐ *delay in return to fertility*
- ☐ *not being able to stop the contraception straight away (it is with you for 3 months)*
- ☐ *friends/relatives have put me off the idea*
- ☐ *other (please specify)*.....

.....

Q6. Would you regard it an advantage if you could reduce the number of visits to your doctor/ clinic for contraception?

yes ☐ ☐ *no*

Q7. Would you consider depo-provera if you were able to give the injection to yourself?

yes ☐ ☐ *no*

PLEASE SEAL YOUR COMPLETED QUESTIONNAIRE IN THE ENVELOPE PROVIDED AND HAND IT IN AT RECEPTION. *THANK YOU.*

Survey : 8710

Family Planning and Well Woman Services, Dean Terrace, Edinburgh, EH4 1NL

Page : 1

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. novel estrogen-free oral contraceptive pill for women: ıulticentre, double-blind, randomized controlled trial of ıifepristone and progestogen-only pill (levonorgestrel)

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ACKGROUND: The acceptability and continuation rate of oral contraceptive steroids are limited by unpredictable eeding and the fear of long-term risks such as breast cancer. By inhibiting ovulation and by altering the receptivity the endometrium, antagonists of progesterone, such as mifepristone, could be developed as estrogen-free novel con-aceptives. METHODS: Multicentre, double-blind, randomized controlled trial comparing frequency of amenor-oea (primary outcome), bleeding patterns, side effects and efficacy in women taking daily 5 mg mifepristone = 73) or 0.03 mg levonorgestrel (progestogen-only pill; POP, $n = 23$) for 24 weeks. RESULTS: More women :re amenorrhoeic while taking mifepristone than POP (49 versus 0% $P < 0.001$), and fewer women bled or otted for >5 days per month (4 versus 39% $P < 0.001$). Forty-eight percent of women who took mifepristone for months had cystic glandular dilatation of the endometrium but none showed hyperplasia or atypia. There were pregnancies in 356 months of exposure in women who used only mifepristone for contraception. Two pregnancies curred in women taking mifepristone who were also using condoms for dual protection. CONCULSIONS: Daily ifepristone (5 mg) is an effective oral contraceptive pill which has a better pattern of menstrual bleeding than an isting POP (levonorgestrel).

ywords: antiprogestins; contraception; levonorgestrel; mifepristone; progestogen-only pill

troduction

fty years have passed since the first clinical trials in Puerto co demonstrated that a daily pill containing ethinyl estradiol d norethynodrel was a highly effective contraceptive (Pincus *al.*, 1958). Contraceptive development since the introduction the pill has been limited to variations on the theme of steroid rmones [new delivery systems, different progestogens, lower ses of estrogen (Baird and Glasier, 1999; Population eports, 2003)]. Oral contraceptives, containing either proges-gen or a combination of estrogen with progestogen, are pular because they are highly effective and easy to use aird and Glasier, 1999; Population Reports, 2003). owever, continuation rates are often disappointingly low. The commonest reason for discontinuation of combined oral contraception (COC) is breakthrough bleeding (Rosenberg and Waugh, 1998) but fear of breast cancer (Collaborative Group on Hormonal Factors in Breast Cancer, 1995; Larsson *et al.*, 1997; Althius *et al.*, 2002), and to a lesser extent venous thromboembolism, are significant disincentives to both uptake and continuation despite the obvious convenience of relief of dysmenorrhoea and other symptoms related to menstruation (ESHRE Capri Workshop Group, 2005). Progestogen-only pills (POP) have the advantage of fewer serious risks but are associated with unpredictable vaginal bleeding which is the commonest reason for discontinuation (McCann and Potter, 1994; Fraser, 1999). In many surveys,

fewer than 50% of women continue using oral contraceptives for >12 months (D'Arcangues *et al.*, 1992).

Even the regular pattern of monthly bleeding characteristic of the combined pill is considered undesirable by some women. When oral contraceptives were first marketed it was assumed that women would prefer to have a monthly cycle because it would be perceived as more 'natural' (Pincus 1965). Recently, it has been shown that many women, regardless of age, prefer to have either predictable bleeding less often than once a month or not to bleed at all (Den Tonkelaar and Oddens, 1999; Glasier *et al.*, 2003).

Mifepristone is a synthetic C19 steroid which is a potent antagonist of progesterone (Ulmann, 2000). There is very little information about the use of mifepristone (or other progesterone receptor modulators) for contraception in spite of the fact that the original clinical publication 23 years ago demonstrated its contraceptive as well as abortifacient properties (Herrman *et al.*, 1982; Baird, 2001). The lack of progress with these compounds has not been due to scientific reasons but relates to the political and religious controversy surrounding RU486 ('the Abortion Pill'). In low daily doses, mifepristone has been shown to inhibit ovulation by suppressing the pre-ovulatory surge of LH and acting directly on the endometrium to induce amenorrhoea in the majority of women (Ledger *et al.*, 1992; Croxatto *et al.*, 1993; Cameron *et al.*, 1995; Brown *et al.*, 2002). We have previously demonstrated in a pilot study that mifepristone at a dose of 2 or 5 mg/day has contraceptive potential (Brown *et al.*, 2002). In contrast to pills containing estrogens and/or progestogens, there is no theoretical risk that such a pill would increase the risk of breast cancer or cardiovascular disease. Rather, experimental data show that antigestogens are antimitotic in breast cancer cell lines and animal models and hence might actually reduce the risk of malignancy (Horowitz, 1992; Klijn *et al.*, 2000; Poole *et al.*, 2006).

A pill which contained no estrogen and which reproducibly induced amenorrhoea in high proportion of women should prove popular. In the present study, therefore, we directly compared the pattern of bleeding and other side effects of mifepristone at a dose of 5 mg/day for 24 weeks with a method of contraception in common use in the UK, the POP (levonorgestrel). We hypothesized that women given mifepristone would have a much higher incidence of amenorrhoea and fewer days of menstrual bleeding.

Materials and Methods

This was a multicentre, double-blind, randomized controlled phase II trial comparing two daily contraceptive pills. The primary outcome was the percentage of women who had amenorrhoea throughout the study. Secondary outcomes included number of days of bleeding, endometrial thickness and histology, and number of pregnancies. Before starting, the trial was approved by the steering committee of the Contraceptive Development Network (MRC Grant No. G9523250). It was performed in accordance with Good Clinical Practice including regular monitoring of centres.

Between June 2003 and January 2004, a total of 97 healthy volunteers with regular menstrual cycles (21–42 days) aged 18–40 years were recruited from four sites (34 in Sagamu, Nigeria; 18 in Cape Town, South Africa; 10 in Hong Kong, People's Republic of China and 35 in Edinburgh, Scotland). The study was approved by local ethical committees at all centres. All women gave written informed consent before enrolment and were screened before entering the study by routine physical and gynaecological examination and measurement of height, weight, blood pressure and pulse rate. Blood samples were collected for measurement of progesterone, clinical chemistry and haematology. The size of the uterine cavity and ovarian follicles were measured by transvaginal ultrasound scan. Urinary hCG was also measured to exclude pregnancy before entering the trial. Women who had used any form of hormonal contraception within the last 3 months of the start of the study were excluded except in Edinburgh where nine women with regular cycles while already using a POP were included without having to undergo a washout period.

Subjects were studied for one pretreatment cycle, six treatment cycles (24 weeks) and for one post-treatment cycle. Subjects were randomly allocated to receive either mifepristone 5 mg/day (one half a 10 mg tablet) (from Laboratoire Exelgyn, 6 rue Christophe Colomb, 75 008-Paris for women in Edinburgh; and from Hualian Pharmaceuticals Ltd, Shanghai, China for women in Shanghai, Cape Town and Sagamu) or levonorgestrel 0.03 mg/day (POP) (Norgeston; Schering Health Care Limited, The Brow, Burgess Hill, West Sussex, RH15 9NE) starting on Day 1 or 2 of the cycle. Randomization was achieved by blocked computer-generated randomization performed individually for each centre to ensure good balance of numbers in the different treatment groups. Each centre was given numerical sequence of coded treatment bottles which were identical generated by statistician in Edinburgh office. Participants were enrolled in each centre by the local investigator. The numerical sequence was determined by the clinical trials manager in Edinburgh. To maximize, the number of cycles observed on mifepristone without greatly reducing the power of the randomized comparison, three times as many subjects were randomized to mifepristone as to levonorgestrel. In Cape Town, Hong Kong and Edinburgh, daily doses were issued at eight weekly intervals in pre-packed identical bottles containing mifepristone (a half tablet) with placebo, or levonorgestrel with one half placebo tablet. In Nigeria, daily doses were issued at weekly intervals. The placebo and active tablets were both white but of slightly different size. Unused medication was returned and new medication dispensed at this time. All subjects were sexually active and intending to use the study drug as their sole method of contraception. However, in Cape Town and Hong Kong some women also used condoms for protection against sexually transmitted infection. Cycles in which dual protection was used were omitted when calculating the months of exposure to the risk of pregnancy.

The women were asked to record every day the amount of vaginal bleeding and adverse events on a diary card. If bleeding occurred it was recorded as spotting (one or less pad/tampon required) or bleeding (more than one pad/tampon required).

Subjects attended the clinic for review at eight weekly intervals during the treatment phase and once more on Day 5–11 of the first menstrual cycle after discontinuation of the study medication. At the end of the treatment phase a vaginal examination was performed on all subjects and an endometrial biopsy performed in a subgroup of volunteers. In order to identify those women who might develop hyperplasia of the endometrium a biopsy was performed at any time during the study on all subjects if the measurement of the uterine cavity was observed to be >12 mm ('safety biopsy'). Biopsies were performed as an outpatient procedure with a Pipelle suction curette (Pipelle de Cornier, Laboratoire C.C.D, 60 rue Pierre Charron, 75 008-Paris-France, Ref. 1103000). Endometrial samples were then fixed in 10% neutral buffered formalin prior to embedding in paraffin wax. Histological examination of the endometrial sections was

ıducted blind by an independent pathologist and classified into five egories [proliferative, secretory, inactive, insufficient and cystic ındular dilatation (CGD) as previously described (Baird *et al.*,)3)]. (i) Proliferative includes 'active' in which glands are ıular, with columnar epithelial cells showing nuclear stratification ł frequent mitoses (> = 5 per 20 gland profiles); and 'weakly'—ıple tubular glands with columnar epithelial cells showing mild :lear stratification (2–4 mitoses per 20 gland profiles). (ii) Inac-e—cuboidal to columnar cells showing focal or diffuse nuclear stra-cation, in glands with simple tubular or undulating profiles without nificant dilatation. Resembles endometrium of basalis in normal :ling endometrium. Glands are not atrophic, and show evidence limited recent growth or function, but lack the findings seen in rmal cycling proliferative endometrium. Mitoses are absent or common (no more than om mitotic figure per 20 gland profiles).) Inactive with CGD—inactive glandular epithelium as described ove, with dilatation of gland lumina. Dilatation is defined as the ınd having an open lumen that forms a space, i.e. greater than ır times the epithelial thickness. (iv) Secretory—glands are variably tuous with cytoplasmic vacuolation that varies according to phase cycle. Cells are columnar, with non-stratified nuclei showing an sence of mitoses (except for infrequent mitoses in the early :retory phase). Stromal decidual change starts around spiral ssels, becoming confluent in the late secretory phase. Some nples contained inadequate amounts of tissue for accurate histologi- evaluation.

Ovarian function was assessed by the measurement of progesterone venous blood collected at screening and at 8, 16 and 24 weeks after rting treatment. The woman was classified as having ovulated at ıst once if the concentration was >15 nMol/l in any of the three nples. Following centrifugation the serum was stored at −20°C labelled sample tubes. Analysis by radioimmunoassay was carried t at the end of the study locally by each centre. Transvaginal ultra-nography was used to assess number and size of follicles or cysts at reening and after 8, 16 and 24 weeks.

ıtistical methods

ıe number of subjects on mifepristone was chosen to give an upper nfidence limit of <5% for the risk of pregnancy over the 6 months treatment if no pregnancies occurred in that group. To maximize, : number of cycles observed on mifepristone without greatly redu-ıg the power of the randomized comparison, three times as many bjects were randomized to mifepristone as to levenorgestrel. ıese numbers were also sufficient to give over 99% power to show significant difference in the rate of amenorrhoea over 6 months if e rate was 50% on mifepristone when compared with the 5% anti-pated in the levenorgestrel group (Collaborative Study Group, 1998). Data analysis was carried out using SPSS version 12 (SPSS, Inc., ıicago, IL, USA) and Excel 2003 (Microsoft Corporation). The 'o randomized groups were compared by chi-squared test with ates' correction and Mann–Whitney or Student's *t*-tests as appropri-e tests for binary and continuous outcomes, respectively. Analysis of ıvariance adjusting for pretreatment values was used to compare the ıdometrial thickness in the two groups at follow-up. Confidence nits were calculated for the incidence efficacy outcomes in the mife-istone group using the Poisson distribution based on person time at sk.

esults

total of 97 women were recruited (Fig. 1) and randomized to eatment [35 in Edinburgh (26M, 9POP); 34 in Nigeria (26M,

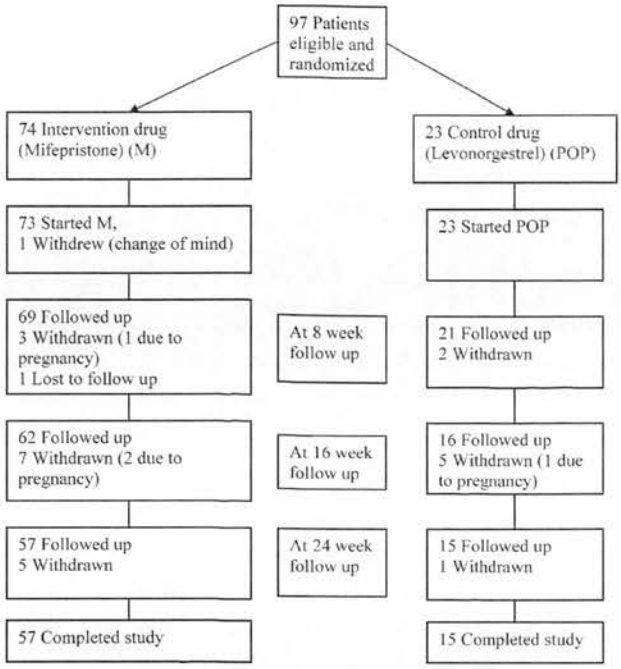

Figure 1: Flow chart of patients through the study

8POP); 18 in Cape Town (14M, 4POP); 10 in Hong Kong (8M, 2POP)]. One subject randomized to mifepristone withdrew for personal reasons and discarded the study drugs without taking any. Therefore, the results of the 96 women who started treatment [73 received mifepristone (M), 23 received levonorgestrel (POP)] were included for analysis.

Of the 87 women who started the study and did not use hormonal contraception in the previous months, 66 were randomized to mifepristone and 21 to POP. An additional nine women in Edinburgh who had regular menstrual cycles while taking POP for contraception were transferred directly to study medication. In this subgroup, seven were randomized to mifepristone and two to POP.

When all centres were combined (Table 1) there were no statistically significant differences in age, weight, height, BMI or parity between the two treatment groups (two-sample Student's *t*-tests). However, there were some differences in the characteristics of the women from different centres. The women in Nigeria were significantly older (years 33.9 ± 4.2 SD, $P = 0.001$) than the women in both Cape Town (26.2 ± 7,7) and Edinburgh (28.7 ± 6.0). The BMI of women in Hong Kong (19.8 ± 1.7 kg/m^2) was significantly lower than that of women in Edinburgh (22.9 ± 1.9, $P < 0.001$), Nigeria

Table 1: Mean (SD) age, weight, height and BMI according to study drug

	M	POP
Age (years)	30.3 (6.3)	30.4 (6.9)
Weight (kg)	60.7 (9.1)	58.4 (6.2)
Height (cm)	161.5 (5.9)	161.3 (6.3)
BMI (kg/m^2)	23.3 (3.6)	22.4 (1.8)

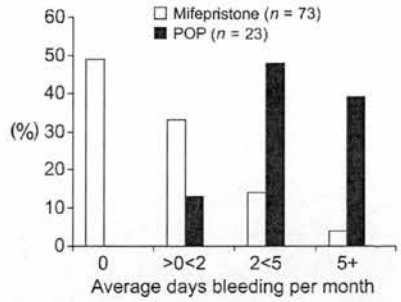

Figure 2: Percentage of women who bled for a given number of days on average per month in 96 women randomized to daily mifepristone or levonorgestrel (POP).
0, amenorrhoea; $> 0 < 2$, any bleeding <2 days per month; $2 < 5$, 2 to <5 days bleeding per month; 5+, 5 or more days bleeding per month

(24.3 ± 3.9, $P = 0.001$) and Cape Town (23.1 ± 3.6, $P = 0.014$).

Twenty-four women (16M, 8POP) did not complete the full 24 weeks of treatment. Eight women withdrew for personal reasons unrelated to the study medication (e.g. moved from the area, relationship ended); six were discontinued by the investigators because of persistent protocol violations (e.g. poor compliance, unavailable for follow-up). Two women withdrew because of anxiety about potential adverse affects of the pills. Four women in the POP group discontinued because of persistent irregular bleeding, no women in the mifepristone group discontinued for this reason ($P < 0.01$). Four women withdrew because of pregnancy: three in the mifepristone group and one while using levonorgestrel.

Seventy-two women completed the study (57M, 15POP) and took the medication for at least 24 weeks (168–192 days).

Menstrual bleeding pattern

There were significant differences in the pattern of menstrual bleeding between the groups (Fig. 2). Of the 73 women who started mifepristone, 36 (49%) were amenorrhoeic for the duration of drug treatment and only three women bled or spotted on average for five or more days per month. In contrast, none of the women in the POP group were amenorrhoeic. Although the number of days of bleeding was within the range of a normal menses in the majority (61%), nine women in this group bled or spotted for five or more days per month and four women withdrew from the study prematurely because of continuous irregular bleeding.

Of the **57** women who took mifepristone for the full 6 months, 25 (44%) were amenorrhoeic and a further 22 (39%) had bleeding or spotting for a total of 10 days or less (Table 2). In comparison, of the 15 women who took POP, none (0%) were amenorrhoeic (compared with mifepristone $\chi^2 = 8.01$, $P = 0.005$).

The pattern of bleeding in those women who continued to menstruate was irregular and unpredictable in the majority in both groups. Although episodes of bleeding in the women who took mifepristone were infrequent and slight, they were mostly unpredictable, except in Nigeria where 4 of the 15 women who continued to bleed had regular monthly periods. Nigerian women were more likely to experience bleeding over the 6 month study duration (13/21; 62%) than those from other centres [Edinburgh 4/22 (18%); Cape Town 3/9 (33%); Hong Kong 1/6 (17%)]. There was a significant difference in bleeding frequency between Nigeria and Edinburgh ($\chi^2 = 6.86$; $P = 0.009$).

Ovarian function

Ovarian function was assessed at baseline, 8, 16 and 24 weeks after starting treatment by measurement of progesterone in blood. In 33 of the 97 pretreatment samples, the concentration of progesterone was >15 nmol/l indicating that in just over a third of the women the blood was collected in the luteal phase after ovulation. At each eight-week review, there was evidence of ovulation in some women in both groups. If a woman showed evidence of ovulation at any of the three review visits she was classified as ovulatory. Ovulation was less likely to occur in women taking mifepristone (14/73; 19%) than in those in the POP group although there was no statistically significant difference (7/23, 30%; $\chi^2 = 0.72$; $P = 0.40$). However, there were significant differences between centres in the incidence of ovulation and amenorrhoea. For example, while taking mifepristone only one of the 26 women in Edinburgh (4%) ovulated when compared with 11/26 women in Nigeria (42%: $\chi^2 = 8.78$; $P = 0.003$). The proportion of women who ovulated while taking the POP was identical in the two centres (37%).

Ultrasound examination revealed the presence of numerous small and medium-sized follicles in the ovaries of women in both groups throughout treatment. Follicular cysts (diameter

Table 2: Distribution of mean (SD) numbers of days bleeding and/or spotting while on drug according to treatment group, in the 72 women who took the treatment for at least 6 months (%)

Number of days	Bleeding/spotting		Bleeding only		Spotting only	
	M	POP	M	POP	M	POP
0	25 (44)	0 (0)	36 (63)	0 (0)	39 (68)	6 (40)
1	4 (7)	0 (0)	0 (0)	0 (0)	6 (10)	2 (13)
2.5	8 (14)	2 (13)	5 (9)	2 (13)	8 (14)	1 (7)
6–10	10 (18)	1 (7)	7 (12)	1 (7)	3 (5)	1 (7)
11–20	5 (9)	3 (20)	5 (9)	5 (33)	1 (2)	2 (13)
21+	6 (11)	9 (60)	5 (9)	7 (47)	1 (2)	3 (20)

ble 3: Mean (SD) endometrial thickness at different times according to atment group

eks	Mifepristone	POP	*P*-value
	6.1 (2.1) [73]	5.8 (2.4) [22]	NS
	6.6 (3.4) [68]	5.3 (2.7) [19]	0.11
	8.0 (5.2) [60]	5.1 (2.8) [15]	0.043
	10.3 (6.8) [58]	4.0 (2.0) [15]	<0.001

e *P*-value is from an analysis of covariance adjusted for the pretreatment ue. Numbers of subjects are shown in square brackets.

30 mm) were detected on eight occasions (six among women ing mifepristone and two in the POP group). The cysts were ymptomatic and resolved spontaneously by the next examin- on without treatment.

idometrial thickness

the mifepristone group, the width of the cavity and the thick- ss of the endometrium increased with time relative to the seline (Table 3). In contrast, there was no statistically signifi- nt change in the POP group, and by 16 weeks the difference the size of the uterine cavity between the groups was statisti- lly significant ($P = 0.043$). There were differences between e centres in the extent of the increase in size with the thickest dometrium at 24 weeks occurring in Edinburgh ($5.2 \pm$ l mm before starting, increasing to 13.8 ± 7.1 at 24 weeks) able 4).

idometrial biopsies

total of 39 women had at least one endometrial biopsy. Six omen in the mifepristone group had two biopsies and one d three biopsies, making a total of 47 biopsies available for amination. Twenty women (19 in the mifepristone group d 1 in the POP group) had a 'safety biopsy' because the endo- etrial cavity on ultrasound was >12 mm. A total of 31 biop- es were available from women who had completed 24 weeks treatment (27 mifepristone and 4 POP).

CGD was found in the endometrium of 13 of the 27 women io had a biopsy at 24 weeks on completion of the study. The mainder were proliferative (4), inactive (3), or insufficient for stological examination (7). Of the 19 women on mifepristone io had a 'safety' biopsy because the uterine cavity was lated above 12 mm, 11 showed CGD on at least one occasion. GD also occurred in four of nine women with normal-sized vity who volunteered to have an endometrial biopsy at 24 eeks. The endometrium in the sole woman in the POP oup who had a safety biopsy (cavity 13 mm) was too scanty for histological examination. None of the women in either group showed evidence of hyperplasia or atypia.

Hysteroscopy was performed as an outpatient procedure on two women (both in Edinburgh) with an enlarged uterine cavity (29 and 16 mm) detected on routine ultrasound at 16 weeks. The uterine cavity was dilated with mucous fluid and a pale oedematous looking endometrium which showed CGD on histological examination (Fig. 3). No serious pathology was detected and one woman opted to continue with the trial. The endometrial cavity returned to normal size after completion of the trial.

ble 4: Mean (SD) endometrial thickness at different times according to ntre for mifepristone only

eeks after starting	Edinburgh	Cape Town	Hong Kong	Nigeria
	5.2 (2.1)	6.7 (1.7)	6.6 (2.6)	6.4 (1.8)
	4.9 (3.4)	7.6 (3.3)	6.4 (4.4)	8.0 (3.2)
	9.2 (5.7)	8.0 (5.0)	8.9 (7.4)	6.5 (4.4)
	13.8 (7.1)	11.5 (8.7)	8.3 (3.6)	6.8 (4.4)

Contraceptive efficacy

Not all the subjects were at risk of pregnancy for the duration of the study. Some women separated from their partner, while a number of women in Cape Town and Hong Kong used barrier methods as well as the study drug for dual protection. In total, there were 356 months of exposure in women who took mifepristone as their sole method of contraception and 85 in women who took POP.

Five women became pregnant during the study: four of them in the group of women randomized to mifepristone. However, there were only three pregnancies while the subjects were taking the study drugs. In the mifepristone group, two pregnancies occurred (both in Capetown) during treatment and were recognized after 62 and 115 days of treatment, respectively. Conception occurred in both within the first 2 months of treatment. One woman underwent a vacuum aspiration and the other opted to continue with the pregnancy and delivered at term of a healthy baby. Both of these women were using condoms for dual protection. Inclusion of these two women in the analysis of efficacy gives an estimate of 0.6% (95% confidence limits 0.07 to 2.0%) for the risk of pregnancy per month while exclusion results in an estimate of 0% (0 and 1.1%). Two additional pregnancies occurred before starting or several weeks after stopping mifepristone. One pregnancy occurred in a woman using levonorgestrel and was only detected because of a routine pregnancy test 8 weeks after starting treatment. She had a blighted ovum and the uterus was evacuated surgically. There was no significant difference in the pregnancy rates between the groups although the study was not powered to detect differences.

Adverse effects

There were no major adverse events in either group. Eight women (35% of those taking POP) and 19 (36% of those on mifepristone) reported a range of symptoms none of which were significantly different between the groups including abdominal discomfort (12% mifepristone versus 4% POP), irregular bleeding (7% mifepristone versus 9% POP), headache (8% mifepristone versus 22% POP), flushes (7% mifepristone versus 0% POP) and mood change (3% mifepristone versus 0% POP).

Discussion

This study has demonstrated striking differences in the pattern of menstrual bleeding between women taking two types of oral

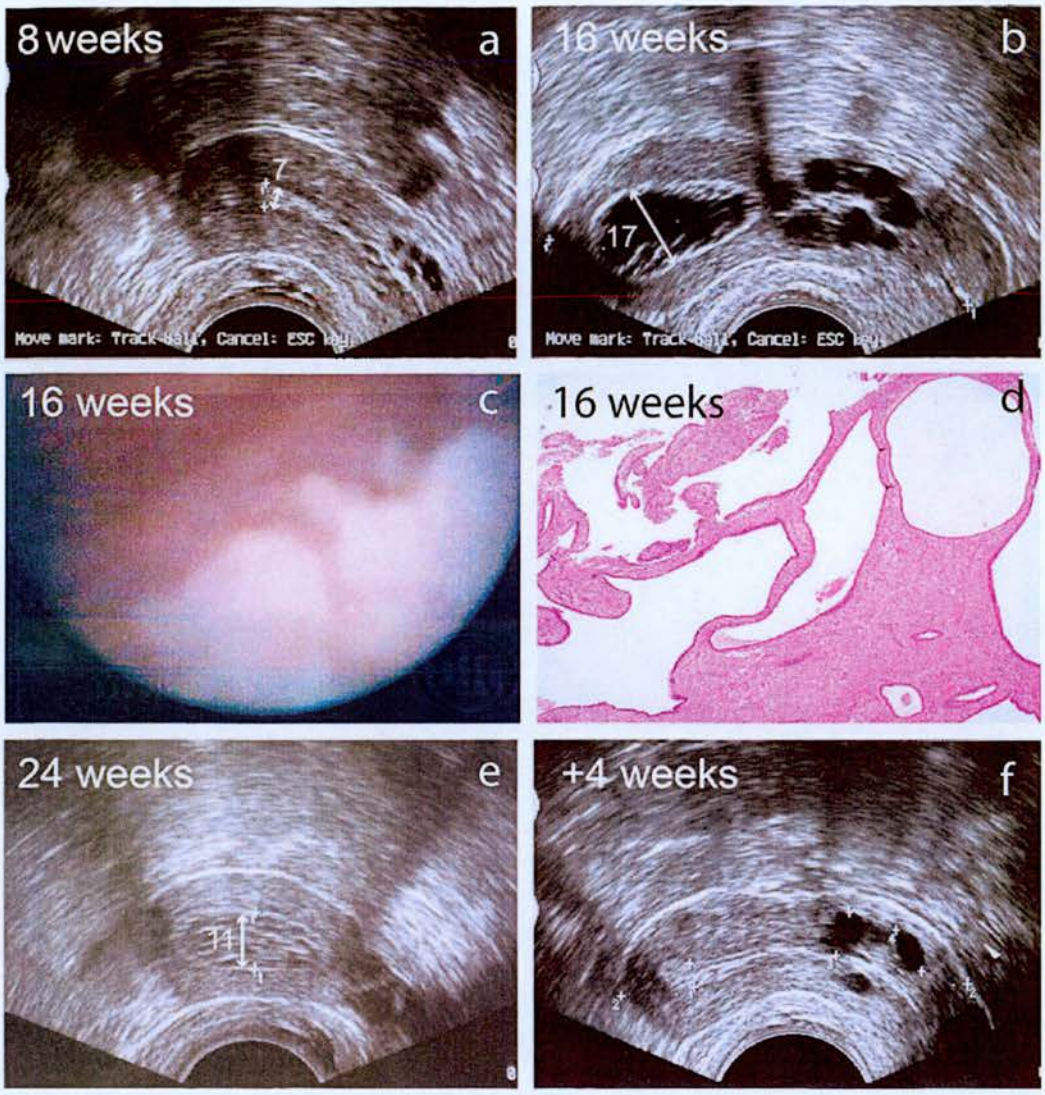

Figure 3: Appearance of the uterus of subject 135 who took 5 mg mifepristone per day for 24 weeks.
The subject remained amenorrhoeic throughout. **(a)** After 8 weeks the thickness of the endometrium and cavity was normal; **(b)** at 16 weeks the cavity was dilated to 17 mm with fluid. Cyst like structures had become apparent in the cervix; **(c)** the cavity was lined by pale dilated endometrium; **(d)** showing CGD on histology (X10); **(e)** by 24 weeks the cavity was still dilated; **(f)** by 4 weeks after stopping the cavity had returned to normal after menses. Note the persistence of dilated cervical glands

contraceptive pills, which contain no estrogen. The results confirm our previous study, which reported that the majority of women taking 5 mg mifepristone every day for four months were amenorrhoeic (Brown *et al.*, 2002). In the present study, over 80% of women who took mifepristone were either amenorrhoeic or had episodes of bleeding or spotting <2 days per month. In comparison, women on levonorgestrel had frequent irregular bleeding (>5 days per month) and none was amenorrhoeic. Four women discontinued the POP because the bleeding pattern was unacceptable.

This study has its strengths and weaknesses. The placebo was similar but not identical to either of the active drugs and hence the study was not truly double blind. It would have been possible for the persistent subject and/or researcher to identify group differences by the size of the treatment tablets. We think that this is unlikely because each woman was supplied with two identical opaque bottles each containing 8 weeks supply of either placebo or active drug prepared by a member of the research team not involved with contact with the subjects. Moreover, the difference in bleeding patterns (the primary end point) was so striking that we think it unlikely that unblinding of an individual subject would have had a significant impact on the overall results.

Nine women (all in Edinburgh) who were taking POP were recruited directly to the study without at least 3 months 'wash-out'. It could be argued that in these women there was

carry over' effect on the ovary and/or endometrium from the ɔvious treatment. We think that it is unlikely because all ɔmen had regular menstrual bleeding while on the POP. ɔreover, at recruitment they had evidence of ovarian activity, . luteal levels of progesterone, follicles >10 mm and/or dometrium >8 mm. Because they were randomized 2:7)P:mifepristone, they would not bias the comparison tween the groups. The seven women in this subgroup who ɪre randomized to mifepristone had amenorrhoea for at ɪst 3 months after starting, illustrating one of the clinical ɔs when the drug becomes available.

There were minor differences between centres in the ɹponse to the drug. However, in all centres the bleeding ttern was better with mifepristone and the contraceptive effi-ɔy was high.

It is arguably in Africa where there is the greatest unmet ed for contraception. The fact that we show that it is effective a range of cultures, including Africa, should help facilitate use world wide particularly in those cultures where ɔmen who are menstruating are subject to social taboos.

In the women in the mifepristone group who continued to ɔed there were differences between the centres in the ttern of menses. Nigerian women were more likely to experi-ce bleeding over the 6 months study and to show biochemical idence of ovulation than women in the other centres. For ɡistic reasons, our assessment of ovarian function by ɔasurement of the concentration of progesterone every weeks was imprecise. We classified a woman as being ʋulatory' if the level of progesterone was in the luteal ase range on any one of the three occasions when it was llected. We argued that there would be about a one in ee chance that the progesterone level will be raised in any ɪgle sample collected at random from normal cycling ɔmen as was found in the control cycle. Although the ɪjority of women in all centres failed to ovulate while ɔing mifepristone, there were significant differences in the ɔidence of anovulation between centres.

These differences between centres are intriguing. We have ɔviously reported that ovulation and menstruation were ɔre easily suppressed by mifepristone in Chinese women in ɜanghai than in Caucasians in Edinburgh (Brown *et al.*, 02). The daily dose of 5 mg mifepristone was chosen cause it resulted in amenorrhoea in over 90% of women in linburgh. We have previously suggested that these differ-ces in response may be due to differences in diet and/or in ɔtabolism of steroids. Alternatively, there could be differ-ces in compliance between centres although there was no idence from the number of returned pills that the women Nigeria omitted more pills than those in other centres. More-ɾr, a large number of tablets would have to be missed before b-therapeutic levels of mifepristone were reached because of e long half-life of mifepristone (Heikinheimo *et al.*, 2003).

One of the main reasons that women discontinue ɪrmonal contraception is because of menstrual irregularity)'Arcangues *et al.*, 1992) and this was true of women using e POP in this study. All four women who discontinued the ɪdy specifically because of menstrual irregularity were king POP. The amenorrhoea or scanty bleeding associated with mifepristone should be perceived as an advantage by many women (Den Tonkelaar and Oddens, 1999; Glasier *et al.*, 2003).

It has been argued that prolonged amenorrhoea is unnatural and even harmful. Monthly menstruation has however been the norm only for the last 100 years. Prior to that, most women spent their short lives either pregnant or breastfeeding and amenorrhoeic. Absence of periods *per se* does no harm. If associated with hypo-estrogenism (as in the menopause or during treatment with analogues of gonadotrophin releasing hormone) it is associated with increased risk of osteoporosis and heart disease; if associated with a high dose of progestogen (as during the use of Depo Provera) or prolonged exposure to COC (as in extended pill use) it may be associated with increased risk of breast cancer and heart disease (Collaborative Study Group, 1998). Amenorrhoea during mifepristone use is not accompanied by hypo-estrogenism and, as stated earlier the risk of breast cancer may be reduced (Horowitz, 1992; Klijn *et al.*, 2000). A recent paper reported that mifepristone prevented the development of breast cancer in transgenic mice with null mutation of BRCA1/p53 (Poole *et al.*, 2006).

Concern has been expressed that with prolonged intake of antiprogestogens the endometrium would undergo hyperplastic or malignant changes due to continued exposure to unopposed oestrogen (Murphy *et al.*, 1995). However, studies in monkeys with mifepristone and other antigestogens have shown evidence of endometrial atrophy rather than hyperplasia (Van Uem *et al.*, 1989; Ishwad *et al.*, 1993; Neulins *et al.*, 1995; Chwalisz *et al.*, 2000). In keeping with our previous report, the endometrial cavity widened progressively with time in women in Edinburgh, but not in the other centres (Brown *et al.*, 2002). We have previously reported that much of the apparent increase in endometrial thickness is associated with cystic dilation of the endometrial glands and the cavity itself (Baird *et al.*, 2003). A recent paper reported the results of a study in which 40 women with fibromyoma were given 5 or 10 mg mifepristone/day for up to 1 year (Eisinger *et al.*, 2005). Simple hyperplasia of the endometrium without atypia was seen after 6 months only in a minority of women (28%) who took 10 mg but none at 5 mg.

In the present study, the commonest histological picture was of inactive CGD. This CGD was only found in those women taking mifepristone. The cause of this unusual change is unknown. It is unlikely to be due to the effects of unopposed estrogen as it has been demonstrated to occur in women who have profound suppression of ovarian follicular development and where oestrogen levels are low (Brown *et al.*, 2002; Baird *et al.*, 2003). Moreover, it occurs more commonly in those women taking 10 mg mifepristone per day than in those taking 5 mg in whom the secretion of ovarian estradiol is higher (Eisinger *et al.*, 2005).

Novel observations in this study were the hysteroscopy finding of atrophic and/or oedematous endometrium. Hysteroscopy revealed that the apparent thickness of the endometrial cavity as measured on ultrasound reflected dilation of the glands and cavity rather than true hyperplasia of the endometrium. It demonstrates the limitation of using measurements of endometrial thickness by ultrasound alone as a marker of

endometrial pathology. The cause of this accumulation of fluid and its nature is unknown but similar findings have been reported in rabbits following treatment with mifepristone (Chwalisz *et al.*, 2000). It may be that the mechanism, which normally allows the passage/and or re-absorption of fluid from the endometrial glands and uterus, is obstructed leading to an accumulation of fluid within the uterus.

This study has confirmed that mifepristone is potentially a highly effective contraceptive. Even in Nigeria where there was biochemical evidence of ovulation in 42% of women there were no pregnancies in the mifepristone group. In our previous study, we reported no pregnancies in 50 women who used mifepristone at a dose of 2 or 5 mg/day for 4 months (Brown *et al.*, 2002). The present study extends our contraceptive experience to a total of 556 women cycles. The two pregnancies in women taking mifepristone occurred in women in South Africa who were also using condoms as protection against sexually transmitted disease including, HIV/AIDS. It is possible that these women may have omitted to take their pills every day because they thought that they were protected from the risk of pregnancy by the use of condoms.

In conclusion, the present study demonstrates that mifepristone at a daily dose of 5 mg is a safe and potentially effective contraceptive. The relatively high incidence of amenorrhoea or reduced amount of scanty bleeding is likely to be better accepted by women than the irregular unpredictable menstrual bleeding that occurs in the majority of women taking the POP. The reduction of menstrual blood loss should convey health benefits to women particularly in developing countries where the incidence of anaemia is high. Because of its antagonism of progesterone the risk of breast cancer may be reduced rather than increased as is the case with COCs containing estrogen and progestogens (ESHRE Capri Workshop, 2005). A large multicentre phase III trial is required further to assess contraceptive efficacy and safety, particularly with respect to endometrial cancer.

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Original research article

Changes in vaginal morphology, steroid receptor and natural antimicrobial content following treatment with low-dose mifepristone☆

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Abstract

Background: We have previously shown that the antigestagen mifepristone is contraceptive when given in a daily dose of 5 mg, po. Epidemiological studies suggest that gestagen-only contraceptives may increase the risk of transmission of human immunodeficiency virus (HIV) due to effects on the vaginal defenses to infection. We investigate the effects of mifepristone on vaginal thickness, steroid receptor and natural antimicrobial content and pharmacokinetics of mifepristone.

Methods: In a pilot study, eight women were given mifepristone 5 mg/day for an average of 33 days. Ovarian function was assessed by measurement of estradiol and progesterone in blood and their metabolites in urine and by serial ultrasound of their ovaries. Vaginal biopsies were collected before (late proliferative) and after taking mifepristone.

Results: All subjects showed a similar pattern of descending serum concentrations of mifepristone. The elimination phase half-life was 18±5.1 h (mean±SD). Mean C_{max} measured at 1 h was 641.7 nmol/L (range, 502–740 nmol/L). All eight women reported amenorrhea for the duration of treatment and seven of eight women showed biochemical and ultrasound evidence of anovulation. There was no significant change in vaginal thickness following treatment [342±40 μm pretreatment, 303±69 μm posttreatment (mean±SEM); $p>.05$]. Estrogen (ERα, ERβ) and androgen receptor were expressed in both vaginal epithelium and subepithelial stroma, whereas progesterone receptor was expressed predominantly in the subepithelial stroma. There was no change in receptor content and distribution following mifepristone treatment. Natural antimicrobial mRNA [secretory leukocyte protease inhibitor, human beta defensins mRNA (HBD1, HBD2, HBD3, HBD5), granulysin and elafin] was extracted from the vaginal tissues, and the content was unaffected by mifepristone treatment.

Conclusion: The absence of changes in vaginal thickness, steroid receptor and natural antimicrobial content and its distribution in this preliminary study suggests that in contrast to other estrogen-free contraceptives, mifepristone is unlikely to be associated with the increased risk of transmission of HIV and other sexually transmitted infections.

Keywords: Mifepristone; Vaginal thickness; Steroid receptors; Natural antimicrobial; Pharmacodynamics

1. Introduction

The state of the vaginal microenvironment affects a woman's risk of human immunodeficiency virus (HIV) transmission. Several human and nonhuman primate studies have shown that long-acting gestagen treatment increases the transmission of HIV [1–3], simian immunodeficiency virus (SIV) [4] and other sexually transmitted infections (STIs) [5,6]. The underlying mechanisms are poorly understood. Experiments on hysterectomized rhesus monkeys suggest that the vagina, rather than the cervix or the uterus, is the main portal of viral entry [7,8].

Epithelial thickness and integrity modulate the ease of access of virus to immune cells and subepithelial vasculature [9]. Estrogen-induced surface keratinization

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and hyperplasia protect rhesus monkeys against SIV inoculation [10,11], whereas estrogen-deficient women such as those who are postmenopausal [12,13] or on long-acting gestagens are at increased risk of HIV, presumably as a result of vaginal thinning. Other biological variables such as vaginal microflora [14–16], immune cell populations [17,18] and natural antimicrobials [19–21] also play an important role in innate defenses of the reproductive tract.

Mifepristone, a potent antagonist of progesterone (P), has the potential to be developed for contraception and other gynecological uses [22–24]. Daily low-dose treatment inhibits ovulation but maintains follicular development, thus exposing reproductive tissues to unopposed estrogen. Since gestagen treatment increases and estrogen treatment decreases HIV/SIV transmission, the aim of the present study was to investigate whether the antigestagen mifepristone modulates underlying mechanisms involved in transmission. We investigated vaginal morphology, steroid receptor and natural antimicrobial [secretory leukocyte protease inhibitor (SLPI), human beta defensins mRNA (HBD1, HBD2, HBD3, HBD5), granulysin and elafin] content. We also report the pharmacokinetics and pharmacodynamics of 5 mg mifepristone supplied by Hualian Pharmaceuticals Co. Ltd. (Shanghai, China), which has been used in the present study as well as in previous studies [23].

2. Subjects and methods

We report two pilot studies using 5 mg mifepristone — the study on vaginal epithelium that was carried out in Edinburgh and the pharmacokinetic study in Helsinki.

2.1. Effects of mifepristone on vagina

A single-center, open, single-group study concerning female volunteers was undertaken. Eight healthy subjects with a mean age of 35 years (range, 27–39 years) and a mean body mass index (BMI) of 23 kg/m^2 (range, 17.3–28.2 kg/m^2) who had regular menstrual cycles (25–42 days) were recruited to the study. The women agreed to refrain from the use of vaginal medications during the study period or from sexual intercourse 48 h prior to vaginal biopsy. Women who had breast-fed or had taken hormonal contraception less than 3 months prior to the study and those with vaginal or pelvic infections (current or past) were excluded. The proposal was approved by the local ethics committee (institutional review board). All women gave written informed consent before enrolment and were screened before entering the study. Screening included a full medical, gynecological history and examination, including measurement of height, weight, blood pressure and pulse. Blood samples were collected for measurement of routine clinical chemistry and hematology (liver function tests, urea and electrolytes, glucose, full blood count). βHCG was measured to exclude pregnant women from the study. Subjects were studied for one pretreatment cycle, one cycle of treatment (approximately 33 days) and one posttreatment cycle. Each subject was reviewed on Day 12 of the pretreatment menstrual cycle (Visit 1), at the end of treatment (Visit 2) and on Day 12 of the posttreatment menstrual cycle (Visit 3). Subjects were given a menstrual record card and were asked to record all vaginal bleeding.

2.2. Assessment of ovarian function

Ovarian function was monitored by measurement of ovarian steroids in urine and plasma and by transvaginal sonography. All subjects collected twice weekly samples of early morning urine during the study period, starting in the early follicular phase (Days 1–5) of the pretreatment cycle. Aliquots were frozen and stored at −20°C until assayed for estrone glucuronide (E1G), pregnanediol glucuronide (PdG) and creatinine (Cr). PdG was measured using a direct enzyme immunoassay, while E1G was measured by direct immunoassay. Ovarian follicular activity during treatment was compared with that during the follicular phase of the pretreatment cycle, and the activity was scored as complete suppression, partial suppression or continued follicular activity. Ovulation was deemed to have occurred if the excretion of PdG exceeded 0.5 mmol/mol Cr and was at least threefold higher than that in the preceding week. A detailed description of this methodology is given in our previous report [23]. Blood samples were collected at all study visits and assayed for estradiol (E2) and P using radioimmunoassay (RIA). Assay characteristics and methodology have been described in our previous reports [23]. A transvaginal ultrasound scan was carried out at all study visits, and ovarian dimensions, follicle number and diameter and presence of ovarian cysts were recorded.

2.3. Vaginal biopsy

A full-thickness vaginal biopsy was taken from the lateral vaginal wall 4 cm proximal to the hymeneal ring on Day 12 of the pretreatment cycle (Visit 1) and, again, after completion of treatment (Visit 2). One ampoule of Citanest with octapressin (3%; prilocaine hydrochloride, 30 mg/mL; felypressin, 0.03 U/mL; Dentsply) was injected into the lateral vaginal wall as a local anesthetic and hemostatic agent. This also elevated the target vaginal tissue sufficiently to permit easy access for a biopsy. Vaginal biopsy was performed using a long Schumacher forceps. Vaginal tissues were stored in RNAlater (Applied Ltd., Cambridgeshire, UK; RNAlater is an aqueous storage reagent to stabilize and protect RNA) and neutral-buffered formalin (NBF; for future preparation of paraffin-embedded tissue for immunohistochemistry). A second biopsy was taken if adequate sample was not obtained from the first biopsy. Vaginal bleeding from the biopsy site was controlled using either silver nitrate or, if necessary, a Vicryl 3-0 suture (Ethicon, UK), depending on the amount of bleeding.

An endometrial biopsy was collected using Pipelle endometrial sampler (Prodimed, Neuilly-en-Thelle, France) at end of treatment (Visit 2), fixed in NBF and embedded in paraffin. Endocervical and posterior fornix swabs were

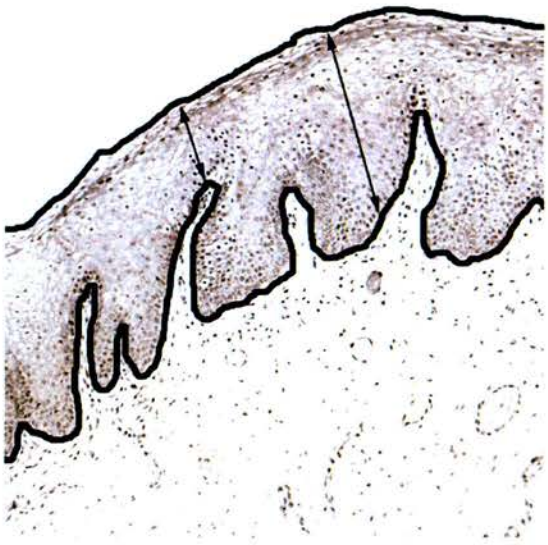

Fig. 1. Vaginal thickness measurement. Average thickness between surface and basement membrane (trace tool, Image Proplus 4.5, Media Cybernetics).

collected at all study visits and cultured for pathogenic organisms, for example, gonococcus, trichomonas and streptococcus, which might influence the parameters studied in target vaginal tissues.

2.4. Safety parameters

At each study visit, blood pressure and pulse were measured, and blood was taken for measurement of routine clinical chemistry and hematology. In addition, each subject was asked to report any health problems or adverse events that had occurred since the last visit.

2.5. Vaginal thickness measurement

Vaginal tissue samples were embedded in paraffin, and serial 5-μm sections were cut at a 90° angle to the vaginal surface epithelial layers. The sections were stained with hematoxylin and eosin, and digital images were captured at ×10 eyepiece magnification using a Spot microscope connected to a Windows PC computer. Image Proplus 4.5 (Media Cybernetics, Silver Spring, CO, USA) software was calibrated to match the eyepiece used to capture the image. The surface and basement membranes of vaginal epithelium were outlined using a trace tool within the software (Fig. 1). The software automatically calculated the average distance between the two traced outlines.

2.6. Immunohistochemical (IHC) localization of estrogen receptor alpha (ERα), estrogen receptor beta (ERβ), progesterone receptor (PR), androgen receptor (AR) and proliferation marker phospho-histone H3 (PH3)

Immunohistochemistry was carried out on both the vaginal and endometrial biopsies for the following proteins of interest: ERα (Novocastra, Newcastle-upon-Tyne, UK), ERβ (Serotec, Oxford, UK), PR (A+B) (Novocastra), AR (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), PH3 (Upstate Biotechnology, Poole, UK) and SLPI (Hycult Biotechnology, Cambridge, UK). All antibodies used were mouse monoclonal, except for PH3, which was a rabbit polyclonal. They were tested individually at a range of dilutions and at different antigen retrieval conditions to determine the protocol that gave the least background and

Table 1
Immunohistochemistry protocol

Protein	Tissue	IHC method	Antigen retrieval	Primary antibody	Negative control	Detection system
ERα	Vagina	Bond-X machine ABC detection	Pressure cook: 0.01 M sodium citrate, pH 6, 5 min	1:100 mouse anti-ERα 3 h at room temperature	MIgG1 1:1300	Biotinylated secondary and ABC detection
	Endometrium			1:1000 mouse anti-ERα 3 h at room temperature	MIgG1 1:13,000	(Vision Biosystems)
ERβ	Vagina Endometrium	Biotinylated secondary and ABC-streptavidin	Pressure cook: 0.05 M glycine/EDTA, pH 8, 7 min	1:40 mouse anti-ERβ Overnight at 4°C	Serum[a]	Biotinylated rabbit antimouse antibody and ABC-streptavidin (both from DAKO, Cambridgeshire, UK)
PR	Vagina Endometrium	Goat antimouse envision system	Microwave: 0.01 M sodium citrate, pH 6, 10 min	1:80 mouse anti-PR 37°C for 60 min	MIgG1 1:2000	Goat antimouse envision system (DAKO)
AR	Vagina Endometrium	Biotinylated secondary and ABC-Elite	Pressure cook: 0.01 M sodium citrate, pH 6, 5 min	1:400 rabbit anti-AR Overnight at 4°C	RIgG 1:2000	Biotinylated goat antirabbit antibody and ABC-Elite (both from Vector Laboratories)
H3	Vagina Endometrium	Goat antirabbit envision system	Pressure cook: 0.01 M sodium citrate, pH 6, 5 min	1:1000 rabbit anti-H3a Overnight at room temperature	RIgG1 1:1000	Goat antirabbit envision system (DAKO)
SLPI	Vagina Endometrium	Biotinylated secondary and ABC-Elite	Microwave: 0.01 M sodium citrate, pH 6, 10 min	1:50 mouse anti-SLPI Overnight at 4°C	MIgG 1:500	Biotinylated horse antimouse antibody and ABC-Elite (both from Vector Laboratories)

[a] The anti-ERβ antibody has been previously preabsorbed with the peptide to which it had been raised [25].

highest specific staining (Table 1). Positive and negative controls were included in every run. In most cases, negative controls were performed by adding a matched IgG control antibody (mouse IgG, Sigma, Poole, Dorset, UK; rabbit IgG, Vector Laboratories, Peterborough, UK) of the same species and at the same antibody concentration as the primary antibody. Protocols were carried out either on the bench or with the use of a Bond-X automated immunohistochemistry staining machine (Vision Biosystems, Newcastle, UK).

All tissue sections were initially prepared in a similar manner. Five-micron paraffin-embedded tissue sections were dewaxed in Histoclear (National Diagnostics, Hessle, UK) and rehydrated in descending grades of alcohol to distilled water (dH_2O). Antigen retrieval was then carried out by heating the sections either in a microwave oven (setting: high) or in a Tefal Clipso pressure cooker (Setting 2/high, Tefal, Nottingham, UK). The buffer concentration and duration of antigen retrieval varied depending on protocol (Table 1). Sections were left to cool in both cases for 20 min. Endogenous peroxidase activity was quenched by immersion in 3% hydrogen peroxide (BDH, Poole, UK) in methanol for 30 min at room temperature. For AR only, sections were incubated in avidin for 15 min at room temperature (Vector Laboratories), followed by incubation in biotin (Vector Laboratories), also for 15 min at room temperature. Nonspecific binding of the primary antibody was blocked by incubating the sections for 20 min at room temperature in a 1:5 dilution of nonimmune serum (Autogen Bioclear, Holly Ditch Farm, Wilts, UK) in phosphate-buffered saline containing 5% bovine serum albumin. All immunostaining methods employed a detection system dependent on visualization of the reaction using a horseradish peroxidase enzyme and the chromagen 3′ 3-diaminobenzidine (DAB). After the DAB step, the sections were counterstained in hematoxylin before dehydrating them in ascending grades of alcohol and mounting them from xylene with Pertex (Cellpath plc., Hemel Hempstead, UK). Full details of each individual protocol are given in Table 1.

2.7. IHC analysis

We used a descriptive methodology as there is no quantitative or semiquantitative methodology established for analysis of vaginal tissue samples in our laboratory. The intensity and distribution of immunostaining for ERα, ERβ, PR, AR, PH3 and SLPI are described for vagina (epithelium and stroma) and endometrium (glands, stroma and surface epithelium), and differences between pre- and posttreatment samples were analyzed.

2.8. RNA extraction

Tissue was minced using a standard sterile surgical scalpel blade and immersed in 2 mL of Tris-buffered saline (Sigma-Aldrich, St. Louis, MO, USA). The mixture was homogenized for 60 s and incubated overnight at 4°C. The following day, tissue sample was warmed to room temperature and 200 μg of bromochloropropane was added. The mixture was centrifuged at 14,000 rpm at 4°C for 15 min, and aqueous-phase RNA (supernatant) was transferred to a fresh tube. Five hundred microliters of isopropanol was added and incubated at 4°C for 60 min. The mixture was centrifuged for 10 min; the supernatant was discarded, and the pellet was washed with 1 mL of 70% ethanol. The mixture was centrifuged for 5 min, and the supernatant was discarded, allowing the pellet to dry for 5 min. The pellet was resuspended in 20 μL of RNA solution. To standardize measurements between the various biopsy specimens, we assessed the same amount of RNA in each sample. The amount of specific amplicon is related to ribosomal 18S, which is constant relative to the amount of cDNA present and, subsequently, to an experimental internal control. The RNA was reverse transcribed (TaqMan Reverse Transcription Reagents Kit, Applied Biosystems, Foster City, USA) and polymerase chain reaction (PCR) amplified (TaqMan Universal Master Mix, No Amp Erase UNG, Applied Biosystems) according to the manufacturer's instructions. PCR amplification of cDNA was performed on an ABI Prism 7700 Sequence Detection System (Applied Biosystems). Specific forward and reverse primers (300 nmol/L) and probe (200 nmol/L, all synthesized by BioSource UK, Nivelles, Belgium) for the natural antibiotic were also added. Ribosomal 18S cDNA was measured using TaqMan Ribosomal RNA Control Reagents (VIC dye, Applied Biosystems) in each sample as an internal control following the manufacturer's protocol. Samples were measured in triplicate, and no template controls were included in all runs. Primers and probes for quantitative PCR were designed using the PRIMER EXPRESS program (Applied Biosystems;

Table 2
Sequences of quantitative PCR primers and probes for natural antimicrobials

	Forward primer	Reverse primer	Probe
SLPI	GCATCAAATGCCTGGATCCT	GCATCAAACATTGGCCATAAGTC	TGACACCCCAAACCCAACAAGGAGG
HBD1	TCAGCAGTGGAGGGCAATG	CCTCTGTAACAGGTGCCTTGAAT	CTCTATTCTGCCTGCCCGATCTTTACCAA
HBD2	CTGATGCCTCTTCCAGGTGTTT	CTGGATGACATATGGCTCCACTCT	AAGGCAGGTAACAGGATCGCCTATACCACCA
HBD3	CAGAGGCGGCCGGTGT	CGAGCACTTGCCGATCTGTT	CTGTGCTCAGCTGCCTTCCAAAGGA
HBD4	GGCAGTCCCATAACCACATATTC	TGCTGCTATTAGCCGTTTCTCTT	TGTCCAATTCAAATTCGCTTCTCACTGGA
HBD5	ACCTCAGGTTCTCAGGCAAGAG	AGAGGGACTCACGGGTAGCA	CTGCTATTGCCGAACCGGCCGT
Granulysin	CAGGGTGTGAAAGGCATCTCA	GGAGCATGGCTGCAAGGA	CGGCTGCCCCACCATGGC
Elafin	TGGCTCCTGCCCCATTATC	CAGTATCTTTCAAGCAGCGGTTAG	ATCCGGTGCGCCATGTTGAATCC

All probes were labeled with 5′ FAM and 3′ TAMRA fluorophores.

Table 2) [26–31], and probes were fluorescently labeled with the proprietary dyes FAM (5′) and TAMRA (3′).

2.9. Pharmacokinetic study

A pharmacokinetic study of 5 mg of mifepristone was carried out among the same cohort of women who had previously participated in another pharmacokinetic study [32]. The study was approved by the Institutional Review Board of the Helsinki Central Hospital and the Finnish National Agency for Medicines; all subjects signed an informed consent document. Subject characteristics, methodology, collection of sample, analysis of serum levels of mifepristone and calculation of pharmacokinetic parameters were as described previously [32]. In brief, six healthy women, with regular menstrual cycles (23–36 days), a mean age of 32 years (range, 21–45) and a BMI that ranges from 19 to 26 kg/m^2, volunteered for the study. The 10-mg mifepristone tablets supplied by Hualian Pharmaceuticals Co. Ltd. were halved, and a dose of 5 mg, po, was ingested on Day 10 or 11 of the menstrual cycle. Blood samples were collected at 0, 1, 2, 4 and 8 h and, thereafter, daily for the next 6 days and on Day 10 following mifepristone ingestion. Mifepristone was measured in serum by RIA after extraction with *n*-hexane:ethyl acetate and separation by column chromatography using Chromosorb® [33]. The detection limit is 0.36 nmol/L, and the intra-assay and interassay coefficients of variation were 8.4% and between 10.3% and 13.6%, respectively.

2.10. Statistical analysis

Statistical analysis was performed using SPSS (SPSS, Inc., Chicago, IL, USA) and Excel 2002 (Microsoft Corporation, Reading, UK). Sex steroid, vaginal thickness and pharmacokinetic data are expressed as mean with either standard error of the mean or standard deviation. Menstrual cycle data are expressed as mean and range. Nonparametric tests (Friedman's test, Wilcoxon Signed Rank Test and Mann–Whitney test) were used to compare sex steroid level, menstrual data, vaginal thickness and natural antimicrobial RNA content before and after treatment.

3. Results

3.1. Effects of mifepristone on vagina

All eight subjects completed the study. The subjects took mifepristone 5 mg/day, po, for an average of 33 days (range, 28–40).

The average length of the control menstrual cycle was 27 days (range, 24–29), while that of the control menstrual period was 5.7 days (range, 4–9). All eight women reported amenorrhea during ingestion of mifepristone. The time from discontinuing the mifepristone treatment to the next bleeding episode was 17 days (range, 10–23); thus, the length of the mifepristone cycle was 50 days (range, 38–63). Average length of the bleeding episode after discontinuation of mifepristone was 5.1 days (range, 4–7).

During the treatment with mifepristone, seven of the eight subjects experienced either complete suppression of ovarian activity (3/8 women, Fig. 2A, Subject 8) or persistent follicular activity but no ovulation (4/8 subjects, Fig. 2B, Subject 4). In the remaining subject (Subject 7, Fig. 2C), there was a threefold rise in the excretion of pregnanediol in the first 10 days of treatment, suggesting the formation of a corpus luteum. However, there was no menstrual bleeding when the level of pregnanediol dropped 14 days after starting the mifepristone. In this subject, a persistent ovarian cyst with a 42-mm diameter was detected at completion of treatment (Day 40) when the level of P (14 nmol/L) was slightly raised, which was consistent with a persistent unruptured partially luteinized follicle.

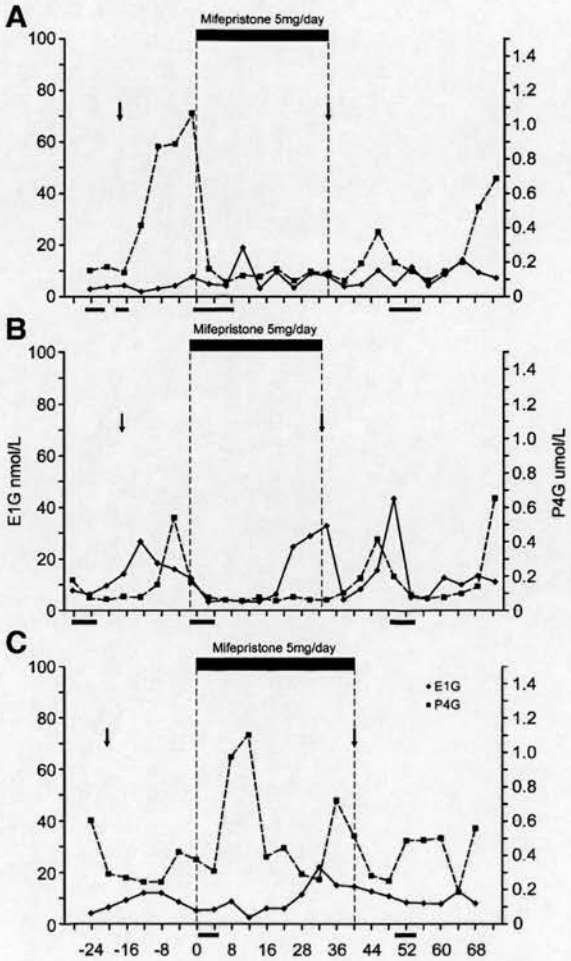

Fig. 2. Excretion of metabolites of ovarian steroids in women taking 5 mg of mifepristone for 31–36 days. Estrone, E1G; pregnanediol, PdG; *x*-axis shows timescale; Day 0, start of treatment. Pretreatment (negative values), treatment (boxed) and follow-up cycles are shown. Black bars represent menstrual episodes; arrow denotes vaginal biopsy. (A) Complete suppression of ovarian activity. (B) Persistent follicular activity but no ovulation. (C) Single ovulatory episode and no menstruation following fall in pregnanediol levels.

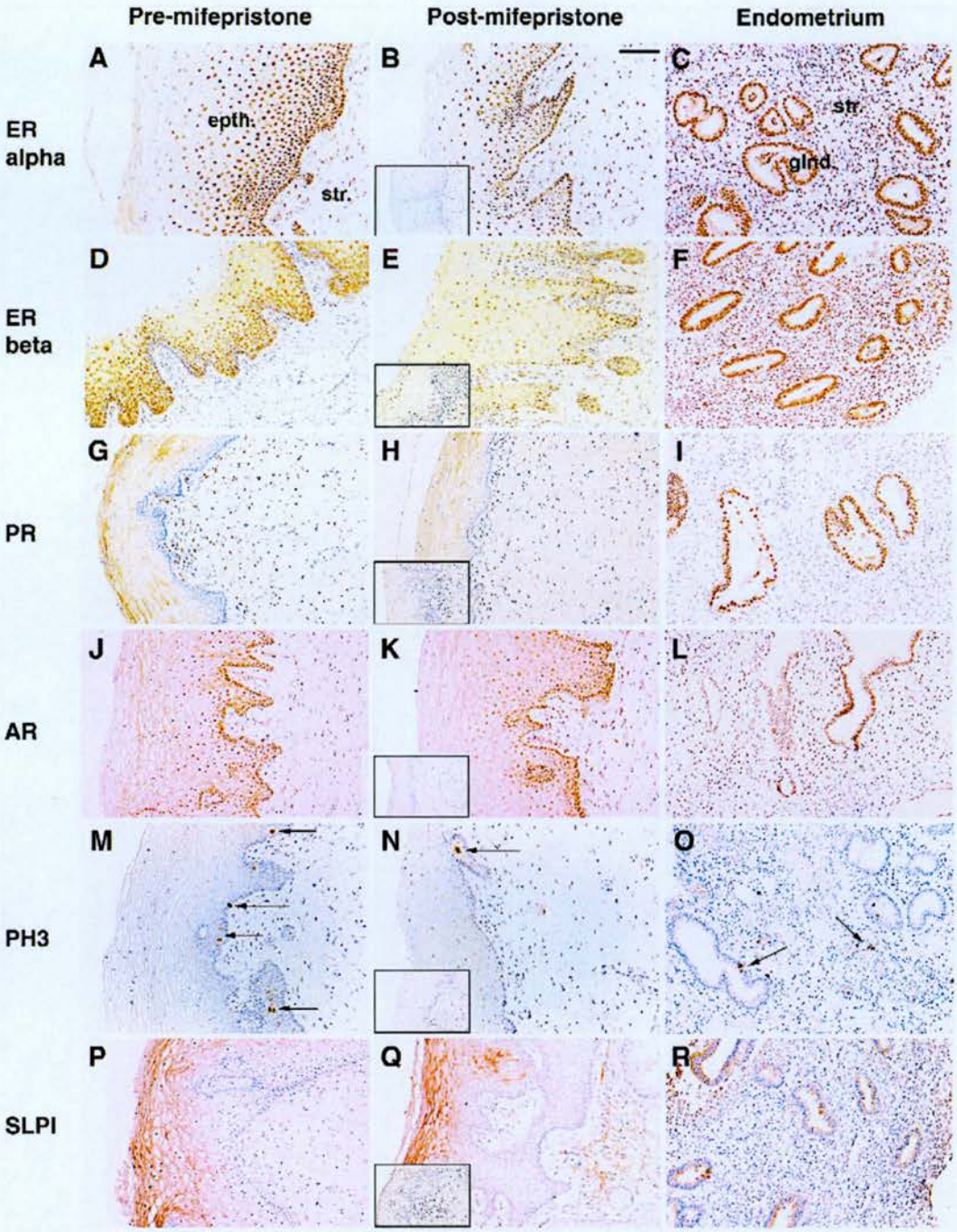

Fig. 3. IHC localization of ERα, ERβ, PR, AR and PH3 in the vagina [epithelium (epth) and stroma (str)] and endometrium [gland (glnd) and stroma (str)] before and after treatment with 5 mg mifepristone. Scale bar=100 μm; inserts denote negative controls. Strong expression of ERα (A and B), ERβ (D and E) and AR (J and K) in the basal and parabasal layers of epithelium and a relative lack of PR (G and H) in the epithelium (there was no observed change following treatment); PH3 expression in the basal layers (M and N; arrows) and endometrium [postmifepristone (O; arrows)]; strong expression of all steroid receptors in the posttreatment endometrium (C, F, I and L); SLPI confined to superficial layers of vaginal epithelium (P and Q) and endometrial glands (R).

Multiple follicles were detected by transvaginal ultrasound (diameter of the largest ranged from 10 to 29 mm) in all eight subjects. The concentrations of E2 in blood samples collected at Visit 2 on the last day of mifepristone treatment were compatible with persistent follicular activity (496±57 pmol/L). In seven of eight women, the concentration of P was <10 nmol/L (3±1 nmol/L), indicating lack of ovulation.

3.2. Endometrial histology

Endometrial biopsies obtained at the end of mifepristone treatment displayed inactive or weakly proliferative endometrium in seven of the eight subjects. In one subject (Subject 9), tortuous glands with evidence of intraluminal secretion were seen. As expected, there was strong immunostaining of ERα and ERβ as well as of PR (A+B) and AR in both glands and stroma. Histological evidence of mitosis was absent or infrequent in all samples.

3.3. Vaginal histology and vaginal thickness

Vaginal biopsy was obtained in all subjects without complications both before and after mifepristone administration. In seven of eight subjects the pretreatment vaginal biopsy was performed between Day 6 and Day 16 of the follicular phase prior to ovulation as confirmed by the hormone levels (mean±SEM: E2, 659±141 pmol/L, P, 4.3±10 nmol/L). In the remaining subject (Subject 5), ovulation had already occurred on the day of biopsy (Day 14 of menstrual cycle) as indicated by high circulating concentration of P (44 nmol/L). Posttreatment vaginal biopsy was performed on Day 33 (range, 28–40) of treatment.

The histology of the vagina showed the expected basal layer of epithelium mounted by up to 20 layers of more superficial desquamating cells (Fig. 1). Vaginal thickness was not altered during administration of mifepristone (342±40 μm vs. 303±69 μm; p=.2, NS).

3.4. Steroid receptor expression in the vagina (ERα, ERβ, AR and PR)

There was nuclear staining of ERα, ERβ and AR in both vaginal stroma and in the epithelium. Immunoreactivity extended through the basal and intermediate layers but not through the superficial layer of vaginal epithelium (Fig. 3). Staining was far more evident in the epithelium as compared with the stroma. There was no significant difference in sex steroid receptor immunostaining after mifepristone administration.

Apart from a few scattered nuclei in the stroma, the basal layer of vaginal epithelium immunostaining for PR was confined to stroma (Fig. 3). There was no significant difference in PR immunoreactivity after administration of mifepristone.

3.5. PH3 immunoreactivity

PH3 immunostaining was confined to scattered nuclei in the basal layers of the vaginal epithelium (Fig. 3). There was no significant change in immunoexpression following treatment with mifepristone.

3.6. Natural antimicrobial mRNA and protein expression

SLPI mRNA was present in vagina, and this epitope was localized to the superficial layer of the vaginal epithelium both before and after mifepristone administration (Fig. 3). SLPI immunostaining was also demonstrated in the superficial layers of luminal and glandular epithelium of the endometrium (Fig. 3). SLPI mRNA expression was unchanged following treatment with mifepristone (p>.05, Wilcoxon test). HBD1, HBD2, HBD3, HBD5, granulysin and elafin mRNA were present in the vagina. The expression was unchanged following treatment with mifepristone (p>.05, Wilcoxon test).

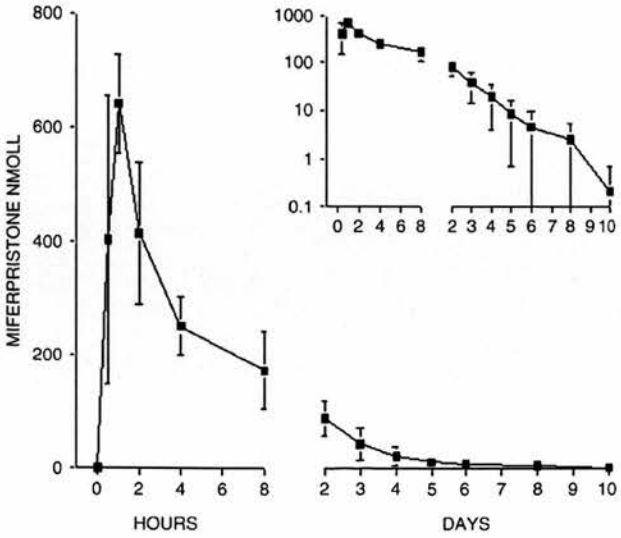

Fig. 4. Serum levels (mean±SD) of mifepristone following ingestion of 5 mg. The data are depicted on both linear (lower) and semilogarithmic (insert) scales.

3.7. Safety parameters

There was no derangement in heart rate, blood pressure or in hematology and biochemistry parameters including liver function tests during the study. Vaginal and cervical bacteriology swab tests cultured negative for pathogenic organisms in all subjects. Vaginal biopsy was well tolerated by all women. One woman had possible low-grade endometritis following endometrial biopsy and was successfully treated with a 7-day course of antibiotics.

3.8. Pharmacokinetics of 5 mg mifepristone

Serum levels (mean±SD) of mifepristone following ingestion of 5 mg mifepristone are summarized in Fig. 4. Mean C_{max} measured at 1 h was 641.7 nmol/L (range, 502–740 nmol/L). All subjects showed a similar pattern of descending serum concentrations of mifepristone. The elimination phase half-life was 18±5.1 h (mean±SD). The mean (SD) areas-under-concentration-curves $AUC_{0-8\ h}$ and $AUC_{0-24\ h}$ were 2.4 (0.5) and 4.8 (1.3) μmol/L, respectively.

4. Discussion

The vagina is a key portal of entry for HIV and other STIs. In this article, we report the effect of a potential new contraceptive pill on different parameters involved in the

natural defenses of the vagina to infection. Vaginal epithelial thickness, steroid receptor and natural antimicrobial content and distribution were unchanged following treatment with mifepristone for 30–40 days. Vaginal epithelial thickness is regulated by the levels of circulating estrogen. Epithelial thickness is maximal at time of ovulation [34–37] and decreases in the luteal phase and postmenopause [36]. There is a significant reduction in circulating estrogen levels following long-term gestagen treatment [37]. Severe vaginal atrophy has been demonstrated in primate studies, and this clearly increases the risk of SIV transmission [4]. However, the response of human vaginal epithelium to gestagen-induced hypoestrogenism is variable. A small but significant decrease (10%) in thickness has been demonstrated in one study [37], whereas most other studies have demonstrated no change [38,39]. Paradoxically, vaginal epithelial hyperplasia has been reported in users of depot medroxyprogesterone acetate (DMPA), oral contraceptive pill and P implants [39–41]. E2 levels in all three treatment groups were significantly lower compared with normal menstruating women. The steroid receptor content and distribution were similar except for PR, which was suppressed in the DMPA group.

It is difficult to reconcile all available data into a working hypothesis due to differences in study designs and methodology. The vagina has diverse embryological origins [42]; Ildgruben et al. used a cross-sectional study design and sampled lateral vaginal fornices, whereas we used a longitudinal study design and sampled lateral midvaginal wall in keeping with other reports [41].

Although vaginal epithelium clearly responds to circulating estrogen, the underlying cellular and molecular mechanisms are poorly understood. We localized PH3, a marker for mitosis and cellular proliferation, to a few scattered nuclei in the basal layers of the vaginal epithelium. The strong nuclear expression of ER and AR in the basal and parabasal layers suggests a role in the regulation of epithelial proliferation. Estrogen treatment induces surface keratinization and hyperplasia of primate vaginal epithelium, and we expected a similar change in mifepristone-treated vaginal samples due to unopposed estrogen effect. The observed epithelial thickness in control samples in the present study (mean pretreatment thickness, 342 μm) is comparable to other reports [38–40]. Thickness was unchanged following mifepristone treatment, and this agrees with similar work in cynomolgus monkeys [43]. There are several possible explanations for our findings. Firstly, mifepristone treatment did not have any effect on level of circulating steroid hormones. Secondly, PR was localized to a few scattered nuclei in the basal layers of vaginal epithelium and, hence, mifepristone, a high-affinity PR ligand, did not have any direct epithelial effects.

Consistent with previous reports, we have demonstrated a strong immunoexpression of ER and AR as well as a relative lack of PR in the vaginal epithelium compared to subepithelial stroma [44–51]. The distribution of steroid receptors has obvious implications for the development of topical and parenteral steroid treatment. Available data, including the present study, indicate that it is likely that estrogen preparations will act via epithelial ER, whereas P preparations are likely to have an endocrine and paracrine effect after binding with the subepithelial PR. The role of AR in the genital tract is unclear, but AR may play a role in regulating endometrial proliferation [52–54] and in modulating vaginal blood flow [50] and female genital sexual arousal [49].

The endometrium sampled at the end of mifepristone treatment showed persistent proliferative histology and strong ER, PR and AR expression, which is consistent with our previous reports [54,55] and which demonstrates that the mifepristone preparation used in this study is comparable to that we have used previously from another source (Exelgyn, Paris).

The expression, regulation and role of natural antimicrobial compounds in the female reproductive tract are extensively reviewed elsewhere [29]. We have investigated natural antimicrobials that regulate innate protection at mucosal interfaces. SLPI has been shown to play an important role in limiting transmission of HIV [20,56,57] and other lower genital tract infection [19]. SLPI mRNA has been demonstrated in vaginal fluid previously [19,20]. In the present article, we demonstrate mRNA in vaginal tissue and immunolocalize the protein to the superficial layers of the vaginal epithelium. The expression in superficial layers of surface and glandular endometrium is in keeping with previous reports [26]. HBD1 [58] and elafin [59] mRNA have been reported in human vagina. We show that HBD2, HBD3, HBD5 and granulysin mRNA are present in vaginal tissue. HBD4 mRNA was not previously demonstrated. Natural antimicrobial expression is modulated by hormonal treatment, and up-regulation of endometrial SLPI by P is attenuated in the presence of mifepristone [31]. Reassuringly, expression and distribution of natural antimicrobials were unchanged following mifepristone treatment.

Low-dose mifepristone suppressed menstruation and ovulation in a majority of women in the present study. This adds to similar observations in our previous reports [23,60] and also demonstrates the biological activity of mifepristone supplied by Hualian Pharmaceuticals Co. Ltd. The pharmacokinetic properties of the 5-mg dose from the above supplier follow a first-order linear kinetic pattern [32,61]. The half-life of 18 h is shorter than the 20 h previously reported for 10 mg mifepristone, whereas the $AUC_{0-24\ h}$ was half that of the 10-mg dose [32]. All women received planned treatment for at least 5 days beyond the expected date of menstruation (33 days) so that any posttreatment vaginal bleeding was due to withdrawal of mifepristone rather than to a spontaneous menstruation. Fortuitously, Subject 7 was administered mifepristone for 40 days. She did not bleed, although there was evidence of ovulation and formation of corpus luteum followed by a significant decrease in pregnanediol levels. This supports our previous

reported findings that mifepristone-induced amenorrhea is a result of direct endometrial effects that are possibly mediated by a rise in glandular and luminal glucocorticoid and AR [62].

In summary, we have shown that low-dose mifepristone does not influence vaginal thickness and proliferation. We have demonstrated that ER (ERα and ERβ), PR, AR, SLPI and other natural antimicrobials are present in the human vagina. There is no change in epitope expression following mifepristone treatment. The presence and distribution of vaginal steroid receptors have implications for the development of topical and systemic preparations to modulate this sex steroid responsive organ in health and disease.

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