much slowly with Europe increasing less than 5%. The largest increase in absolute numbers will occur in India, China and Nigeria totalling nearly 500 million people.

Contraceptive usage

The use of certain types of contraceptives is not suited to some categories of women. For example, women above 35 years of age and who are habitual smokers are at a greater risk to cardiovascular disorders, stroke, thrombosis and embolism if they use some of the older generation of high-dose, steroidal oral contraceptives; such women should use an alternate method of contraception. Nevertheless, the use of oral contraceptives can also confer reproductive-health benefits such as lowering the risks of reproductive cancer (i.e., those of the cervix, endometrium, ovary and breast). The availability of a wide range of contraceptives to suit individual needs, a widening in the number of contraceptive users, concern and appropriate legislative steps by governments to reduce risk-factors affecting reproductive health are the most obvious steps that need to be taken urgently to stem the threats to personal reproductive health and to our planet by unbridled growth rates of our population.


Antiprogestins: Useful investigative tools and novel contraceptives

Chander P. Puri

Institute for Research in Reproduction, (ICMR), Parel, Bombay 400 012, India

The development of antiprogestational drugs such as, RU 486, ZK 98.299, ZK 98.734 and HRP 2000, is one of the most significant contributions to science in recent years. These drugs were originally synthesized to intercept progesterone action at the molecular level of receptor binding. They have found use as a tool to understand mechanisms regulating progesterone action. Antiprogestins also have direct effect on the endometrium as well as on the hypothalamo-hypophyseal axis and therefore have a potential use for interrupting a wide range of progesterone-dependent reproductive processes. This potential is being explored to develop new methods of birth control. Treatment with antiprogestins during the follicular phase of the menstrual cycle impairs gonadotropin release in primates, as a consequence of which folliculogenesis is either retarded or arrested and ovulation is blocked. When administered in the luteal phase, the secretory activity of the endometrium is inhibited and the corpus luteum regresses. Interestingly, the effects of antiprogestins on the gonadotrophs and the endometrium are dose-related, the endometrium being more sensitive than the gonadotrophs. The doses at which ovulation is blocked also retard endometrial development. However, at lower doses endometrial development is impaired but ovulation is not blocked. Treatment of bonnet monkeys with 2.5 mg or 5 mg ZK 98.299 administered every third day for four to six consecutive cycles does not block ovulation but the endometrial glands were regressed, atrophied and rendered non-secretory. Since conception did not occur in 39 out of the treated and mated cycles it would appear that the desynchronization of the endometrium by antiprogestins treatment is incompatible with the establishment of pregnancy. These results clearly suggest that the antiprogestins can be developed as contraceptives by inhibition of ovulation or by rendering the endometrium out of phase with respect to embryonic implantation.
Estrogen and progesterone are the two main sex hormones which act synergistically to regulate the growth and differentiation of the female reproductive tract and breast. Estrogen stimulates cell proliferation while progesterone modulates the functional differentiation of the cells formed as a result of estrogen stimulation. In the non-fertile menstrual cycle, progesterone is secreted by the granulosa cells of the corpus luteum and as much as 40 to 50 mg of the steroid is secreted daily during the mid luteal phase. During early pregnancy, both the corpus luteum and the syncytiotrophoblast cells of the placenta contribute to circulating levels of progesterone; however, the placenta takes over the production of progesterone after six weeks of gestation and it produces as much as 250 mg of the steroid daily.

The major physiological role of progesterone is to prepare the uterus for implantation of the fertilized ovum and thereafter provide nutrients for its development. These progestogenic effects are regulated through the secretion of specific proteins by the endometrial glands such as those belonging to the family of lactoglobulins and integrins. Progesterone regulates the expression of endometrial cytokines and growth factors which, in turn, have a facilitory role in ovum implantation and development of embryo in primates. The steroid promotes proliferation of the endometrial stromal tissue and thus facilitates embryonic implantation.

The steroid also has a direct role in the intra-ovarian regulation of folliculogenesis and ovulation in primates. Progesterone receptors have been localized in the stromal, thecal and granulosa cells of primate ovaries. The steroid is known to facilitate transport of the fertilized ovum through the oviduct.

Progesterone also participates in the neuroendocrine triggering of the mid-cycle LH surge and thus induces ovulation.

Progesterone acts synergistically with estrogen in the normal development of breast and it causes the glandular tissue of the mammary gland to grow and differentiate into secretory tissue to facilitate milk production.

Progesterone also has several antifertility effects which depend on the dose and the stage of the menstrual cycle during which the steroid is administered. It inhibits ovulation when administered during the follicular phase—an effect primarily mediated by inhibiting the release of gonadotropins. The hormone causes a thickening of the uterine cervical mucus and thus impairs trans-cervical sperm movement. It decreases the motility and secretory activity of the fallopian tubes and uterus.

In view of the fact that progesterone is necessary for the ductal transport of the embryo, its uterine implantation and maintenance of pregnancy, a number of approaches have been developed to either block the bioavailability of progesterone or its action and thus prevent conception or terminate pregnancy. Immuno-neutralization of circulating progesterone by administering monoclonal progesterone antibodies prevents pregnancy in laboratory animals. However, this immunological approach does not seem to be of practical use in women. The search has therefore shifted to develop compounds which would either inhibit progesterone biosynthesis or block its action at the target site. The availability of such compounds would have many applications to regulate fertility and also provide important tools for understanding the intricate physiological mechanisms controlling the action of progesterone.

This paper reviews some of the strategies developed to inhibit the action of progesterone through the use of antiprogestins, and their use for fertility regulation as well as to understand the mechanism of the biological action of progesterone.

Inhibition of progesterone biosynthesis

Suppression of progesterone biosynthesis by specific enzyme inhibitors is a possible method for birth control. The steroid is synthesized in the ovary, adrenal and the placenta by the conversion of its precursor, pregnenolone, by the enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD). Azastane, Trilostane and Epostane inhibit 3β-HSD activity and consequently progesterone biosynthesis is blocked. These enzyme inhibitors lack tissue-specificity and therefore progesterone biosynthesis is blocked indiscriminately in all the steroid-producing tissues. Since progesterone is an intermediate for cortisol production in the adrenal glands, the inhibition of progesterone biosynthesis impairs corticosteroid biosynthesis and thus adversely affect physiological homeostasis. However, these three enzymic inhibitors do not have similar potency. Azastane and Epostane preferentially inhibit biosynthesis of progesterone in the ovary and placenta rather than in the adrenal. Epostane is more potent than Azastane and it has been evaluated for its ability to block ovulation and also to terminate pregnancy. Treatment with Epostane during the early follicular phase inhibits folliculogenesis, and treatment during the luteal phase induces premature menstruation in women. Epostane terminates pregnancy in monkeys and women. However, the compound has to be administered in unacceptably high doses for several days to elicit this interception. A dose of 800 mg for seven consecutive days induced abortion in 84% women who were amenorrheic for less than 49 days. The abortifacient efficacy of Epostane appears to be similar to that observed with the antiprogestin, RU 486 (Mifepristone) when administered alone. However, the limited clinical experience with Epostane indicates that the incidence of subjective side effects is higher compared with RU 486.

The incidence of complete abortion is increased significantly by complementing Epostane treatment with
a prostaglandin (PG) analogue in a sequential manner\textsuperscript{17}. The effect is similar to that of RU 486 when used in combination with a PG analogue\textsuperscript{16}. However, the inhibitory effects of Epopean on adrenal corticosteroid biosynthesis limit its use for chronic administration. Efforts need to be made to develop more potent \( \beta \)-HSD inhibitors, which are also tissue-specific. The sequential treatment with an enzymic inhibitor and a receptor blocker, such as RU 486, also needs to be evaluated as preliminary studies in the guinea pig indicate a synergistic effect on uterine myometrial contractility\textsuperscript{18}.

**Progesterone receptor blockers**

**Progesterone action**

Progesterone effects occur in cells containing progesterone receptors (PRs). PRs are present in the ovary, uterus, fallopian tube, pituitary, hypothalamus, endocochial cells of decidual blood vessels and mammary glands\textsuperscript{19,20}. PRs in the chicken oviduct and human breast cancer cells exist as two distinct subtypes, PR-A and PR-B, which are respectively 94 and 120 kD. This has been shown to be true for the untransformed cytotoxic PRs as well as the nuclear bound PRs. In this respect, PRs are different from receptors for estrogen, androgen and glucocorticoid, which are a single class of binding proteins.

Human PR-A and PR-B isoforms are identical except that PR-B contains an additional 164 amino acids at the N-terminal. Both the isoforms have similar binding affinities and are important for eliciting biological responses. The synthesis of the two homologous PR-A and PR-B could possibly be due to gene duplication, alternate transcription from a single gene, synthesis of multiple messages by processing of a single precursor RNA, or use of alternate translation 'Start' sites from a single message\textsuperscript{21}.

PRs comprise of a ligand-binding domain (LBD) of about 250 amino acids, a DNA-binding domain and two zinc fingers. The LBD resides in the carboxyl terminal end of PRs whereas the DNA-binding domain is located more or less in the centre of the molecule. One of the two zinc fingers is important for interaction with DNA and the other is involved in the dimerization of the receptor molecules. Although the amino terminal region of PRs is not required for either ligand binding or DNA binding, it is essential for transcriptional activation. In addition to A- and B-proteins, PRs contain at least two nonsteroid-binding proteins of 90 kD and 70 kD, both of which are termed as heat-shock proteins (HSPs). In the absence of the ligand, HSPs are bound to the receptor and keep the untransformed receptor in an inactive form.

Following the entry of the ligand into the cell, it binds to the LBD of the PRs. The conformational change induced in the receptor provokes dissociation of PR-HSP complex and thereby imparts to the transformed receptor the ability to interact with the progesterone responsive elements (PREs), with high affinity. The ligand binding also promotes additional phosphorylation and receptor dimerization. The binding of the receptor complex to PRE leads to transcription of mRNA and gene expression.

This brief summary of the mechanism by which progesterone interacts with its receptors in the target organ to induce gene expression is rather simplistic view. Many questions still remain unanswered. For example, why are there two isoforms of PRs when a single receptor seems adequate for the other steroid hormones? What is the origin and functional role of these two PRs proteins? What is the physiological role of HSPs? It has been shown that under in vitro conditions, steroid binding to the receptor dissociates HSP to expose the DNA-binding sites. Does this also happen in vivo? Not much is known about the mechanism of transcription activation of the steroid receptors. Membrane-bound PRs have been demonstrated in several tissues\textsuperscript{22,23}, the functions of which are still not known. Antiprogestational compounds such as RU 486 or ZK 98,299 which have high affinity for the classical PRs, do not compete for binding with the membrane receptors. Do these membrane-bound receptors interact with the classical nuclear receptors and affect gene expression? The availability of specific molecules which block the action of progesterone at the receptor level would provide an excellent tool to understand the various steps involved in progesterone action.

**Synthesis of progesterone receptor blockers**

It has been conceptualized that the blockade of PRs by other compounds would prevent the binding of progesterone to target tissue; such an approach would thus prevent the biological effects of progesterone. A number of receptor-blocking compounds have been synthesized and screened for their antiprogestational activities by various pharmaceutical companies on the basis of this concept.

Substitution of estrogen-like molecules with an extra cycle (dimethylaminooxoyphenyl) at the C-11\( \beta \) position transformed the estrogen into an antiestrogen with higher binding affinity for estrogen receptors\textsuperscript{24}. It was speculated that the PR may also have an extra pocket to accommodate bulky substituents at the C-11 position of the progesterone molecule, with a possible consequence that the conformation of the corresponding receptor complexes would differ from that of the agonist-receptor complexes and be biologically inactive. This hypothesis was substantiated by scientists at Roussel Uclaf (Paris), who successfully synthesized RU 486 through the use of the 11\( \beta \)-epoxidation pathway\textsuperscript{25}. RU
RU 486, a 19-norsteroid with \( p \)-dimethylaminophenyl group at the 11\( \beta \)-position and 1-propynyl chain at C-17 position, binds both to progesterone and glucocorticoid receptors with high affinity. Excitement in this area was further aroused with the findings that RU 486 binding to the receptors results in antagonistic instead of agonistic responses\(^{11,26}\).

After this initial discovery, a large variety of compounds with 11\( \beta \)-aryl substitution have been synthesized of which ZK 98,734, ZK 98,299 and HRP 2000 are under closer investigation (Figure 1). ZK 98,734 and ZK 98,299 have been developed by Schering AG, Berlin\(^{27}\), whereas HRP 2000 has been developed by Research Triangle Institute, USA\(^{28}\) and is being evaluated for its antifertility effects under the aegis of the Human Reproduction Programme of the World Health Organization.

ZK 98,734 represents a minor variation of the lead compound RU 486 and has a \( Z \)-configured 17\( \alpha \)-(3-hydroxy-1-propenyl)-group instead of a 17\( \alpha \)-(1-propynyl)-side chain. Although ZK 98,299 appears to be similar to the other two compounds on a two-dimensional picture, it has a distinctly different molecular shape due to the configurational inversion at positions C-13 and C-17 relative to natural steroids. HRP 2000 is a 17\( \alpha \)-acetoxy derivative with a 17\( \beta \)-progesterone side chain and a 11\( \beta \) RU 486-like substituent. It is also an antiprogestin and an antiglucocorticoid. These drugs form 'lock and key' fits with the PRs. They are unique in the sense that while they bind to the receptor they do not trigger agonistic hormonal actions. Administration of these drugs results in potent antiprogestational effects.

**Mechanism of action**

The precise mechanism by which antiprogestins block progesterone action at the receptor level is not known. It is also not known whether they bind only to the PRs and/or they have their own distinct binding sites. Progesterone antagonists enter the cell freely and they compete with progesterone for binding\(^{15,29,30}\). The binding of an antiprogestin to the receptor may block any of the steps involved in progesterone action. It may alter or block the conformational changes associated with the binding, block dissociation of HSP from the receptor, alter or block dimerization of the receptor, alter or block DNA binding or alter interaction with other factors to produce transcriptionally active receptors\(^{31}\). Recent studies have shown that RU 486 does not block the dissociation of HSP from the receptor, it permits receptor dimerization and DNA binding\(^{32}\). However, it appears that the conformation of the dimers and of the DNA complexes are different from those produced following the binding with an agonist\(^{33}\). It is possible that the action of the antisteroid is at the level of transconformation of the receptor, involving change in the chromatin and activation of the transcription factor. On the other hand, the mechanism of action of ZK 98,299 could be different from that of RU 486. Studies have shown that ZK 98,299 does not bind to DNA, and it does not induce conformational changes in the DNA required for receptor binding\(^{34}\). Our studies have shown that ZK 98,299 binds to PR in the human myometrial cytosolic and nuclear fractions\(^{35}\). However, when compared with the binding of progesterone to PRs in the nucleus, the extent of binding of ZK 98,299 to the
PR was relatively low. Conversely, the DNA-cellulose binding of progesterone-bound PRs was also greater than that of ZK 98.299-receptor complexes. We therefore believe that the differences in the extent of nuclear accumulation of the agonist or antagonist–receptor complexes and to bind to DNA-cellulose may account for different responses. However, it is also possible that different antagonists may act at different steps in receptor function and a single antagonist may affect more than one stage of receptor activation. Since these progesterone antagonists can intercept progesterone action they may be used as a tool to understand the various steps involved in progesterone action. This information can be of further value to develop more potent agonists and antagonists.

**Antifertility effects**

The synthetic hormone antagonists referred above have proven to be potentially good antifertility agents. Anti-progestins can be used as menstrual inducers, to terminate early pregnancy, as an emergency method of post-coital contraception and also as a regular contraceptive either as a daily or once-a-week pill\(^{26}\) (Figure 2).

It is now well accepted that treatment with a single oral dose of RU 486 (200 mg) in conjunction with a uterotonic PG analogue, Gemeprost (May and Baker, UK), Sulprostone (Schering AG, Berlin), or 9-methylene-PGE\(_2\) vaginal gel results in complete expulsion of the conceptus in almost 95% women with an amenorrhea of up to 63 days\(^{36-38}\). However, in a few cases myocardial infarction occurred when Sulprostone was used in conjunction with RU 486 for termination of early pregnancy. In view of this finding, intramuscular preparation of sulprostone has been removed from the market and is not recommended for use in combination with RU 486. Recent studies suggest that these PG analogues can possibly be replaced with an orally active, inexpensive and more stable PG, Misoprostol (Searle Pharmaceuticals), without compromising the abortifacient efficacy\(^{39}\).

The abortifacient efficacy of ZK 98.734 has been evaluated in a limited clinical trial conducted under the aegis of the World Health Organization. The dose-
finding study conducted on 95 women with an amenorrhea of less than 49 days showed that the overall abortifacient efficacy with the different dosages, 12.5 mg, 25 mg, 50 mg and 100 mg administered twice daily for four consecutive days, was 68% (ref. 40), which is quite comparable to that observed with RU 486 using similar protocols. The abortifacient efficacy of ZK 98.299 and HRP 2000 has been evaluated in monkeys. Administration of 25 mg of ZK 98.299 for three consecutive days terminated early pregnancy in about 65% of the animals41, and this efficacy is less than that observed for ZK 98.734.

HRP 2000 has been evaluated in the guinea pig and in the lion-tailed macaque (Macaca fascicularis). In the guinea pig, 30 mg HRP 2000 administered s.c. on days 43 and 44 of gestation terminated pregnancy in about 75% animals. The abortifacient efficacy at this dose level was similar to that observed with RU 486 and ZK 98.734. However, at lower doses HRP 2000 is less effective compared to the other antiprogestins42. The abortifacient efficacy of HRP 2000 was more by the i.m. route in monkeys compared to oral administration. The abortifacient efficacy of HRP 2000 following i.m. administration is comparable to that of RU 486 (ref. 43). These preliminary results suggest that ZK 98.734 and HRP 2000 are potent abortifacients, which merit further evaluation. However, as in the case with RU 486, these antigestagens may also need to be supplemented with PG analogues.

Besides having a direct effect on the endometrium or the decidua, antiprogestins also impair gonadotropin release. Since FSH and LH are required for normal follicular development and LH is required for normal progesterone secretion during the luteal phase, antiprogestins can affect folliculogenesis, corpus luteum function and endometrial development. They thus have several potential applications in fertility control as summarized below.

Effect on folliculogenesis

Folliculogenesis is primarily influenced by the gonadotropins, FSH and LH. Progesterone also affects follicular growth. These effects could either be direct on the ovary or mediated through the release of gonadotropins. The presence of PR in the stromal, thecal and granulosa cells very early in the follicular development4, the high concentrations of intrafollicular progesterone44, the additive effects of progesterone on the hCG-stimulated growth of small antral follicles45, the additive effects of progesterone on gonadotropin-stimulated secretion of progesterone46, and the presence of PRs in the hypothalamus of the monkeys47 are suggestive of a role for progesterone in folliculogenesis. The observations that the levels of progesterone start to increase about 12 h before the LH surge48, and treatment with progesterone in the late follicular phase advances the timing of gonadotropin surge49 indicate that progesterone facilitates ovulation. On the other hand, treatment with progesterone on day six of the menstrual cycle terminates folliculogenesis in the rhesus monkeys49. These inhibitory effects of progesterone are due to the blockade of gonadotropin release. Progesterone, therefore, has both facilitatory and inhibitory effects on the follicular development, which seem to depend on the preexisting hormonal environment. Treatment with antiprogestins at various stages of follicular development would thus not only provide an insight on the role of progesterone in follicular development but would also provide information as to whether these drugs can be used to block folliculogenesis and consequently ovulation.

Treatment with antiprogestins either in the early or mid-follicular phase invariably interrupts follicular development50-52. The preovulatory rise in serum levels of estradiol and LH is either attenuated or completely blocked. However, these effects are dependent on the dose and the frequency of administration of the antiprogestin. Treatment of bonnet monkeys with ZK 98.299 (20 mg/daily, s.c.) either from days 5 to 8 or days 5 to 15 significantly increases the length of the menstrual cycle due to the prolongation of the follicular phase51-53. The expected rise in the pre-ovulatory levels of estradiol and bioactive LH is either completely blocked or suppressed during treatment. Blood concentrations of gonadotropins increase after cessation of treatment and the animals ovulate normally, as indicated by progesterone levels during the luteal phase.

When the frequency of treatment with ZK 98.299 was reduced to once-a-week, none of the three doses (5, 10 or 20 mg) used had any effect on folliculogenesis or ovulation54. The menstrual cycle length was of normal duration, but vaginal bleeding was considerably reduced. Luteal function was normal at the 5 and 10 mg dose but at the 20 mg dose luteal insufficiency was evident. Although ZK 98.299 at these dose-regimem had no effect on folliculogenesis or ovulation it delayed endometrial development.

The effects of ZK 98.299 on folliculogenesis are similar to those reported with RU 486 (Table 1). Treatment for a period of a few days during the mid- to late-follicular phase arrests folliculogenesis and delays ovulation in monkeys55,56, and in women57. However, if the treatment is initiated very early in the cycle RU 486 has no effect on folliculogenesis or ovulation58. Similar effects are observed59,60 in women treated throughout the menstrual cycle with RU 486 at an oral, daily dose of 1, 2 or 5 mg. However, with the low doses the effects on follicular development are attenuated as reflected by the normal increase in the urinary excretion of estrone glucuronide during treatment69. In spite of normal estrogen levels, RU 486 at such low doses blocked the positive feed-back effect of estrogen on the
Table 1. Effects of RU 486 administered during the follicular phase on ovulation and follicular phase length of women

<table>
<thead>
<tr>
<th>Dose</th>
<th>Day of treatment</th>
<th>Ovulation</th>
<th>Follicular phase length</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mg/kg body wt/day (n = 5)</td>
<td>Day 1-3 of m c *</td>
<td>OV+</td>
<td>Not affected</td>
<td>58</td>
</tr>
<tr>
<td>50 mg/day (n = 7)</td>
<td>Day 7-10 of m c.</td>
<td>OV−</td>
<td>Prolonged</td>
<td>52</td>
</tr>
<tr>
<td>100 mg/day (n = 6)</td>
<td>Day 10-17 of m c</td>
<td>OV−</td>
<td>Prolonged</td>
<td>50</td>
</tr>
<tr>
<td>3 mg/kg body wt/day (n = 6)</td>
<td>Day 10-12 of m c.</td>
<td>OV−</td>
<td>Prolonged</td>
<td>57</td>
</tr>
<tr>
<td>25 mg/day (n = 3)</td>
<td>Day 1-14 of m c.</td>
<td>OV−</td>
<td>Prolonged</td>
<td>97</td>
</tr>
</tbody>
</table>

*Treatment was initiated after the emergence of dominant follicle, as judged by ultrasonography. M.C. Menstrual cycle.

hypothalamo-pituitary axis and the LH surge was absent. Croxatto et al.\(^6\) have shown that treatment with 5 or 10 mg RU 486 once-a-day throughout the menstrual cycle suppressed ovulation, whereas, a dose of 1 mg did not consistently block ovulation. Ovulation was blocked only in one of the five cycles treated with 1 mg dose. The effects of antiprogestins on folliculogenesis, therefore, depend upon the dose and the frequency of administration.

If treatment of bonnet monkeys with ZK 98.299 or RU 486 was delayed until the late follicular phase or initiated during the preovulatory period when the serum estradiol levels have increased beyond 150 pg/ml, no effect on the follicular phase or serum levels of estradiol and bioactive LH was observed\(^51,62\). The length of the luteal phase and blood levels of progesterone were comparable to the control cycles. Similar observations have been reported with RU 486 in women\(^57\). The oocyte released in such instances is probably normal in terms of its maturation and ability to be fertilized\(^63\). However, in monkeys, treatment with RU 486 on days 10-12 of the menstrual cycle delays the expected surges of FSH and LH, but estradiol levels continue to rise during the period of drug administration\(^55\).

It is interesting to note that treatment of bonnet monkeys with ZK 98.299 arrests folliculogenesis as long as the treatment is continued. LH surge and ovulation occur 2 days after stopping treatment in bonnet monkeys\(^51,53\), and in women, 4 to 6 days after stopping treatment with RU 486 (ref. 60). The resumed reproductive-endocrine profile of bonnet monkeys is similar to control cycles with respect to estradiol levels, magnitude of LH surge and the duration of the subsequent luteal phase. In normal cycling bonnet monkeys with a menstrual cycle length of 28 days, the mid cycle peak in estradiol levels is generally observed on day 11 ± 1, indicating that in a cycle of 28 days, 13 to 14 days are the time taken from recruitment of follicles to ovulation. Since LH surge and ovulation are observed as early as two days following the cessation of the antiprogestin treatment initiated during the follicular phase in monkeys; this suggests that the growth of the dominant follicle was arrested during the treatment period and that the same follicle recovered soon after the treatment was stopped. It is also possible that a new leading follicle is recruited immediately after the treatment is stopped because collapse of the dominant ovarian follicle has been observed by ultrasonography after treatment with RU 486 (ref. 57).

The inhibitory effects of antiprogestins on folliculogenesis and on ovulation could be due to impaired gonadotropin release. Our studies in bonnet monkeys have shown that the ZK 98.734-induced blockade of folliculogenesis is restored by treatment with LH and FSH (hMG) or by 'pure' FSH alone\(^64\). RU 486 inhibits, in a dose-dependent manner, the GnRH-induced LH and FSH secretion by the rat pituitary cells grown in vitro, and which had been primed with estradiol to maintain the PRs\(^65\). Evidence of a direct effect of RU 486 on gonadotropin release is provided by the observation of inhibition of the mid-cycle FSH and LH surges without affecting the estradiol levels following treatment with RU 486 (1 mg/day) during the mid follicular phase and that these effects can be reversed by the exogenous administration of progesterone\(^6\).

The inhibitory effects of antiprogestins on follicular development could also be due to their direct effects on the ovary. Treatment with RU 486 inhibits the secretion of β-HSD\(^66\) and aromatase\(^67\), the key enzymes involved in the steroidogenic pathway.

The agonadotrophic effects of antiprogestins appear to be specific to their antiprogestational activity rather than due to their antiglucocorticoid activity. The following observations support this conclusion: i) RU 486-induced blockade of the preovulatory LH surge in monkeys cannot be reversed by simultaneous treatment with dexamethasone\(^55\); ii) The ZK 98.734-induced decrease in testosterone levels in adult male rats can be reversed by simultaneous treatment with hCG but not with dexamethasone\(^68\); iii) The ability of RU 486 to inhibit gonadotropin release is diminished in the presence of progesterone\(^69\).

**Effects of corpus luteum function**

The corpus luteum, consisting primarily of granulosa and thecal cells, synthesizes and secretes progesterone and estrogen. Progesterone prepares the uterine endometrium for implantation and maintains early pregnancy.
thereafter the function of corpus luteum is taken over by
the placenta. LH is generally considered to be necessary
for maintaining the normal corpus luteum function.
Reduced availability of LH during the luteal phase
contributes to luteolysis. The presence of LH receptors
in the corpus luteum and the luteotropic effects of LH
support the view that at least LH is required for normal
corpus luteum function. Since the administration of
antiprogestins during the follicular phase of the
menstrual cycle impairs the release of gonadotropins, it
is logical that the treatment during the luteal phase
might have similar effects on LH, and consequently may
cause premature decline of luteal function. FSH is
probably not involved in luteolysis.\textsuperscript{70}

Treatment with ZK 98.299 or ZK 98.734 during the
luteal phase induces luteolysis in bonnet monkeys\textsuperscript{31,72}
and common marmosets\textsuperscript{73} as indicated by the decrease
in serum progesterone levels. The antiprogestin-induced,
preadapted decrease in progesterone levels results in
induction of menstruation and shortening of the luteal
phase. The induction of menstruation is not only due to
the premature decrease in progesterone levels but is also
due to the direct effects of antiprogestins on endo-
metrium. The effects of RU 486 on corpus luteum func-
tion in the human are similar to those of ZK 98.299 or
ZK 98.734 in monkeys. Treatment with RU 486 during
the mid-luteal phase provokes luteolysis and uterine
bleeding in a dose-dependent manner.\textsuperscript{74,75} At lower
doses, a transient decline in luteal function is more
frequently observed, with a rebound increase in pro-
esterone levels followed by a second bleeding episode
corresponding to the time of the spontaneous decrease in
circulating progesterone levels. On the other hand, when
RU 486 is administered during the post-ovulatory phase
to monkeys it does not cause any change in luteal
function.\textsuperscript{76}

The precise manner in which antiprogestins induce
luteolysis is not known. In the rat, treatment with RU
486 or ZK 98.734 during early pregnancy induces
structural changes in the corpora lutea which are
characteristic of luteolysis.\textsuperscript{77,78} Under in vitro
conditions, RU 486 inhibits the activity of 3\beta-HSD, thereby
reducing progesterone production in human granulosa
cells.\textsuperscript{86} However, when pieces of human corpus luteum
of mid-luteal phase are incubated with RU 486, ZK
98.299 or ZK 98.734 no significant change in the syn-
thesis of progesterone is observed (unpublished personal
observations). Gonadotropin-receptor binding and gon-
adotropin-stimulatable adenyI cyclase activity in mem-
brane preparations of the human corpus luteum are also
not affected by RU 486 (ref. 79), further supporting the
observations that the antiprogestins may not have a
direct effect on the primate corpus luteum.

Since progesterone production by the primate corpus
luteum is LH-dependent and the available evidence
suggests that the antiprogestins have inhibitory effects
on hypothalano-pituitary function, it is possible that the
luteolytic effects of antiprogestins are mediated through
the withdrawal of gonadotropin support to the corpus
luteum. That ZK 98.299 or ZK 98.734-induced pre-
mature decrease in serum progesterone levels during the
luteal phase can be prevented by simultaneous treatment
with hCG\textsuperscript{72} supports the possibility of gonadotropin-
mediated effects of antiprogestins on corpus luteum
function. Similar effects have been observed with RU
486 in women.\textsuperscript{80} Studies in which frequent blood
sampling has been done after the administration of RU 486
during the mid-luteal phase show an initial increase in
the frequency and amplitude of LH pulses\textsuperscript{81} followed by a
decrease in LH secretion.\textsuperscript{77} The responsiveness of
pituitary to GnRH also diminishes in women treated
with RU 486, thereby indicating the pituitary as the
probable site of action.

Effects on the endometrium

The effects of antiprogestins on the endometrium depend
on the time of treatment during the menstrual
cycle, as well as on the dose administered. Treatment
during the proliferative phase does not induce premature
bleeding and the morphology of the endometrium is not
altered. On the other hand, treatment during the secre-
tory phase induces regressive changes in the endo-
metrium and its premature shedding.

The inhibitory effects of the antiprogestins on
endometrial development could be due to direct action
on the endometrium and as a consequence of inhibition
of progesterone during the luteal phase. Antiprogestins
also induce menstruation without affecting circulating
levels of progesterone. Endometrial biopsy taken from
bonnet monkeys treated with ZK 98.299 once-a-week
(20 mg/week) throughout the menstrual cycle showed a
few glands which were distinctly atrophic and narrow,
the others were enlarged and sometimes cystically
dilated, lined with small low cuboidal epithelial cells.
The stroma showed spotted edema accentuated by
nedaematous parts containing dense small or spindly
cells. The glands and stroma failed to develop comple-
tely indicating asynchrony.\textsuperscript{54} Similarly, the light micro-
scopic studies indicate that treatment with a single dose
of RU 486 (between 5 and 200 mg) during the secretory
phase inhibits glandular secretory activity, accelerates
degenerative changes, induces vascular changes, and
increases stromal mitotic activity. In this study,
menstrual induction after the administration of RU 486
occurred independent of luteolysis, which is suggestive
of the direct effect of the PRs blocker on the endo-
metrium.\textsuperscript{82} Treatment with RU 486 also inhibits
the normal down-regulation of progesterone and estrogen
receptors in the endometrium.\textsuperscript{83} The serum concentra-
tions of plasma protein 14, a glycoprotein synthesized
by the progesterone-induced secretory endometrium and
which is proposed as a marker for endometrial function, is also suppressed after treatment with RU 486 (ref. 59). These RU 486-induced changes in the morphology and function of the endometrium are typical of those seen in the infertile women with luteal phase defects.

In view of the direct inhibitory action of RU 486 on the endometrium, it may be possible to alter the endometrial receptivity to the blastocyst without provoking changes in the endocrine function of gonadotrophs or corpus luteum, or endometrial bleeding. The use of very low doses of RU 486 during the entire luteal phase or a single dose within a few days after ovulation may interfere with the development of secretory endometrium sufficiently enough to prevent nidation.

Contraceptive strategy

The results presented above clearly suggest that the gonadotropic cells of the pituitary, the endometrium and/or decidua are the primary target organs of antiprogestins. The inhibitory effects on gonadotropin release are suggestive of their possible use as a regular contraceptive by the blocking of gonadotropin-dependent events such as, folliculogenesis, ovulation or maintenance of corpus luteum function. On the other hand, the direct inhibitory effects of these compounds on the secretory activity of endometrium have possible contraceptive potential based upon prevention of implantation by rendering the endometrium out of phase. Moreover, the effects of antiprogestins are dose-related. There is a differential threshold of the gonadotropic cells of the pituitary and the endometrium. The doses which affect follicular development and ovulation also retard endometrial growth, whereas the lower doses impair endometrial development without affecting ovulation. The antiprogestins, therefore, have the ability to interrupt endometrial development regardless of whether ovulation is blocked or not.

Inhibition of ovulation

The property of antiprogestins to block ovulation can be explored for their possible development as a method of contraception, where the drug could be administered at regular intervals to block ovulation. Since antiprogestins are devoid of estrogenic activity their use as an 'estrogen-free' method of contraception would be preferable over the combination pill containing estrogen and progestin. In spite of anovulation induced with antiprogestins, the estrogen secretion is maintained sufficient enough to avoid complications associated with a hypoestrogenic state. Moreover, the low doses of these antiprogestins have no effect on the adrenal function, and the effects on folliculogenesis and ovulation are readily reversible. However, the lowest dose of antiprogestins which blocks ovulation also induces an amenorrheic state during the period of treatment. As long as women are assured that the state of amenorrhea has no deleterious effects, particularly on the endometrium, the antiprogestins may be accepted as an oral contraceptive. An alternate approach suggested is to use antiprogestins in a sequential contraceptive pill regimen with a potent progestin, which would allow formation of a normal secretory endometrium and the occurrence of timely, well-controlled bleeding. The administration of progestin in this study is suggested to prevent endometrial hypertrophy due to unopposed estrogen and to produce regular menstrual bleeding. However, the total dose of RU 486 and medroxyprogesterone acetate used in one cycle is very high, which may not be accepted and may also not be cost effective. Studies therefore need to be carried out to determine the minimum effective dose of RU 486 which would prevent ovulation, preferably without affecting the estrogen biosynthesis, and the pattern of menstruation and without having any deleterious effects on the endometrium.

Prevention or disruption of implantation

The doses at which the antiprogestins arrest gonadal function also impair endometrial development. However, the endometrium is sensitive to low doses of the drugs, the doses which do not affect folliculogenesis and ovulation. The antiprogestins, therefore, have the ability to interrupt endometrial development regardless of whether ovulation is blocked or not. In view of the direct inhibitory effects of antiprogestins on the endometrium, at least three approaches can be envisaged to use antiprogestins as contraceptive.

Treatment during the early luteal phase. Oral administration of RU 486 during the early or mid-luteal phase induces premature bleeding within 2 to 4 days of the initiation of treatment in normal cycling women. In about 60–70% women, the first bleeding episode is followed by another episode around the time of expected menstruation. The antiprogestin-induced bleeding is due to a direct inhibitory effect of the compound on the endometrial growth and in some cases by the inhibition of luteal progesterone secretion. In women in whom treatment with RU 486 causes premature luteolysis, one bleeding episode with complete shedding of the endometrium is observed. In these cases the menstrual cycle length of the treatment cycle is shortened and the post-treatment cycles are generally longer. On the other hand, in women in whom treatment does not provoke luteolysis or luteolysis is not complete, a rebound in the levels of LH, estradiol and progesterone is observed, followed by spontaneous luteolysis terminating the cycle with a second episode of uterine bleeding. Treatment during the early luteal phase may inhibit implantation, but if such a treatment is associated with disturbances in
Table 2. Antiprogestin RU 486 as a late luteal phase contraceptive

<table>
<thead>
<tr>
<th>Drug*</th>
<th>Dose (mg)</th>
<th>No of women</th>
<th>No pregnant</th>
<th>On-going pregnancies</th>
<th>Failures (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>RU 486</td>
<td>400</td>
<td>24</td>
<td>7</td>
<td>1</td>
<td>14.3</td>
<td>88</td>
</tr>
<tr>
<td>RU 486</td>
<td>600</td>
<td>115</td>
<td>41</td>
<td>8</td>
<td>19.5</td>
<td>88</td>
</tr>
<tr>
<td>RU 486</td>
<td>600</td>
<td>30</td>
<td>18</td>
<td>1</td>
<td>5.6</td>
<td>89</td>
</tr>
<tr>
<td>RU 486</td>
<td>600</td>
<td>20**</td>
<td>22</td>
<td>4</td>
<td>18.2</td>
<td>90</td>
</tr>
</tbody>
</table>

*RU 486 was given once on the day before the expected menses. Pregnancy was confirmed by positive hCG test at the time of RU 486 treatment.
**Twenty women participated for a total of 137 cycles (ref. 90).

menstrual cycle length, it will not be accepted as a regular anti-conceptive agent.

Once again, these effects of antiprogestins on the endometrium and on the corpus luteum function are dose-related. In view of the direct inhibitory action of RU 486 on the endometrium, which is more sensitive, it may be possible to alter the endometrial receptivity to the blastocyst without provoking changes in the endocrine function of gonadotrophs or corpus luteum, or endometrial bleeding. The use of very low dose of RU 486 during the entire luteal phase or a single dose within a few days after ovulation may interfere with the development of secretory endometrium, just enough to prevent nidation without disturbing the normal menstrual cycle rhythm. This has indeed been demonstrated by our studies in the bonnet monkeys. Treatment with a single dose of RU 486 during the early luteal phase prevented pregnancy in bonnet monkeys62. None of the 20 animals treated with different doses of RU 486 (5, 10 or 20 mg) became pregnant whereas, three of eight animals in the control group became pregnant in one cycle of exposure.

The contraceptive efficacy of a single dose of 200 mg RU 486 given on cycle day +2 following the day of LH peak has been tested in 21 sexually active women63. Each participating woman monitored her own LH surge in the urine by using the Clearplant or Ovulstick method. One pregnancy occurred in the 157 ovulatory cycles, corresponding to a Pearl Index of 7.6. Only in a few women was spotting observed. These observations suggest that once a month treatment with antiprogestins during the luteal phase can render the endometrium out of phase and prevent nidation.

However, the practical difficulties that one can envisage from this approach include the need for a correct timing of drug administration as relative to ovulation. It should be essential to ensure that treatment is initiated within two to three days after ovulation and not prior to ovulation. Treatment during the late follicular phase may delay ovulation and expose women to a higher risk of pregnancy due to ovulation occurring later in the extended cycle. Secondly, the prolongation of menstrual cycle length due to treatment in the late follicular phase may create a false alarm of pregnancy in women with delay in menses. On the other hand, if the treatment is delayed to mid- or late-luteal phase it would induce uterine bleeding starting a few days after commencement of therapy and would thus cause menstrual irregularities.

**Treatment during the late luteal phase.** RU 486 has also undergone clinical evaluation as a post-coital method of birth control following its administration either during the late luteal phase or close to the time of expected menses88,89. Oral administration of RU 486 (100 mg/day) for four consecutive days before the expected menses consistently produces menstrual bleeding in women who are not exposed to the risk of pregnancy. However, similar treatment to women who had unprotected intercourse failed to prevent conception in at least two women out of 12 with positive pregnancy tests at the time of expected menses. A single oral dose of 400–600 mg RU 486 administered to pregnant women on the day before the expected menses also failed to interrupt pregnancy (Table 2). Taken together the results of these clinical trials with similar protocols suggest that at the dose regimens used RU 486 cannot be recommended for use as a late post-coital treatment against pregnancy.

The drug is well tolerated by volunteers and no side effects are observed. In one study, 8 of 12 volunteers received treatment for 12 consecutive cycles90. Treatment did not disturb the menstrual cycle rhythm nor did it cause any alterations in the follicular or luteal phase of the following menstrual cycles. The degree and duration of bleeding in these subjects was similar to each woman's own pretreatment menstrual period. Since such an approach would not be influenced by the time elapsed following unprotected intercourse and by the number of sexual exposures, further attempts to improve the efficacy of this approach would be a useful addition to the other hormonal approaches being presently used for emergency post-coital contraception.

Since the abortifacient efficacy of RU 486 is improved significantly following its concomitant administration with PG analogues, it is possible that a similar combination regimen may be more efficacious for regular, monthly use also. For such a once-a-month pill to be acceptable, it will be necessary to develop an orally active PG which is stable at room temperature. The treatment also has to be cost-effective. The most commonly used PG analogues gemeprost and sulpro-
Table 3. Effect of ZK 98 299 on ovulation and corpus luteum function in bonnet monkeys

<table>
<thead>
<tr>
<th>Treatment cycle</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZK 98 299 (2.5 mg every third day)</td>
<td>OV+</td>
<td>OV+</td>
<td>OV+/LI</td>
<td>OV+/LI</td>
<td>OV−</td>
<td>OV−</td>
</tr>
<tr>
<td>232</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>104</td>
<td>OV+</td>
<td>OV+</td>
<td>OV+/LI</td>
<td>OV−</td>
<td>OV−</td>
<td>OV−</td>
</tr>
<tr>
<td>186</td>
<td>OV+</td>
<td>OV+</td>
<td>OV+/LI</td>
<td>OV+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>266</td>
<td>OV+/LI</td>
<td>OV+/LI</td>
<td>OV+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZK 98 299 (5 mg every third day)</td>
<td>OV+</td>
<td>OV+</td>
<td>OV+</td>
<td>OV+/LI</td>
<td>OV−</td>
<td>OV−</td>
</tr>
<tr>
<td>168</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>162</td>
<td>OV+</td>
<td>OV+</td>
<td>OV+</td>
<td>OV+/LI</td>
<td>OV+</td>
<td>OV−</td>
</tr>
<tr>
<td>214</td>
<td>OV+</td>
<td>OV+/LI</td>
<td>OV−</td>
<td>OV+</td>
<td>OV−</td>
<td>OV−</td>
</tr>
<tr>
<td>170</td>
<td>OV+/LI</td>
<td>OV−</td>
<td>OV+/LI</td>
<td>OV−</td>
<td>OV+</td>
<td></td>
</tr>
<tr>
<td>230</td>
<td>Pregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LI Luteal insufficiency, serum levels of progesterone did not rise about 3 ng/ml in any of the blood samples collected after the mid-cycle peak in estradiol levels.

Table 4. Effects of ZK 98 299 on menstrual cycle length and duration of menses (mean ± SD) in bonnet monkeys

<table>
<thead>
<tr>
<th>Pretreatment cycle</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>L</td>
<td>M</td>
<td>L</td>
<td>M</td>
<td>L</td>
<td>M</td>
</tr>
<tr>
<td>2.5 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>28.7</td>
<td>4.5</td>
<td>27.7</td>
<td>4.5</td>
<td>27.0</td>
<td>3.7</td>
</tr>
<tr>
<td>SD</td>
<td>2.2</td>
<td>1.2</td>
<td>2.5</td>
<td>1.0</td>
<td>1.8</td>
<td>0.9</td>
</tr>
<tr>
<td>5 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>28.0</td>
<td>3.5</td>
<td>27.5</td>
<td>1.7</td>
<td>27.0</td>
<td>2.5</td>
</tr>
<tr>
<td>SD</td>
<td>2.2</td>
<td>1.3</td>
<td>1.9</td>
<td>0.9</td>
<td>5.9</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Treatment throughout the menstrual cycle

In view of the practical difficulties to administer antiprogestins during the early luteal phase, it may be a more practical method to administer the drug in small doses either daily or at regular intervals throughout the menstrual cycle. Treatment with ZK 98.299 in small doses throughout the menstrual cycle of bonnet monkeys have been reported following unsuccessful treatment with RU 486 alone in early human pregnancy. Misoprostol is on the market in some countries for the treatment and prevention of gastric ulcers. However, some PGs used for termination of pregnancy are potentially capable of causing teratogenic effects as a result of anoxia due to uterine contractions and constriction of the uterine blood vessels. It would therefore be essential to rule out the possibility of any teratogenic effects of misoprostol in the combination regimen, particularly after long-term use.

Antiprogestin was administered (s.c.) every third day, starting on day five of the first menstrual cycle. L: Cycle length (days), M: Duration of menses (days). N.S.: Not significant with respect to pretreatment cycle.
the eight animals which did not conceive, the first three consecutive treatment cycles of six animals were ovulatory, whereas in the other two animals, two cycles of each were ovulatory (Table 3). The incidence of inhibition of ovulation was increased on prolonged treatment. In some of the ovulatory cycles, prolonged treatment also suppressed luteal activity (Figure 3). The mean menstrual cycle length of the animals was not affected (Table 4). Although the mean duration of menses was not altered significantly, the bleeding was scanty in some of the treated cycles. Treatment with antiprogestins inhibited endometrial glandular secretory activity, increased the degenerative changes in gland cells and induced several changes in the vessels of the stroma (Figure 4). It seems probable from these observations that treatment with antiprogestins interferes with the development of endometrium so as to alter its receptivity to the blastocyst.

The effects of RU 486 and related antiprogestins, administered at regular intervals throughout the menstrual cycle, depend upon the dose administered (Table 5). Treatment with low doses of RU 486 (1, 5 or 10 mg/day) administered once daily throughout the menstrual cycle has been shown to disturb endometrial maturation in women. While 5 and 10 mg doses retarded follicular development and prolonged the cycle length in all the treated cycles, 1 mg dose suppressed ovulation in only one of the five cycles. Even in women in whom ovulation was not suppressed; RU 486 either delayed endometrial morphological characteristics or induced dysynchrony between glandular and stromal tissue. However, it is still not known whether the changes induced in the endometrium with low doses of RU 486 are incompatible with the establishment of pregnancy. Since the doses tried so far did not have a selective effect on the endometrium only, and as ovulation was also inhibited in some women (about 20%), it is likely that either still lower doses of RU 486 administered once daily or decrease in the frequency of administration would consistently block endometrial development without altering the follicular and endocrine profile of gonadal and pituitary hormones.

**Post-coital contraception**

Post-coital or emergency contraception refers to a procedure to prevent pregnancy following unprotected intercourse. Since the administration of RU 486 and related antiprogestins during the luteal phase induces premature bleeding, it is likely that it would prevent pregnancy in case of an unprotected intercourse during this period. Similarly, treatment with antiprogestins during the follicular phase is likely to prevent pregnancy in view of the inhibition of ovulation. Therefore, the efficacy of RU 486 as emergency contraception derives from their ability to prevent ovulation and retard

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**Figure 3.** Serum levels of estradiol and progesterone during the pretreatment and four treatment cycles of a bonnet monkey (#186) treated with 2.5 mg ZK 98.299. Treatment was initiated on day five of the first treatment cycle and thereafter it was administered every third day. The profile of gonadal hormones during the first two treatment cycles was similar to that of pretreatment cycle. The menstrual cycle length of the third cycle was prolonged which is due to the shift in the mid cycle peak in estradiol levels. Progesterone levels increased above 1 ng/ml after the estradiol peak but the levels did not rise above 3 ng/ml, which is suggestive of luteal insufficiency. In the fourth treatment cycle also progesterone levels on day 8 after the peak in estradiol levels were lower than those seen in pretreatment and two treatment cycles.

is very effective to prevent pregnancy. In this study five bonnet monkeys were treated with vehicle, and another nine with 2.5 mg (n = 4) or 5 mg (n = 5) ZK 98.299 once every third day for four to six consecutive menstrual cycles. All the five animals treated with the vehicle became pregnant, one in first cycle, three in second cycle and one in the third mated cycle. On the other hand, only one animal out of nine treated with ZK 98.299 became pregnant. Four animals treated with 2.5 mg ZK 98.299 for 17 cycles, and another four treated with 5 mg dose for 21 cycles did not conceive. In
Figure 4. a, Endometrium from control animals showing glands with secretions in their lumen (×1250). b, Endometrium from a monkey (#186) treated with 25 mg ZK 98 299 showing matosis in the pseudostatified glandular epithelium. Stroma shows spindle shaped cells (×1250). c, Endometrium from monkey (#162) treated with 5 mg ZK 98 299 showing small, narrow, tubular inactive glands with accumulation of secretory material in the glandular lumen and stromal cells (×1250).
endometrial development. In two WHO-supported clinical trials, the women (402 at one centre and 195 at the other) who were exposed to the risk of pregnancy were given a single dose of 600 mg RU 486 within 72 h of unprotected intercourse. No pregnancy occurred. Analysis of the data from the centre where 195 women were enrolled showed that at least 72 women had had intercourse within three days after ovulation. Yuzpe regimen (0.1 mg ethinylestradiol and 0.5 – 1.0 mg levonorgestrel), two doses of levonorgestrel alone (0.75 mg) and Danazol have been extensively used as post-coital contraceptive. However, in comparison to these methods, the use of RU 486 appears to be more effective and safe (Table 6). In two identical trials comparing the efficacy of RU 486 with the Yuzpe regimen, no pregnancy occurred in any of the 597 women treated with RU 486 as against nine pregnancies in 589 women treated with the Yuzpe regimen. Moreover, compared to the Yuzpe regimen, the incidence of vomiting, nausea, headache and breast tenderness was much less common in RU 486-treated women. However, in 42% women treated with RU 486, the menstrual cycle length was prolonged by three or more days, compared to 13% in women treated with Yuzpe regimen. This prolongation of menstrual cycle length could be due to the extension of follicular phase resulting in delayed ovulation. These initial trials have provided very encouraging results on the possible use of antiprogestins as a post-coital or

### Table 5. Effects of antiprogestins administered at regular intervals throughout the menstrual cycle on ovulation and endometrial development

<table>
<thead>
<tr>
<th>Antiprogestin</th>
<th>Species</th>
<th>Administration schedule</th>
<th>Dose</th>
<th>Effects on Ovulation</th>
<th>Endometrium</th>
<th>M.C. length</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZK 98 734</td>
<td>Bonnet monkeys</td>
<td>Once-a-week</td>
<td>25 mg</td>
<td>OV-</td>
<td>Atrophic</td>
<td>Prolonged</td>
<td>64</td>
</tr>
<tr>
<td>ZK 98 299</td>
<td>Bonnet monkeys</td>
<td>Once-a-week</td>
<td>20 mg</td>
<td>OV+</td>
<td>Atrophic</td>
<td>Prolonged</td>
<td>54</td>
</tr>
<tr>
<td>ZK 98 299</td>
<td>Bonnet monkeys</td>
<td>Once-a-week</td>
<td>10 mg</td>
<td>OV+</td>
<td>Atrophic</td>
<td>Not affected</td>
<td>54</td>
</tr>
<tr>
<td>ZK 98 299</td>
<td>Bonnet monkeys</td>
<td>Once-a-week</td>
<td>5 mg</td>
<td>OV+</td>
<td>Atrophic</td>
<td>Not affected</td>
<td>54</td>
</tr>
<tr>
<td>RU 486</td>
<td>Cynomolgus monkeys</td>
<td>Once-a-week</td>
<td>25 mg</td>
<td>OV-</td>
<td>Not reported</td>
<td>Prolonged</td>
<td>98</td>
</tr>
<tr>
<td>RU 486</td>
<td>Cynomolgus monkeys</td>
<td>Once-a-week</td>
<td>12.5 mg</td>
<td>OV+</td>
<td>Not reported</td>
<td>Prolonged</td>
<td>98</td>
</tr>
<tr>
<td>RU 486</td>
<td>Women</td>
<td>Once-a-week</td>
<td>10 mg</td>
<td>OV-/+</td>
<td>Not reported</td>
<td>Prolonged</td>
<td>99</td>
</tr>
<tr>
<td>RU 486</td>
<td>Women</td>
<td>Once-a-week</td>
<td>50 mg</td>
<td>OV-/+</td>
<td>Not reported</td>
<td>Prolonged</td>
<td>99</td>
</tr>
<tr>
<td>RU 486</td>
<td>Women</td>
<td>Once daily</td>
<td>1 mg</td>
<td>OV-</td>
<td>Out of phase</td>
<td>Prolonged</td>
<td>59</td>
</tr>
<tr>
<td>RU 486</td>
<td>Women</td>
<td>Once daily</td>
<td>1 mg</td>
<td>OV+</td>
<td>Out of phase</td>
<td>Not affected</td>
<td>61</td>
</tr>
<tr>
<td>RU 486</td>
<td>Women</td>
<td>Once daily</td>
<td>2 mg</td>
<td>OV-</td>
<td>Not reported</td>
<td>Prolonged</td>
<td>60</td>
</tr>
<tr>
<td>RU 486</td>
<td>Women</td>
<td>Once daily</td>
<td>5 mg</td>
<td>OV-</td>
<td>Not reported</td>
<td>Prolonged</td>
<td>60</td>
</tr>
<tr>
<td>RU 486</td>
<td>Women</td>
<td>Once daily</td>
<td>5 mg</td>
<td>OV-</td>
<td>Out of phase</td>
<td>Prolonged</td>
<td>61</td>
</tr>
<tr>
<td>RU 486</td>
<td>Women</td>
<td>Once daily</td>
<td>10 mg</td>
<td>OV-</td>
<td>Out of phase</td>
<td>Prolonged</td>
<td>61</td>
</tr>
</tbody>
</table>

*Ovulation did not occur at the expected time and the follicular phase was extended and ovulation was delayed. OV+-/- In some volunteers ovulation was inhibited while in others it was not.

### Table 6. Efficacy of steroidal post-coital contraceptives in women

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Schedule of administration</th>
<th>No. of women</th>
<th>No. of pregnancies</th>
<th>Failure (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>RU 486</td>
<td>600 mg</td>
<td>Single dose, given within 72 h of intercourse</td>
<td>402</td>
<td>0</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>Yuzpe regimen</td>
<td>100 μg EE + 1 mg LNG</td>
<td>Given twice, separated by 12 h, given within 72 h of intercourse</td>
<td>398</td>
<td>4</td>
<td>1</td>
<td>95</td>
</tr>
<tr>
<td>RU 486</td>
<td>600 mg</td>
<td>Single dose, given within 72 h of intercourse</td>
<td>195</td>
<td>0</td>
<td>0</td>
<td>96</td>
</tr>
<tr>
<td>Yuzpe regimen</td>
<td>100 μg EE + 1 mg LNG</td>
<td>Given twice, separated by 12 h, given within 72 h of intercourse</td>
<td>191</td>
<td>5</td>
<td>2.6</td>
<td>96</td>
</tr>
<tr>
<td>Danazol</td>
<td>600 mg</td>
<td>Given twice, separated by 12 h, given within 72 h of intercourse</td>
<td>193</td>
<td>9</td>
<td>4.7</td>
<td>96</td>
</tr>
<tr>
<td>Levonorgestrel</td>
<td>0.75 mg</td>
<td>Given twice, separated by 12 h, given within 48 h of intercourse</td>
<td>331</td>
<td>8</td>
<td>2.4</td>
<td>100</td>
</tr>
<tr>
<td>Yuzpe regimen</td>
<td>100 μg EE + 1 mg LNG</td>
<td>Given twice, separated by 12 h, given within 72 h of intercourse</td>
<td>336</td>
<td>9</td>
<td>2.6</td>
<td>100</td>
</tr>
<tr>
<td>Danazol</td>
<td>400 mg</td>
<td>Given twice, separated by 12 h, given within 72 h of intercourse</td>
<td>990</td>
<td>17</td>
<td>1.7</td>
<td>101</td>
</tr>
<tr>
<td>Levonorgestrel</td>
<td>0.75 mg</td>
<td>Given twice, first tablet given within 8 h of intercourse, and the second 24 h later</td>
<td>259</td>
<td>2</td>
<td>0.8</td>
<td>102</td>
</tr>
</tbody>
</table>

LNG: Levonorgestrel, EE: Ethinyl estradiol.
emergency method of contraception. These studies need to be extended to determine the minimum effective dose and to find out whether the treatment would still be effective in women presenting with a delay of up to 5 or 6 days after unprotected intercourse. It also needs to be determined as to how often these antigestagens can be used in one cycle without causing disturbances in the menstrual cycle rhythms.

Concluding statements

In brief, the antiprogestins like RU 486 and ZK 98.299 block the action of progesterone at the receptor level, impair gonadotropin release and retard endometrial development. Moreover, they do not have estrogenic or androgenic activity. The availability of these drugs has provided an excellent tool to probe into the mechanisms by which progesterone regulates various reproductive processes, as well as a new approach to regulate fertility. The antiprogestins are being developed as contraceptives either by using the strategy to block ovulation and as an endometrial contraceptive, where the treatment might alter the endometrial receptivity to the blastocyst. In addition to developing antiprogestins as a regular method of contraception, requiring administration throughout the menstrual cycle, they are also being developed as a method of postcoital contraception or an emergency method of contraception. The initial studies in various animal models and the limited clinical trials carried out so far have provided very encouraging results; however, extensive basic and clinical work needs to be carried out before any of these possibilities can become a reality. To develop antiprogestins as an endometrial contraceptive, studies need to be carried out to determine the minimum effective dose and the frequency of administration which would alter the endometrial receptivity to the blastocyst without affecting folliculogenesis, adrenal function and menstrual cyclicity. It also needs to be determined whether drug intake is required throughout the menstrual cycle or whether treatment during the early luteal phase is sufficient. To develop antiprogestins as an emergency method of contraception there is a need to determine the minimum effective dose, and how often the antiprogestin can be administered in each cycle without causing menstrual disturbances and delay in the onset of next menses. Another most important issue which needs to be looked into critically is the teratogenic effects of antiprogestins. How safe are these drugs? Can a woman using these antiprogestins as a method of contraception continue safely with the pregnancy in case of a failure? It is absolutely essential to rule out the possibility of any teratogenic effects of these drugs, particularly after long-term use. Long-term, large-scale clinical trials on the efficacy and safety can only provide answer as to how far these drugs are effective, safe and superior to the existing hormonal methods of contraception.

Contraceptive vaccines

D. K. Giri and G. P. Talwar

National Institute of Immunology, JNU Complex, Aruna Asaf Ali Marg, New Delhi 110 067, India

Fertility control by immunological approaches is no longer a fantasy. It can be achieved in both male and female with no significant side-effects. Besides the feasibility of using these vaccines in animal fertility control, birth control vaccines for human use, have reached the stage of clinical trials. A vaccine directed against hCG is at the most advanced stage and is the first to provide evidence on the ability of the vaccine to prevent pregnancy in women at and above 50 ng/ml hCG antibody titre. The vaccine is safe and reversible.

On the grounds that the hormonal subunits made ectopically by such tumours act as autoerote growth factors for the cancer cells, the recombinant hCG vaccine is under trial in Mexico in lung cancer patients of the type that secrete hCG. Another vaccine directed against LHRH is under trial in hormone-dependent prostate carcinoma patients. Two CMI vaccines employing purified extract of neem seed hold promise for regulating female and male fertility without impairment of sex steroid production, vigour and libido.

VACCINES were traditionally developed against pathogens ‘foreign’ to the body. It has, however, been possible to devise vaccines that can induce either antibody or cell-mediated immune (CMI) response selectively directed against a hormone or a gamete antigen involved in reproduction. What began as an idea in the mid-seventies has become a feasible reality today. Vaccines against three hormones, viz. hCG, LHRH and FSH, have reached the stage of clinical evaluation. A vaccine against the human chorionic gonadotropin (hCG) has not only passed through Phase I clinical trials in India and four countries abroad, but has also completed successfully Phase II clinical trials in three major centres in the country, demonstrating the safety, reversibility and efficacy of the vaccine to prevent pregnancy in women. Novel CMI vaccines for males and females are on the anvil employing immunomodulators from neem (Azadirachta indica). Finally, some vaccines originally made for fertility control are likely to find applications in hormone-dependent cancers. The hCG vaccine is in clinical trial in Mexico in lung cancer patients carrying a type of tumour that secretes hCG and its subunits. Similarly, LHRH vaccine is approved for trials in prostate carcinoma patients. What follows is a brief review of these developments. In the context of this special issue, discussion will be largely confined to the vaccines developed by us.