

Mechanism of action of hormonal preparations used for emergency contraception: a review of the literature

Horacio B. Croxatto^{a,*}, Luigi Devoto^b, Marta Durand^c, Enrique Ezcurra^d, Fernando Larrea^c, Carlos Nagle^c, Maria Elena Ortiz^a, David Vantman^b, Margarita Vega^b, Helena von Hertzen^d

^a*Instituto Chileno de Medicina Reproductiva, Santiago, Chile*

^b*Instituto de Investigaciones Materno Infantil, Hospital San Borja-Arriarán, Universidad de Chile, Santiago, Chile*

^c*Instituto Nacional de la Nutrición S. Z., Ciudad de México, México*

^d*Human Reproduction Program, World Health Organization, Geneva, Switzerland*

^e*Centro de Educación Médica e Investigaciones Clínicas (CEMIC), Buenos Aires, Argentina*

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1. Introduction

This review brings together research efforts done to understand how emergency contraception (EC) methods act to prevent pregnancy and to identify what is known and what are the important gaps that need to be addressed. We expect this review will enroll more scientists in this quest and will stimulate further research to fully elucidate the mode of action. Pursuing more knowledge in this area is needed to attain informed choice. In addition it should contribute to overcome barriers in many settings, facilitate the widespread utilization of preparations for EC, and lead toward further improvements. The mode of action is important for some users, health providers, policy makers, developers and manufacturers because of sensitive ethical issues. These issues resolve in either 1 of 2 questions. For some, it is whether EC acts before or after fertilization, while for others it is whether it acts before or after implantation.

From the biologic perspective, the scenario is far more complex because full understanding of the mode of action implies defining how EC methods act, directly or indirectly, at various levels: the molecular level at which the exogenous steroid initiates its action, the signaling level at which regulation of reproduction takes place, the target organs where the signals act to elicit responses and finally the level of the reproductive entities, i.e. the gametes and the developing zygote up to at least the implanting blastocyst stage. This is represented in Table 1.

Some of the discrete steps of the reproductive process whose theoretical interference by EC could prevent pregnancy are:

- follicle maturation
- the ovulatory process
- sperm migration into and through the fallopian tube, including adhesion of spermatozoa to the epithelium needed to acquire and maintain their fertilizing capacity
- fertilization
- zygote development in the fallopian tube
- zygote transport through the fallopian tube
- preimplantation development within the uterus
- uterine retentiveness of the free laying morula or blastocyst
- endometrial receptivity
- blastocyst signaling, adhesion and invasiveness
- corpus luteum sufficiency and responsiveness to hCG

Figure 1 illustrates the chronology of some of these steps within the normal conceptional cycle of women and the time period within which EC needs to act to interfere with each one of them.

1.1 Historical background

The first major trial carried out in women with “modern” contraceptive methods for “emergency purposes” was undertaken in Yale in 1963, using diethylstilbestrol 25–50 mg/day or ethinyl estradiol 0.5–2 mg/day for 4–6 days following intercourse. No pregnancies occurred among the first 100 cycles reported [1]. It was not until the mid-1970’s that larger studies, conducted in Holland with estrogens alone [2] and in Canada with an estrogen-progestin combination [3], opened the way for a more widespread use of emergency contraception. Attempts to develop a progestin-only post-coital method for regular use, conducted in Perú

* Corresponding author. Tel.: +56-2-632-1998; fax: +56-2-633-6204.
E-mail address: icmer@terra.cl (H.B. Croxatto).

Table 1
Spectrum of possibilities for the mode of action of emergency contraceptive drugs.

SPECTRUM OF POSSIBILITIES FOR THE MODE OF ACTION OF EMERGENCY CONTRACEPTIVE DRUGS		
LEVEL OF ORGANIZATION AFFECTED BY DRUG	PROCESS AFFECTED BY DRUG	SITE OF ACTION
MOLECULAR	RECEPTOR ACTIVATION ENZYME ACTIVITY	ANY CELL IN REPRODUCTIVE SYSTEM
CELLULAR	CELL-CELL COMMUNICATION VIA NEURAL, ENDOCRINE OR PARACRINE MECHANISM	HYPOTHALAMIC-PITUITARY-OVARIAN-GENITAL TRACT AXIS
REPRODUCTIVE ORGANS, PROCESSES OR ENTITIES	FOLLICULAR DEVELOPMENT OVULATORY PROCESS SPERM MIGRATION, COMPETENCE AND PERSISTENCE FERTILIZATION ZYGOTE DEVELOPMENT EMBRYO TRANSPORT MORULA-BLASTOCYST DIFFERENTIATION ENDOMETRIAL RECEPTIVITY IMPLANTATION	OVARY CERVIX, UTERUS, FALLOPIAN TUBE FALLOPIAN TUBE FALLOPIAN TUBE, UTERUS UTERUS

[4], provided the basis for its application to emergency situations two decades later. In addition, the post-coital insertion of an intrauterine device [5] and the administration of the antiprogesterin RU486 [6], have also been used to prevent pregnancy in the context of “emergency situations”.

1.2. Definition of emergency contraception

A comprehensive definition of EC is the following: “specific contraceptive methods that can be used as emergency measures to prevent pregnancy after unprotected intercourse; emergency contraception is used after coitus but before pregnancy has become established; as such, it is considered a back-up method for occasional rather than

regular use” [7]. The Consortium for Emergency Contraception [8] has adopted a similar definition: “a number of methods used by women within a few hours or a few days following unprotected intercourse to prevent pregnancy.” Both definitions essentially highlight that EC is to be used within a given period after unprotected intercourse to prevent pregnancy and that it differs from most fertility regulation methods in that it is not intended for regular use.

There are a variety of situations where EC is indicated, among them, condom rupture, unplanned unprotected intercourse (particularly in case of young adults engaging in sexual experiences), incidental misuse of regular contraceptive methods, and sexual assault.

1.3. Current hormonal EC regimens

A brief description of the mode of use, efficacy, and most common side effects of the 2 hormonal methods most widely used for EC, the Yuzpe regimen and levonorgestrel, and a promising method under research, mifepristone (RU486), follows.

1.3.1. Yuzpe regimen

In recent years this has been the most commonly used method. It consists of 2 doses, given 12 h apart, each providing 100 ug ethinyl estradiol (EE) plus 500 ug levonorgestrel. The pills usually contain 50 ug EE and 250 ug levonorgestrel or 500 ug dl-norgestrel, so that a total of 4 tablets need to be taken. The first dose should be administered within 72 h after unprotected intercourse. Nausea and vomiting are the most common side effects of this method. The effectiveness of the Yuzpe regimen for EC is now well established [3,9–15]. A recent meta-analysis found effectiveness rates of 56–89%, with a weighted average of 74% [16]. This does not mean that 26% of users actually get pregnant since of all women who take EC pills, only about 2% get pregnant [8]. It means that it prevents 74% of pregnancies that are to be expected based upon the time of the cycle in which intercourse took place.

1.3.2. Levonorgestrel

Norgestrel or DL-norgestrel is a racemic mixture of d-norgestrel and l-norgestrel. d-Norgestrel, the biologically active enantiomer, is designated as levonorgestrel (LNG). The focus of this section is on LNG used alone for EC.

Hoffman [17] looked for the first time at the efficacy of LNG as an emergency contraceptive. He administered a single dose of 0.6 mg within 12 h of unprotected intercourse and observed a failure rate of 2.9%, not different from the failure rate in a parallel group treated with the Yuzpe regimen.

Ho and Kwan [18] carried out a randomized study to compare the standard Yuzpe regimen with 0.75 mg LNG administered twice, 12 h apart, starting within 48 h after a single unprotected intercourse. Again, the efficacy of LNG was similar to that of the combined estrogen-progestin regimen with failure rates of 2.4% and 2.6%, respectively.

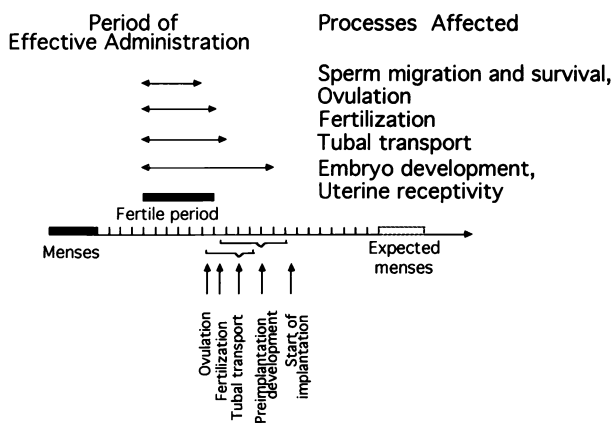


Fig. 1. Period of administration and processes interfered with by emergency contraception products.

Recently, a randomized, double-blind clinical trial of LNG versus the Yuzpe regimen that enrolled nearly 2,000 women at 21 centers world-wide was reported by the WHO Task Force on Post-Ovulatory Methods for Fertility Regulation [19]. In this study, one tablet containing 0.75 mg LNG plus a placebo tablet were taken no later than 72 h after unprotected intercourse. A second tablet containing the same dose plus a placebo tablet were to be taken 12 h later. The crude pregnancy rate was 1.1% (11/976) in the LNG group compared with 3.2% (31/979) in the Yuzpe group. The proportion of pregnancies prevented, compared with the expected number without treatment, was 85% with LNG and 57% with the Yuzpe regimen. The efficacy of both treatments declined significantly with increasing time since unprotected intercourse. The pregnancy rates were 0.4%, 1.2%, and 2.7% when LNG was given within the first, second or third 24 h period since unprotected coitus.

In all studies, LNG has been associated with a significantly lower incidence of side effects than the Yuzpe regimen [18–20].

Following single oral administration of 0.75 mg, LNG serum concentration reach a maximum (5–10 ng/mL) approximately at 2 h and decline rapidly during the first 24 h with considerable inter- and intra-individual variation [21–23]. The scientific literature examined does not allow for assertion that the current dose being used is optimal or that the second tablet contributes significantly to the contraceptive effectiveness of this method.

1.3.3. Mifepristone (RU486)

Mifepristone is an 11-dimethyl-amino-phenyl derivative of norethindrone with high affinity for progesterone receptors and less so for glucocorticoid receptors. At low doses suitable for EC, it exhibits antiprogestin but no antiglucocorticoid action. The pharmacokinetics following single oral administration are characterized by rapid absorption, peak serum concentrations in the micromolar range and a long half-life of 25 to 30 h [24].

Glasier et al. [6,12] and Webb et al. [13], compared the efficacy and side effects of a single dose of 600 mg of mifepristone with those of the Yuzpe regimen. None of the nearly 600 women who received mifepristone became pregnant, whereas, nine pregnancies were observed among those given the Yuzpe regimen. Significantly more women who were treated with mifepristone had a delay in the onset of menstruation but other than that, this treatment was associated with minimal side effects.

A recent multicenter study, which included 1,717 women seeking EC, showed that reducing the mifepristone dose from 600 to 50, and even to 10 mg, did not decrease its efficacy (pregnancy rates 1.3%, 1.1%, and 1.2%, respectively); overall, 84 to 86% of expected pregnancies were prevented. Lower doses were associated with less disturbance of the menstrual cycle and lower incidence of unpleasant side effects; overall, 12–17% of the subjects reported nausea, headache or dizziness [25].

In summary, the results of these clinical studies, indicate that mifepristone is a highly effective EC agent, and its only apparent disadvantage from the user perspective is that the next menstrual period comes after the expected date in nearly half of the cases.

2. Effects of post-coital administration of steroids upon fertility in non-primate animal models

Numerous studies have shown that pregnancy can be prevented in a variety of mammalian species by post-coital administration of sex steroid hormones, their synthetic agonistic and antagonistic analogs as well as non-steroidal drugs that share in part their pharmacologic properties. The species that comprise this group, which are most commonly used in the laboratory, e.g., rabbit, rat, mouse, hamster, and guinea pig, differ from the human and other primates in many aspects of their reproductive process. One of them which is crucial for studying the mode of action of post-coital contraception, is the fact that at variance with the human, coitus always precedes ovulation by 12 h or less. This means that coitus either comes after the ovulatory stimulus has taken place (spontaneous ovulators) or induces the gonadotropin surge immediately thereafter (reflex ovulators), leaving little or no chance for post-coital treatments to interfere with pre-fertilization events. Only a few examples, which illustrate what sex steroids do in these species when used in a manner comparable to that of EC, were arbitrarily selected.

2.1. Estrogens

Greenwald [26] compared the response of the rabbit, rat, mouse, hamster, and guinea pig, to a single post-coital injection of estradiol cyclopentylpropionate and showed that post-coital treatment with estrogens caused either tubelocking of embryos or accelerated transport to the uterus. Although other effects were also detected, the alteration in oviductal transport accounted by and large for the contraceptive effect. Embryos that entered the uterus prematurely were expelled whereas those whose sojourn through the oviduct was prolonged, degenerated.

Further work in which a single injection of estradiol was given at different times after coitus revealed that a wide range of effectiveness can be achieved and different mechanisms can account for the contraceptive effect when the same steroid is given at different times post-coitum [27,28].

2.2. Progestins and estrogen-progestin combinations

Post-coital administration of progesterone 5 mg on day 2 or 3 (ovulation = day 1) [29] or LNG 10 µg on day 1 (Croxatto, unpublished) has no effect upon fertility in the rat whereas depot medroxyprogesterone acetate (DMPA) 12.5 mg on day 1 prevents the nidatory estrogen surge, hence

blastocysts do not implant [30]. Post-coital single administration of DMPA in the hamster, which does not require the estrogen surge for implantation, has no contraceptive effect (Croxatto, unpublished).

Immediate pre-coital or up to 5 h pre-coital administration of progesterone 1–20 mg s.c. reduces by 20–50% the rate of fertilization in the hamster [31]. Indirect evidence indicates this effect is achieved by interference with sperm migration from the uterus to the oviduct or within the oviduct. While administration of progestins to rabbits shortly before insemination and ovulation greatly reduces the rate of implantation mainly because it disturbs sperm and egg transport and interferes with fertilization [32], the same treatment given post-coitally has little or no antifertility effect [33–36]. Rabbits failed to become pregnant in spite of having ovulated normally only when a very high single oral dose of LNG (2.25 mg or more) was given immediately after coitus [37]. The mechanism involved has not been reported.

Very few papers report the effects of post-coital treatment with estrogen-progestin combinations in this group of experimental animals. In the rabbit and the rat, two species in which post-coital estrogen reduces the pregnancy rate mostly through altered oviductal embryo transport, the concomitant administration of progesterone can partially reverse this effect [38,39].

2.3. Mifepristone

Post-coital administration of mifepristone reduces the pregnancy rate in the rat and the mouse in a dose-related fashion. In the rat, this effect is associated with accelerated oviductal transport of embryos, delayed development beyond the morula stage, loss of embryos from the uterus and postponement of the window for endometrial receptivity [40]. Blastocysts recovered from mifepristone-treated rats do implant in untreated pseudopregnant recipients but not vice versa [41]. In the mouse, the inhibition of pregnancy is associated with retention of some embryos in the oviduct and accelerated transport and expulsion of others. This is accompanied by a notable delay in embryonic development [42,43]. Development of rat and mouse embryos in vitro in the presence of mifepristone in the culture medium from 2-cell up to blastocyst stage is not impaired unless millimolar concentrations are used [44,45].

Several general conclusions derive from the analysis of the mode of action of post-coital contraception in non-primate animal models. First, this group is fairly sensitive to post-coital treatments that involve a relative estrogen excess or progesterone deficit and is fairly insensitive to an excess of progestins. This last feature is at variance with the human. Secondly, not only the contraceptive effectiveness, but also the mode of action is in some cases dose- and time-dependent. Third, in any given experimental situation there is usually not a single well-defined mode of action but various effects that account for the reduced pregnancy rate. Fourth,

marked species differences in the mode of action of the post-coital treatments force the conclusion that to learn how they work in the human, the studies need to be done in women.

3. Studies in non-human primates

Hormonal compounds currently used for EC have not been tested, as such, in non-human primates, therefore, this review focuses on the effects of estrogens, progestins or the antiprogestin mifepristone, administered in the periovulatory period to macaques and the New World monkey *Cebus apella*.

3.1. Estrogens

Morris and van Wagenen [1] found that every compound that was uterotrophic in the mouse, prevented pregnancy in the macaque, and Morris [46] concluded that estrogens given during the immediate postovulatory period were a highly effective method to prevent pregnancy. Without knowing exactly how, and assuming the step of the reproductive process interfered with was one that takes place after fertilization, but before established implantation, they designated this mode of action as “interception” to differentiate it from contraception. They believed the main target was the endometrium where they observed stromal edema, hemorrhage, and loss of decidua, all of which was considered unsuitable for implantation [38]. High doses of estrogen given in the preovulatory phase depressed endometrial growth and angiogenesis through a negative influence on the vascular endothelial growth factor [47]. This suggested that estrogens might interfere with endometrial receptivity even if given before ovulation.

Other mechanisms for the contraceptive effect of post-coital estrogen can operate in monkeys. If given in the follicular phase, so as to advance the preovulatory increment in plasma estrogen, they evoke a premature LH surge that does not trigger ovulation and the formation of a functional corpus luteum, and the spontaneous LH surge is delayed or suppressed [48,49]. Estrogen receptors (ER) present in the granulosa cells of antral and preovulatory follicles and in luteal cells [50–52] allow for a diversity of effects in the ovary. In rhesus monkey, supraphysiological doses of estrogen given in the mid or late follicular phase induce atresia or luteinization without rupture of the dominant follicle, reduce the viability of granulosa cells, reduce the synthesis of estradiol and progesterone and are detrimental to the oocyte [51,53,54].

Although administration of estradiol during the mid-luteal phase, induces premature luteal regression [12,55,56], this is not the case when macaques and Cebus monkeys are treated in the early luteal phase [57,58], at a time when ER levels in the corpus luteum are low [52]. Thus, luteolysis is not involved in the prevention of pregnancy by post-coital estrogen in monkeys.

3.2. Estrogen-progestin combinations

Concomitant administration of estradiol (E_2) and progesterone (P) before the LH surge, effectively inhibits ovulation [59] whereas administration in the early luteal phase induces premature luteolysis [58,60]. These effects explain the high contraceptive efficacy of estrogen-progestin combinations given to monkeys either before or after the LH surge. In attempting to extrapolate this observation to the human, it should be taken into consideration that premature luteolysis is more critical in the monkey than the human because in the former the embryonic signal for corpus luteum rescue arises 2 or 3 days later.

3.3. Progesterone, progesterone synthesis inhibitors, and antiprogestins

The effect of levonorgestrel administered in the periovulatory period in non-human primates has not been reported. Since its binding affinity to progesterone receptor (PR) and its progestomimetic activity are several-fold higher than P itself [61], one can expect it will share many of the effects of the natural progestin. The effects of P upon fertility, when administered in the periovulatory phase, have not been reported in the monkey. However, exogenous progesterone antagonizes estrogen-induced gonadotropin release required for ovulation [59,62] and causes asynchronous development of glands and stroma in the endometrium [47,63,64].

Progesterone plays a pivotal role in periovulatory and luteal events through receptor-mediated pathways [65–68]. Thus, a reduction of P bioavailability by inhibition of its synthesis or by competition with its receptor might interfere with ovulation, fertilization, luteal function, and subsequent endometrial development.

Preovulatory administration of inhibitors of 3β -hydroxysteroid dehydrogenase, a key enzyme for progesterone synthesis, before ovulation, leads to failure of follicular rupture and to the “luteinized unruptured follicle syndrome” [67]. Although the mechanism underlying the dissociation of luteinization from rupture is unknown, recent studies suggest that progesterone regulates the ovarian production and effects of cytokines thought to be involved in the rupture of the follicular wall [69]. On the other hand, inhibition of progesterone synthesis during the follicular phase or during the periovulatory period markedly impaired the fertilization rate of oocytes inseminated in vitro [67]. Administration of 3β -hydroxysteroid dehydrogenase inhibitors after ovulation, acutely reduces progesterone production. This in turn favors the invasion of the corpus luteum by macrophages and results in a reduction or disappearance of the luteal cell population [66].

In macaques, mifepristone is effective in preventing pregnancy when it is administered either before or after ovulation. Growth of the leading follicle is temporarily arrested and ovulation is delayed or blocked when given in the follicular phase [70–72]. The resulting luteal phase

is usually shortened. Administration in the preovulatory period also impairs the midcycle gonadotropin surge [70], probably due to a direct action at the pituitary level [73].

Treatment in the early luteal phase inhibits implantation in the rhesus monkey [74,75]. Multiple actions of this antiprogestin on nidatory endometrium are exemplified by the following: administration of mifepristone in the early luteal phase increased the levels and secretion of leukemia inhibitory factor and transforming growth factor β , it decreased vascular endothelial growth factor in the glandular and vascular compartments, and inhibited the expression of Le^y oligosaccharide in the epithelial compartment [76,77]. Another study showed that the concentration of endometrial prostaglandins PGE_2 and $PGF_{2\alpha}$ as well as the ratio of $PGF_{2\alpha}$ to PGE_2 , were increased after giving mifepristone in the early luteal phase [78].

In addition, administration of mifepristone in the early luteal phase, delayed embryo development [75]. Since no developmental abnormalities were evident in preimplantation embryos exposed to the antiprogestin in vitro, the effects seen in vivo are likely to be secondary to the alterations induced by mifepristone in the genital tract milieu [79].

It appears plausible that the contraceptive effect of mifepristone in monkeys, when given before ovulation, is due to deferral of ovulation and when given in the early luteal phase, it is mediated first by an alteration in the oviductal and uterine microenvironment which becomes detrimental to embryonic development and subsequently by abnormal expression of molecules involved in endometrial receptivity or implantation.

4. Clinical studies

4.1. The Yuzpe regimen

Rowlands et al. [80], gave the combination EE-dl-norgestrel to 14 women requesting EC up to 120 h after unprotected intercourse. LH, pregnanediol glucuronide, hCG, and creatinine were monitored in daily urine samples. Normal parameters were observed in 4 subjects, suppression or deferral of the LH peak in 3, and shortening or insufficiency of the luteal function in the remaining 7. In no case was hCG detected. The timing of treatment within the cycle was not reported.

4.2. Effects of the Yuzpe regimen administered before the LH surge

Swahn et al. [81] investigated the effect of the combination EE-LNG administered on day 12 of the cycle. The concentrations of LH, pregnanediol, and estrone glucuronide were followed daily in the first morning urine of 8 women during one control, one treatment and one recovery cycle. Treatment affected the timing and/or the amplitude of

the LH peak: in 3 women, it was not possible to identify the LH peak, in 2 it was postponed to days 38–39 while in the remaining 3, it was detected on days 13, 13, and 22. The mean area under the curve of LH was significantly lower than in control cycles and in 5 subjects excretion of pregnanediol glucuronide was significantly lower than in control cycles. These findings suggest that when treatment precedes the LH peak, the Yuzpe regimen inhibits or delays ovulation and/or causes insufficient luteinization to support normal progesterone secretion. These assumptions need to be confirmed by ultrasonographic determination of the outcome of the dominant follicle and by hCG challenge tests.

Ling et al. [82] conducted a study with the combination EE-dl-norgestrel. Eleven subjects were studied during a placebo-treated and a drug-treated cycle. Ovarian and gonadotropin function were monitored by hormone assays in daily serum samples. The timing of treatment was aimed to be before the LH peak based upon basal body temperature records of previous cycles. Endometrial biopsies were performed 7 days after treatment or on the first day of the menstrual bleeding. The effect of treatment on plasma hormone levels and cycle length within this group varied considerably between individuals.

In short, 3 of the 11 subjects, had an endocrine profile compatible with anovulation and one with postponed ovulation. In 4 subjects who had presumably ovulated, endometrial maturation appeared altered and did not coincide with the expected day of the cycle as related to the LH peak. Gland development was lagging 2 to 6 days behind stromal maturation. In the remaining 3 subjects, luteal phase hormone levels were altered but it is unclear if enough to affect the establishment of pregnancy.

4.3. Effect of the Yuzpe regimen administered after the LH surge

Ling et al. [83] administered the combination EE-dl-norgestrel 18 h after the LH peak in serum and again at 30 h, to five volunteers. Each subject was studied during a placebo-treated and two consecutive drug-treated cycles. Ovarian and gonadotropic function were monitored by hormone measurements in daily serum samples. One subject had P and E₂ levels significantly decreased in both drug-treated cycles. Progesterone levels were decreased in one drug-treated cycle of one subject and in the two drug-treated cycles of another. In the remaining five cycles, the treatment did not alter E₂ and P levels during the luteal phase.

Ling et al. [84] administered the combination EE-dl-norgestrel 36 h after the LH peak in serum and again at 48 h in 12 volunteers. Each subject was studied during a placebo-treated and a drug-treated cycle. Ovarian function was monitored by steroid assays in daily serum samples. The endometrium of six subjects was biopsied on the 9th day after the LH peak. Five subjects showed no significant change in the steroid levels but their luteal phase was shortened by a mean of 3 days. Three subjects had P and E₂ levels significantly

decreased from day LH +2 to LH +7 and their luteal phases were 2 days shorter. In two subjects, only E₂ levels diminished. The remaining two subjects had lower P levels from day 4 to 9 after the LH peak and their E₂ levels presented a fluctuating pattern throughout the luteal phase. The endometrium of drug-treated cycles showed varying degrees of out of phase and asynchronous development of the stroma and epithelial components.

Swahn et al. [81] investigated in 8 women the effect of administering the Yuzpe regimen on day LH +2 upon the endometrium. The treatment did not affect estrone and pregnanediol glucuronide excretion. An endometrial biopsy was obtained on day LH +6 to +8. The morphometric dating did not differ enough from the chronological dating to characterize the endometrium as out of phase. A significant increase in the number of vacuolated cells and a wider diameter of the glandular lumen was observed in comparison with control cycles. These data neither prove nor exclude the possibility that the endometrial alterations observed would be sufficient to prevent implantation.

The above studies [80–84] show that the Yuzpe regimen can alter in some cases some of the physiologic parameters measured, e.g., the signaling from the pituitary to the ovary and from the corpus luteum to the endometrium, as well as the morphology of this target tissue.

4.4. Effect of the Yuzpe regimen on progesterone-regulated endometrial proteins

Kubba et al. [85] administered the Yuzpe regimen 48 h after the onset of the LH surge and found significantly decreased ER and PR concentrations in the endometrium 24 h after the first dose in 7 of 8 subjects. The effect on NAPD-dependent isocitrate dehydrogenase, a progesterin sensitive enzyme, was quite variable.

Two studies [86,87] investigated the effect of the Yuzpe regimen administered on day 9 of the luteal phase on progesterone-regulated endometrial proteins. Although interesting for their design and their findings, these two studies are unfortunately not relevant to the mechanism of action of the Yuzpe regimen used for EC since they mimic a situation in which intercourse would have taken place 72 h prior to luteal phase day 9. This is definitely outside the fertile period of the menstrual cycle and, therefore, fails to address the issue at stake. It would be worth repeating these studies giving EC in the periovulatory period.

4.5. Levonorgestrel

There are few studies designed to look at the mechanism of action of LNG in EC and its exact mode of action remains unknown. Moggia et al. [88] proposed that the post-coital contraceptive effect of LNG is due to changes in the endometrium that prevent implantation. Kesseru et al. [89] provided evidence that single administration of LNG 0.4 mg 3 to 10 h post-coitum: a) decreased the number of

sperm recovered from the uterine cavity beginning 3 h after treatment; b) caused pronounced alkalization of the intra-uterine fluid beginning at 5 h, which immobilized the sperm, and; c) increased the viscosity of the cervical mucus, beginning at 9 h, which denied further passage of sperm to the uterus. Seregély [90] suggested that disturbances in LH pulse frequency following LNG administration were involved. Landgren et al. [21] examined the effects of repeated doses of LNG (0.75 mg) given before (days 2, 4, 6, and 8), during (days 9, 11–13, 15, 16, and 19) or after ovulation (days 16, 18, 20, and 22). Administration in the early follicular phase increased the duration of the follicular phase. Treatment around ovulation resulted in varying effects ranging from anovulation or deficient luteal function in some women to normal ovarian function in others. The administration of LNG during the luteal phase was not followed by changes in cycle length or endometrial morphology. Spona et al. [91] administered single or multiple doses of 0.4 mg LNG before or after the LH peak to 6 subjects. Treatment prior to the LH peak suppressed the gonadotropin surge, reduced the levels of E₂ and P, and markedly lowered the cervical mucus score, whereas treatment after the LH peak did not alter these parameters. The effects of these multiple administrations cannot be freely extrapolated to the current EC regimen.

Wang et al. [92] compared the effects of 0.75 mg LNG given twice, 12 h apart, when the first dose was administered on day LH-2 versus LH+2. The main endpoints were timing and incidence of ovulation and the status of the endometrium at the time of implantation (LH+7). Preovulatory administration had no effect on ovulation, whereas at the level of the endometrium, it caused divergent effects depending on the time of drug intake. Factors believed to be critical for implantation, such as integrins, steroid receptors, or leukemia inhibitory factor, among others, were changed in ways which are likely to alter endometrial receptivity.

4.6. Appraisal of possible modes of action of Yuzpe regimen and LNG for EC

The results presented above [80–84] indicate that pre-ovulatory treatment with the Yuzpe regimen has the ability to postpone, quench or suppress the ovulatory stimulus in a fair proportion of cases, but not in all. What makes the difference, e.g. timing of treatment administration or individual bioavailability profiles, has not been determined. Ultrasound assessment of the stage of follicular development at the time of treatment and subsequent verification of the fate of the leading follicle is missing. A 24-h variation in the timing of treatment after the LH peak did not change the proportion of cycles with altered endocrine pattern [83, 84] but such variation in the timing of treatment may be more critical when it is given before ovulation.

Other effects observed were on ovarian secretion of estrogen and progesterone but it is unclear if and how they

contribute to prevent pregnancy. These effects also present wide inter-individual variation and the source of this variation has not been addressed. It may be worth attempting to correlate plasma levels of the components of the Yuzpe regimen and other EC methods with the outcome of the treatment in mechanism of action studies.

Whether or not the insufficient luteal function seen in some treated cycles can be rescued by hCG, should an embryo reach the appropriate stage to produce it, has not been determined.

Effects described on the endometrium are also not consistent, sometimes minimal or absent and their mediation of the contraceptive effect is impossible to ascertain at the present time.

The only study that provides a large enough data base to examine the relationship between coitus-treatment interval and outcome shows that LNG as well as the Yuzpe regimen exhibit an inverse relationship between contraceptive efficacy and the length of time from intercourse to treatment. Pregnancy rates increased from 0.5% when treatment was given within the first 12 h period after intercourse to 4.1% when it was given within the fifth 12 h period (61–72 h) [93]. While this fact does not allow for discriminating between possible modes of action, it does lend support to a significant role of pre-fertilization mechanisms in their contraceptive effectiveness, albeit not necessarily the same ones for both methods. Our hypothesis is that the earlier the Yuzpe regimen is given, the better the chances it will have to prevent ovulation, while in the case of LNG the earlier it is given, the better the chances it will interfere with sperm migration and function at all levels of the genital tract.

4.7. Mifepristone

The effects of mifepristone on the human menstrual cycle are highly dependent on the stage of the cycle at the time of treatment and the dose administered. Here we deal only with treatments given in the segment of the menstrual cycle that is relevant to EC. Administration of mifepristone 3 mg/Kg for 3 days beginning after the emergence of the dominant follicle, delayed ovulation, prolonged the follicular phase and increased the length of the menstrual cycle to an average of 42 ± 9 days [94]. Similar results were obtained when mifepristone was given either as a single dose of 5 mg at the time the leading follicle had reached a diameter between 12 and 14 mm or as a multiple dose of 5 mg/day for 3 days when follicles were 14 to 16 mm but not 6 to 11 mm in diameter [95]. These findings indicate that mifepristone can interrupt normal follicular development after the selection of the dominant follicle. Moreover, mifepristone 2 mg/day for 30 days [96] inhibits the positive feedback effect of estrogens, therefore, blocking or delaying the preovulatory LH surge.

In studies in which mifepristone was given for EC, menses delay has been frequently reported and some women

have become pregnant from intercourse 10–15 days after treatment [12,13]. These observations are in keeping with the ovulation-delaying effect of mifepristone.

Messinis and Templeton [97] examined the possibility that mifepristone might interfere with gonadotropin-induced oocyte maturation in vivo. Following a 5-day course of clomiphene to stimulate follicular growth, 100 mg mifepristone was given on day 16, one hour before injecting 5000 IU of hCG to 20 women. At laparoscopy for tubal sterilization done 34 h after hCG, all follicles >15 mm were aspirated and the collected oocytes were submitted to in vitro fertilization. Another 20 women not given mifepristone served as control. Egg recovery was close to 80%, the rate of fertilization was close to 60% and the rate of cleavage was not different between the two groups. These data practically exclude the possibility that preovulatory administration of this antiprogesterin acts by reducing the fertilizability of the oocyte.

Another target of mifepristone is the endometrium. Several studies have shown that administration of mifepristone during the luteal phase interferes with the development of a normal secretory endometrium causing ultrastructural changes in decidual capillaries, vascular damage and decidual necrosis. Presumably, these alterations should prevent or disrupt implantation [98]. A single dose of 200 mg of mifepristone administered on cycle day LH +2, close to the end of the fertile period (early luteal phase), curtails the expected increase in the expression of leukemia inhibitory factor during the mid-luteal phase [99]. Progesterone is also a key hormone in the regulation of the plasminogen/plasmin system in the human endometrium, believed to play a pivotal role in implantation and ensuing embryonic development [100]. In an in vitro model of decidualization of endometrial stromal cells, mifepristone blocked and reversed progestin-inhibited plasminogen activator expression, suggesting a role of this system in mifepristone-induced endometrial extracellular matrix dissolution and bleeding [101].

4.8. Appraisal of probable modes of action of mifepristone for EC

Mifepristone treatment during the mid to late follicular phase interrupts further growth of the dominant follicle, probably by lowering its sensitivity to FSH and counteracts the positive feedback of estradiol preventing the gonadotropin surge and postponing the time of ovulation. Under these circumstances, spermatozoa derived from the single act of unprotected intercourse have to wait too long for the oocyte to be released and fertilization cannot take place.

When treatment falls within the 1- or 2-day window in which follicular rupture has not taken place yet but it is too late to stop, the contraceptive effect of low doses may rest upon hitherto undisclosed mechanisms. High doses, such as 50 mg, given late within this window, may partially act as a post-ovulatory treatment, due to the long half-life of this compound. They may cause insufficient progesterone sup-

port of tubal and uterine functions required for normal embryo development, transport and implantation.

5. Cogitation

Numerous attempts to determine the involvement of selected steps of the reproductive process in the mechanism by which EC prevents pregnancy have been done. In spite of that, a wide gap of information persists that hinders a clear-cut answer to the question.

With few exceptions, the fact that an entity or a process is altered by the treatment does not necessarily mean that it explains how pregnancy is prevented in real life situations. In this respect, ovulation inhibition can explain by itself how pregnancy is prevented whereas abnormal expression of a given molecule in the endometrium lacks that strength until it is shown that its normal expression is essential for pregnancy to occur.

It is now well recognized that one of the complexities that researchers have to deal with to find a thorough answer is that the mechanism may differ for the same EC treatment depending upon when it is given relative to time of intercourse and also relative to time of ovulation. A single act of intercourse that takes place up to 5 days before ovulation may result in pregnancy in the human. Therefore, many women who request EC receive the treatment before ovulation and possibly before fertilization if ovulation has occurred. Neither the minimum length of time from coitus to fertilization, when the oocyte is waiting for the sperm, nor the shortest interval from ovulation to fertilization, when the sperm is waiting for the oocyte, have been determined in the human. Therefore, the exact theoretical amplitude of the window for acting before fertilization is undetermined, less so the actual window in real cases.

The contraceptive effectiveness of LNG and the Yuzpe regimen has been shown to depend on the intercourse-treatment interval (the easy one to obtain), whereas there is no data for the ovulation-treatment interval (the difficult one to obtain). Given that in 15–25% of the cycles treated with EC, the expected pregnancy is not prevented, chances are that there is a specific window in the cycle in which treatment is more likely to fail. Attempts to pinpoint the stage of the menstrual cycle at which treatment is given to women requesting EC for subsequent correlation with the contraceptive outcome may shed some light on the mode of action of a particular method. Admittedly, even at a research center, it is difficult to get informed consent for such a study given the anxiety that surrounds every case. Since the intercourse-treatment and ovulation-treatment intervals are inter-related, should information about both become available, complex analyses will be needed to estimate how each one relates to the contraceptive outcome.

Most mechanistic studies have attempted to assess to what extent ovulation inhibition is involved. However, none has used ultrasound to confirm follicular rupture and to pinpoint at what stage of follicular development

treatment was given. It is clear that EC appears to prevent ovulation in many cases but not so clear what the conditions are in terms of timing of treatment relative to the stage of follicular development. The criteria used to time treatment lacks this precision in practically all studies reviewed. Because ultrasound has not been used, the occurrence of ovulatory dysfunctions, such as luteinized unruptured follicle, has not been determined.

Both logistic and ethical constraints prevent designing and performing experiments that can directly address what in fact happens to the crucial biologic entities -sperm, oocyte, zygote or preimplantation embryo- in the genital tract of women who receive EC in comparison to those who receive placebo. The fate of spermatozoa and of the oocyte can be studied without risking the occurrence of conception if either one is absent from the genital tract. It is easy to avoid the presence of sperm for this purpose, without altering the biologic environment. In order to suppress the presence of the oocyte, one could inhibit ovulation using a GnRH-antagonist and give appropriate sex steroid replacement therapy to provide a “normal environment for sperm”. The effect of EC treatment on sperm could then be studied at centers where retrieval of spermatozoa from the site of fertilization is feasible. In fact, with the exception of Kessler et al. [89], no other study has focused on the effects of EC upon spermatozoa.

Alterations in embryo transport through the fallopian tube or uterus following EC, are also difficult to explore. Delayed transport or retention in the tube cannot be excluded a priori, although no increased incidence of tubal pregnancy has hitherto been reported with the current methods. Accelerated transport through the tube appears unlikely since neither estradiol nor progesterone given in high doses right after ovulation have this effect in women [102]. Expulsion of the egg from the uterus could result from myometrial effects of wide steroid oscillations.

Several studies have focused attention on alterations of the endocrine profile during the luteal phase. Luteal insufficiency, caused by EC, cannot be claimed to contribute to pregnancy prevention until it is shown to persist through a hCG challenge test.

The most difficult parameter to assess with certainty is endometrial receptivity. Endometrial markers of receptivity have been established so far with certainty only in rodents. Even if endometrial receptivity is shown to be altered by EC, other steps that precede implantation may also be altered enough to interrupt the process at an earlier stage.

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