The Postovulatory Mechanism of Action of Plan B

A Review of the Scientific Literature

Rebecca Peck, MD, and Rev. Juan R. Vélez, MD

Abstract. Levonorgestrel is widely used as emergency contraception, yet much confusion surrounds its use. Consensus statements and reviews typically attribute its efficacy to prefertilization mechanisms of action (MOAs), such as suppression of ovulation and interference with cervical mucus or sperm function, yet studies do not rule out a postovulatory MOA. To yield greater clarity, the authors review recent scientific studies examining the MOAs of LNG-EC. They conclude that LNG-EC exerts minimal effects on cervical mucus and sperm function and that suppression of ovulation is not the dominant MOA accounting for the contraceptive efficacy of LNG-EC. Luteal deficiencies and endometrial changes reported in the literature strongly suggest a postovulatory MOA when LNG-EC is given during the critical preovulatory (or fertile) period. *National Catholic Bioethics Quarterly* 13.4 (Winter 2013): 000–000.

The question is, does the emergency contraceptive levonorgestrel (LNG-EC) prevent pregnancy by suppressing ovulation or by other prefertilization means (e.g., impairment of cervical mucus or inhibition of sperm function), or does it have an abortifacient effect? This paper reviews the evidence to date on the mechanism of action (MOA) of LNG-EC.

Rebecca Peck, MD, is a family physician and clinical assistant professor at the Florida State University College of Medicine. Rev. Juan R. Vélez, MD, PhD (Theology), is a Roman Catholic priest and former board-certified internist in Berkeley, California. The authors can be reached at rbamer2@yahoo.com and jrvg98@gmail.com, respectively. The authors thank Patrick Yeung, MD, Bruno Mozzanega, MD, Chris Kahlenborn, MD, Walter Rella, MD, and philosophy professors Thomas Cavanaugh and Christopher Tollefsen for helpful comments as well as Rev. Deacon Thomas J. Davis Jr., JD, LLM, MA (Theology), for his comments and editorial assistance.

Levonorgestrel is a widely used emergency contraceptive.[1] In the United States, it is generally known by a trade name—Plan B One-Step or Next Choice One Dose. In these forms, LNG-EC is now taken as a single 1.5 mg dose within one hundred twenty hours of unprotected intercourse, although it is most efficacious when taken within seventy-two hours.[2] Prescribing information for Plan B One-Step from the US Food and Drug Administration states that the drug is "believed to act as an emergency contraceptive principally by preventing ovulation or fertilization (by altering tubal transport of sperm and/or ova). In addition, it may inhibit implantation (by altering the endometrium)."[3]

LNG-EC is to be taken after unprotected intercourse or contraceptive failure irrespective of when in the cycle intercourse occurred. However, in a woman's monthly cycle there is a fertile window of only six days during which the ovum may be fertilized.[4] Sperm can live up to five days in the cervical crypts or the fallopian tubes, waiting to fertilize the ovum when released.[5] Since the ovum can survive just one day after ovulation, the fertile window thus comprises the five days before ovulation and the day of ovulation (day -5 through day 0). Sperm survival may be affected by changes in the woman's cervical mucus, hormone levels, and endometrial environment. To be able to fertilize the ovum when released, sperm must also undergo capacitation, sperm hyperactivation, and the acrosome reaction.[6]

---

[1] Task Force on Postovulatory Methods of Fertility Regulation, "Randomized Controlled Trial of Levonorgestrel versus the Yuzpe Regimen of Combined Oral Contraceptives for Emergency Contraception," *Lancet* 352.9126 (August 8, 1998): 428–433.

[2] L. Cheng, Y. Che, and A. M. Gülmezoglu, "Interventions for Emergency Contraception," *Cochrane Database of Systematic Reviews 2012*, no. 8 (August 15, 2012): CD001324; and H. von Hertzen et al., "Low Dose Mifepristone and Two Regimens of Levonorgestrel for Emergency Contraception: A WHO Multicentre Randomised Trial," *Lancet* 360.9348 (December 7, 2002): 1803.

[3] US Food and Drug Administration, Prescribing Information for Plan B, July 2009, http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/021998lbl.pdf.

[4] D. B. Dunson et al., "Day Specific Probabilities of Clinical Pregnancy Based on Two Studies with Imperfect Measures of Ovulation," *Human Reproduction* 14.7 (July 1999): 1835–1839; and A. J. Wilcox, C. R. Weinberg, and D. D. Baird, "Timing of Sexual Intercourse in Relation to Ovulation: Effects of the Probability of Conception, Survival of the Pregnancy, and Sex of the Baby," *New England Journal of Medicine* 333.23 (December 7, 1995): 1517–1521.

[5] Wilcox et al.,"Timing of Sexual Intercourse," 1520.

[6] S. S. Suarez and A. A. Pacey, "Sperm Transport in the Female Reproductive Tract," *Human Reproduction Update* 12.1 (January–February 2006): 23–37; and A. Hermanny et al., "In Vitro Assessment of Some Sperm Function following Exposure to Levonorgestrel in Human Fallopian Tubes," *Reproductive Biology and Endocrinology* 10 (January 30, 2012): art. 8, http://www.rbej.com/content/10/1/8. Sperm capacitation occurs in the female genital tract, producing structural and functional changes in the sperm that make them capable of fertilization. Hyperactivation causes them to swim faster and more forcefully. The acrosome reaction, which occurs as sperm approach the ovum, consists of changes at the head that make the sperm able to penetrate the tough outer layer of the ovum during fertilization.

> Scientists have proposed that LNG-EC may work in a number of ways:[7] (1) by affecting cervical mucus or sperm function; (2) by preventing sperm–ovum binding, thus preventing fertilization; (3) by delaying or inhibiting ovulation; (4) by impairing formation of the corpus luteum, which is essential for adequate progesterone support of the endometrium; and (5) by decreasing endometrial receptivity, thwarting the embryo's implantation.

The majority of studies we reviewed conclude that LNG-EC works by a preovulatory MOA and does not have postfertilization effects. However, these conclusions conflict with many of the actual findings of the studies. We now turn to the scientific evidence for each of the possible MOAs mentioned above.

First Proposed MOA: Cervical Mucus, Sperm Transport and Sperm Capacitance

Cervical Mucus

Cervical mucus is critical for sperm survival.[8] Levonorgestrel used as emergency contraception was originally believed to prevent fertilization by the impairment of cervical mucus, on the basis of data obtained from a study of continuous oral progestin-only pills.[9] In women using *long-term* progestin-only contraceptives (pills or the LNG-secreting intrauterine device), cervical mucus is of diminished quality and is inhospitable to sperm; this is one of the main mechanisms by which these agents exert their antifertility action.[10] However, comparing the long-term effects of LNG to the effects of one-time administration of LNG-EC is not valid, for several reasons. First, the LNG-secreting IUD (20 mcg continuous daily secretion) provides a very high local *uterine* drug concentration, compared with a one-time *oral* dose of LNG-EC (1.5 mg). Research shows that "the endometrial tissue concentration of LNG is approximately one hundred times higher in IUD-releasing LNG than in a single dose of LNG-EC by either the oral or vaginal route."[11] Second, multiple studies

---

[7] K. Gemzell-Danielsson, C. Berger, and P. G. L. Lalitkumar, "Emergency Contraception—Mechanisms of Action," *Contraception* 87.3 (March 2013): 300–308; and K. Gemzell-Danielsson, "Mechanism of Action of Emergency Contraception," *Contraception* 82.5 (November 2010): 404–409. Two other possible postfertilization mechanisms of action have been described in the literature—(1) altered fallopian tube transport of the zygote, and (2) altered pH environment of the fallopian tubes and uterus—but available evidence for them is limited and they will not be reviewed in this paper.

[8] E. Odeblad, "Cervical Factors," in *Female Infertility*, ed. P. J. Keller (Basel: Karger, 1978), 132–142.

[9] K. S. Moghissi, F. N. Syner, and L. C. McBride, "Contraceptive Mechanism of Microdose Norethindrone," *Obstetrics and Gynecology* 41.4 (April 1973): 585–594.

[10] D. Africander, N. Verhoog, and J. P. Hapgood, "Molecular Mechanisms of Steroid Receptor-Mediated Actions by Synthetic Progestins Used in HRT and Contraception," *Steroids* 76.7 (June 2011): 636–652; and R. A. Lewis et al., "Effects of the Levonorgestrel-Releasing Intrauterine System on Cervical Mucus Quality and Sperm Penetrability," *Contraception* 82.6 (December 2010): 491–496.

[11] W. A. Palomino, P. Kohen, and L. Devoto, "A Single Midcycle Dose of Levonorgestrel Similar to Emergency Contraceptive Does Not Alter the Expression of the L-Selectin Ligand

show an effect on cervical mucus with use of daily continuous progestin-only pills or an LNG intrauterine system that explains their efficacy in reducing pregnancy risk,[12] but the one-time postcoital use of oral LNG as an emergency contraceptive is a very different scenario.

What does the research show on this subject? An in vivo study by Josiane do Nascimento and colleagues shows no impairment in the quality of cervical mucus after administration of LNG-EC: "Viable spermatozoa were found in the genital tract thirty-six to sixty hours after coitus and twenty-four to forty-eight hours after LNG administration."[13] These findings expressly contradict those of a much older study, by Estaban Kesserü et al., which reported impaired cervical mucus and reduced numbers of sperm measured between three and ten hours after a related compound was given (d-norgestrel, 400 mcg).[14] In a major review of emergency contraceptives, Kristina Gemzell-Danielsson and colleagues note that "the observations described by Kesserü et al. of LNG effects on cervical and intrauterine mucus are probably of importance when LNG is used as a regular contraceptive but unlikely to be the main mechanism of action of LNG used for [emergency contraception], since sperm can be retrieved from the fallopian tube within 5 min after insemination of semen in the vagina."[15]

In a large study of LNG-EC, Gabriela Noé and colleagues propose this MOA to explain the efficacy of LNG-EC in the face of their finding of an extraordinarily high ovulation rate among their subjects. However, their reference to "increased cervical mucus viscosity caused by LNG," which they assert "impedes the migration of sperm,"[16] is not based on data derived from their study but relies solely on the

---

or Molecular Markers of Endometrial Receptivity," *Fertility and Sterility* 94.5 (October 2010): 1592. This is important because several researchers will hypothesize that oral LNG-EC will increase glycodelin-A (which is thought to impair sperm fertilization of ova) since it is well known that the LNG-IUS (IUD) increases glycodelin-A as one of its contraceptive mechanisms of action. It is this higher endometrial exposure that may trigger this specific effect for the IUD and will be shown not to trigger this MOA for oral LNG-EC.

[12] M. F. Natavio et al., "Temporal Changes in Cervical Mucus after Insertion of the Levonorgestrel-Releasing Intrauterine System," *Contraception* 87.4 (April, 2013): 426–431; and X. F. Li, G. C. Davies, and J. Newton, "A Review of the Effects of Long-Acting Progestin-Only Contraceptives on Ovarian Activity," *Advances in Contraception* 8.1 (March 1992): 1–19.

[13] J. A. do Nascimento et al., "In Vivo Assessment of the Human Sperm Acrosome Reaction and the Expression of Glycodelin-A in Human Endometrium after Levonorgestrel-Emergency Contraceptive Pill Administration," *Human Reproduction* 22.8 (August 2007): 2190.

[14] E. Kesserü et al., "The Hormonal and Peripheral Effects of d-Norgestrel in Postcoital Contraception," *Contraception* 10.4 (October 1974): 417. This study did not report on the cervical mucus or sperm effects of d-norgestrel beyond ten hours but did observe that altered intrauterine pH persisted for forty-eight or more hours after drug intake.

[15] Gemzell-Danielsson et al., "Emergency Contraception," 302, referring to observations reported in E. Kesserü et al., "In Vitro Action of Progestogens on Sperm Migration in Human Cervical Mucus," *Fertility and Sterility* 26.1 (January 1975): 57–61.

[16] G. Noé et al., "Contraceptive Efficacy of Emergency Contraception with Levonorgestrel Given Before or After Ovulation," *Contraception* 81.5 (May 2010): 419–420; hereafter, Noe et al., "Contraceptive Efficacy" 2010.

dated Kesserü findings—the same ones powerfully contradicted by do Nascimento's more modern study. Strikingly, although the Noé study was originally published in 2010, it makes no mention of the 2007 do Nascimento study, which provides the best evidence on the topic. In a 2011 study based on the same data as their 2010 study, Noé et al. acknowledge do Nascimento's work but suggest that Kesserü's findings still account for a "transient" MOA which is "more pronounced in the 12 [hours] following LNG intake but vanishes after 24 to 48 [hours]."[17]

If we consider that LNG-EC is only "needed" to prevent conception in the late follicular phase (i.e., during the fertile window), any effect on cervical mucus would exert very little influence on sperm survival, as it is likely that sperm would already have reached the fallopian tubes.

Sperm Mobility

Fertilization requires sperm movement into the fallopian tubes. This occurs in two stages: first, some spermatozoa are "aided by propulsive contractions of the genital tract to the fallopian tube"; second, "over a period of several days, spermatozoa that have been stored in the uterine cervix migrate in successive cohorts to the fallopian tube."[18] Kesserü and others have proposed that findings related to "interference with sperm migration … could play a role in the contraceptive mechanism" of postcoital emergency contraception.[19] Several studies have shown that sperm can be retrieved from the fallopian tubes within five minutes to two hours after insemination in the vagina,[20] and it is known that sperm can survive in the fallopian tubes for up to five days.[21]

Georg Kunz and colleagues show that during the late follicular phase, uterine contractions direct sperm from the cervix into the fallopian tube on the same side as the dominant follicle—that is, on the side of the follicle that will release an ovum.

---

[17] G. Noé et al., "Contraceptive Efficacy of Emergency Contraception with Levonorgestrel Given Before or After Ovulation," *Contraception* 84.5 (November 2011): 491; hereafter, Noé et al., "Contraceptive Efficacy" 2011.

[18] K. S. Brito et al., "The In Vitro Effect of Emergency Contraception Doses of Levonorgestrel on the Acrosome Reaction of Human Spermatozoa," *Contraception* 72.3 (September 2005): 225–228.

[19] Kesserü et al., "Hormonal and Peripheral Effects," 422.

[20] The following studies confirm that sperm reach the fallopian tubes in minutes to hours: E. Kesserü et al., "Hormonal and Peripheral Effects"; G. Kunz et al., "The Dynamics of Rapid Sperm Transport through the Female Genital Tract: Evidence from Vaginal Sonography of Uterine Peristalsis and Hystero-salpingoscintigraphy," *Human Reproduction* 11.3 (March 1996): 627–632; M. E. Ortiz and H. B. Croxatto, "Copper-T Intrauterine Device and Levonorgestrel Intrauterine System: Biological Bases of Their Mechanism of Action," *Contraception* 75.6 suppl. (June 2007): S16–S30; D. S. F. Settlage, M. Motoshima, and D. R. Tredway, "Sperm Transport from the External Cervical Os to the Fallopian Tubes in Women: A Time and Quantitation Study," *Fertility and Sterility* 24.9 (September 1973): 655–661; and M. Ahlgren, "Sperm Transport to and Survival in the Human Fallopian Tube," *Gynecologic Investigation* 6.3–4 (1975): 206–214.

[21] Wilcox et al., "Timing of Sexual Intercourse," 1520.

They note that "these data indicate that rapid transport of the spermatozoa through the female genital tract is under the endocrine control of the dominant follicle, ensuring the preferential accumulation of spermatozoa at the site of fertilization."[22] If sperm can arrive in the fallopian tubes in minutes, then the action of emergency contraception (even if taken within twenty-four hours) would occur too late to affect this phenomenon, even if it had an effect on subsequent waves of sperm migration.[23]

What does the research show? In 1974, Kesserü et al. showed that after a single dose of d-norgestrel, there was a rapid decrease of spermatozoa in the uterus—an observation still used by others to suggest that the phenomenon explains LNG-EC efficacy.[24] However, the recent in vitro and in vivo studies we reviewed report no significant effect of LNG-EC on sperm functions at doses that would actually be achieved in vivo.[25] The in vivo study by do Nascimento et al. shows that it is possible to recover an adequate number of viable and motile human spermatozoa from both the cervix and the uterine cavity thirty-six to sixty hours after coitus in women who were treated with LNG-EC within twelve to thirty-six hours after coitus.[26]

---

[22] Kunz et al., "Dynamics of Rapid Sperm Transport," 627.

[23] LNG did not affect numbers of motile spermatozoa or their adhesion to the fallopian tubes after LNG exposure, thus indicating that these rapid sperm remain viable adhered to the tubal epithelium. Hermanny et al., "In Vitro Asessment of Some Sperm Function," 1. See also W. S. Yeung et al., "Effects of Glycodelins on Functional Competence of Spermatozoa," *Journal of Reproductive Immunology* 83.1–2 (December 2009): 26–30.

[24] Kesserü et al., "Hormonal and Peripheral Effects," 411–424. See also D. Hapangama, A. F. Glasier, and D. T. Baird, "The Effects of Peri-ovulatory Administration of Levonorgestrel on the Menstrual Cycle," *Contraception* 63.3 (March 2001): 123–129; A. Tirelli, A. Cagnacci, and A. Volpe, "Levonorgestrel Administration in Emergency Contraception: Bleeding Pattern and Pituitary-Ovarian Function," *Contraception* 77.5 (May 2008): 328–332; Noé et al., "Contraceptive Efficacy" 2011, 491; and F. Davidoff and J. Trussell, "Plan B and the Politics of Doubt," *JAMA* 296.14 (October 11, 2006): 1776.

[25] The studies showing no LNG-EC effect on sperm at doses found in vivo are do Nascimento et al., "In Vivo Assessment of the Human Sperm Acrosome Reaction"; W. S. Yeung et al., "The Effects of Levonorgestrel on Various Sperm Functions," *Contraception* 66.6 (December 2002): 453–457; and Hermanny et al., "In Vitro Assessment of Some Sperm Function."

[26] Do Nascimento et al., "In Vivo Assessment of the Human Sperm Acrosome Reaction," 2194. It should be noted that the do Nascimento study was a double-blind placebo-controlled study, which is considered one of the strongest types of studies in an evidence-based review. Kesserü et al. hypothesized that increased alkalinization correlating with LNG use, beginning four to five hours after intake and remaining constant for at least forty-eight hours, explained his finding of interference with sperm migration. ("Hormonal and Peripheral Effects," 422). If alkalinization affected sperm quality or concentration and remained constant for forty-eight hours, then this effect should have been discovered by do Nascimento when measured at twenty-four hours. Do Nascimento's contrary findings strongly debunk Kesserü's pH-related migration theory. See Thomas J. Davis Jr., letter, *National Catholic Bioethics Quarterly* 10.4 (Winter 2010): 641–643; and Allison LeDoux and Kathleen M. Raviele, letters, *National Catholic Bioethics Quarterly* 11.1 (Spring 2011): 11–15.

The in vitro studies we reviewed do not show effects on sperm mobility or other functions at doses found in vivo.[27] In 2012, Alexia Hermanny et al. examined the effects of LNG on sperm in vitro by perfusing human fallopian tubes with suspensions of spermatozoa that did or did not contain LNG. The results show no effect of LNG "at a similar dose to that observed in serum following oral intake for EC … on the number of motile spermatozoa recovered from the human fallopian tubes in vitro, on their adhesion to the tubal epithelium, distribution, or [acrosome reaction] rate."[28] Hermanny et al. note that "the LNG concentration in uterine flushing after oral intake was less than 2% of that found in serum, and concentrations at the tubal lumen are probably similar."[29] Do Nascimento's data on in vivo LNG concentration are nearly identical.[30] This indicates that many of the in vitro studies actually exposed sperm to much higher concentrations of LNG than would be expected in vivo.

Sperm Capacitance and the Acrosome Reaction

Sperm capacitance and the acrosome reaction are essential processes that ready the sperm cell for fusion with the hard outer membrane of the ovum. Capacitance is triggered by the release of endogenous progesterone and possibly other triggering substances that are present in the follicular fluid.[31]

---

[27] Yeung et al., "Effects of Levonorgestrel"; and Hermanny et al., "In Vitro Assessment of Some Sperm Function."

[28] Hermanny et al., "In Vitro Assessment of Some Sperm Function," 6. This study used a dose of LNG in vitro similar to what would be observed in serum following the 1.5 mg oral dose. For example, do Nascimento et al. report that the mean serum dose of LNG-EC achieved twenty-four hours after the 1.5 mg oral dose was 3462.9 pg/ml, yet the uterine flushing amount was only 47.9 pg/ml. Do Nascimento et al., "In Vivo Assessment of the Human Sperm Acrosome Reaction," 2193. This is important for two reasons. First, since do Nascimento was an in vivo study, the actual oral administration of the drug was able to be assessed via uterine flushing. Second, it raises the question whether the doses to which the sperm were exposed in vitro in other studies were actually too high. For example, Yeung et al. (who did not find any effect on sperm motility but did find an effect on sperm function at the highest drug exposure) used relatively high concentrations (1 ng/ml, 10 ng/ml, 100 ng/ml); even their lowest concentration (1 ng/ml) was orders of magnitude higher than what sperm would actually experience in vivo in the uterine environment. Yeung et al., "Effects of Levonorgestrel," 457.

[29] Hermanny et al., "In Vitro Assessment of Some Sperm Function," 6.

[30] "The LNG in uterine flushing medium represented 1.38% of the values observed in serum 24 [hours] after the LNG intake." Do Nascimento et al., "In Vivo Assessment of the Human Sperm Acrosome Reaction," 2190.

[31] Progesterone in follicular fluid strongly stimulates capacitance. There may also be different hormonal or secreting signals. M. J. Munuce, "In Vitro Effect of Levonorgestrel on Sperm Fertilizing Capacity and Mouse Embryo Development," *Contraception* 72.1 (July 2005): 71–76; and T. T. Sun, C. M. Chung, and H. C. Chan, "Acrosome Reaction in the Cumulus Oophorus Revisited: Involvement of a Novel Sperm-Released Factor NYD-SP8," *Protein Cell* 2.2 (February 2011): 92–98. This initially led researchers to believe that LNG-EC as a progestin could mimic the effects of progesterone on the sperm receptors and activate the

It was originally hypothesized that if LNG were able to induce a premature acrosome reaction, then spermatozoa would be unable to fertilize ova.[32] Previous research showing "that human acrosome-reacted spermatozoa do not further bind to the [zona pellucida] suggests that by increasing the number of acrosome-reacted cells, LNG may decrease sperm fertilizing capacity."[33] It was also found that "those spermatozoa that complete [the acrosome reaction] precociously are unable to penetrate the zona pellucida because they lose enzymatic content."[34]

What do the actual data show? Six recent studies address the question of whether LNG-EC administration can hinder sperm functions or fertilizing capacity.[35] None of these studies show that LNG-EC triggered the acrosome reaction after spermatozoa were exposed to it at doses similar to those found in vivo. The most recent of these studies, from 2012, confirms what others showed previously: that LNG-EC at relevant doses "failed to show any effect … on [the acrosome reaction] in spermatozoa in vitro or in spermatozoa recovered from the uterus."[36]

In summary, the totality of scientific evidence shows that LNG-EC has little or no effect on cervical mucus or sperm functions. Its effects on these processes cannot explain its effectiveness in reducing pregnancy risk. Nevertheless, many contraceptive experts persist in asserting the existence of these effects,[37] despite

acrosome reaction. However, LNG-EC is a weak agonist on sperm progesterone receptors. Hermanny et al., "In Vitro Assessment of Some Sperm Function," 1; Brito et al., "In Vitro Effect of Emergency Contraception Doses," 225. Thus, the research shows no ability of the drug to induce the acrosome reaction at usual doses found in vivo.

[32] Munuce, "In Vitro Effect of Levonorgestrel," 72.

[33] L. Bahomondes et al., "The In Vitro Effect of Levonorgestrel on the Acrosome Reaction of Human Spermatozoa from Fertile Men," *Contraception* 68.1 (July 2003): 55–59.

[34] Hermanny et al., "In Vitro Assessment of Some Sperm Function," 2.

[35] Hermanny et al., "In Vitro Assessment of Some Sperm Function"; Brito et al., "In Vitro Effect of Emergency Contraception Doses"; Bahamondes et al., "In Vitro Effect of Levonorgestrel"; do Nascimento et al., "In Vivo Assessment of the Human Sperm Acrosome Reaction"; Yeung et al., "Effects of Levonorgestrel"; and Munuce, "In Vitro Effect of Levonorgestrel."

[36] Hermanny et al., "In Vitro Assessment of Some Sperm Function," 7.

[37] For example, Davidoff and Trussell assert that "levonorgestrel-induced interference with sperm entry into and migration through the uterine cavity develops too late to prevent the first wave of sperm migration, which begins in minutes; that delay is not relevant to Plan B's contraceptive activity, however, since sperm in the first wave are not yet capacitated and hence are not capable of fertilizing ova. However, when Plan B is taken immediately after sexual intercourse, these effects on the cervical and uterine environment can prevent later waves of migration of capacitated sperm, which begin about 10 hours after intercourse and continue for several days. Conversely, the loss of Plan B's contraceptive effectiveness with delay in use can be explained at least in part by the arrival of capacitated sperm in the fallopian tube before the drug has had a chance to assert its effects on sperm migration." Davidoff and Trussell, "Plan B and the Politics of Doubt," 1776. Significantly, these authors cite two sources for their claims about migration, both of which refer in turn to the Kesserü study: H. B. Croxatto, "Emergency Contraception Pills: How Do They Work?" *IPPF*

the fact that the results of *all* the studies since Kesserü in 1974 show the opposite.[38] Specifically, sperm migration, capacitation, and the ability to undergo the acrosome reaction were not found to be affected by LNG-EC administration at doses found in vivo. Although the first wave of sperm reaching the fallopian tubes in minutes still requires capacitation, there is no reason—based on the findings of the studies reviewed here—to suspect that these sperm may not achieve capacitation and subsequent fertilization after administration of LNG-EC.

Moreover, since Kesserü et al. studied changes in cervical mucus and sperm function up to only ten hours after d-norgestrel administration, it seems conjectural at best to state that the drug could affect subsequent waves of sperm for several days.[39] Most importantly, in 2007, do Nascimento et al. provided evidence that LNG-EC did not impede cervical mucus or sperm function, and these researchers studied the drug effects for up to forty-eight hours after LNG administration. As far back as 2004, well-vested LNG-EC researchers concluded that the drug was unlikely to have effects on sperm function.[40] In a recent review on emergency contraceptives, these same researchers acknowledge that "LNG does not influence sperm acrosome reaction. It inhibits spermatozoa–oocyte fusion as well as decreases the curvilinear velocity of spermatozoa *only at high concentration*, and the contribution of these effects to [emergency contraception] is unlikely to be significant."[41]

Second Proposed MOA: Prevention of Sperm–Egg Binding

Another proposed prefertilization MOA is impairment of sperm–egg binding. This theory is based on the observation of inappropriately high expression of glycodelin-A by sustained delivery of LNG in users of LNG intrauterine systems and subdermal implants.[42]

In 2005, Marta Durand and colleagues postulated that increased levels of glycodelin-A expression in serum and human endometrium may result from peri-

Medical Bulletin 36 (2002): 2, appendix 1–1; and K. Gemzell-Danielsson and L. Marions, "Mechanisms of Action of Mifepristone and Levonorgestrel When Used for Emergency Contraception," *Human Reproduction Update* 10.4 (July–August 2004): 342.

[38] Do Nascimento et al., "In Vivo Assessment of the Human Sperm Acrosome Reaction"; Munuce, "In Vitro Effect of Levonorgestrel"; Brito et al., "In Vitro Effect of Emergency Contraception Doses"; Bahomondes et al., "In Vitro Effect of Levonorgestrel"; Hermanny et al., "In Vitro Assessment of Some Sperm Function"; and Yeung et al., "Effects of Levonorgestrel."

[39] Davidoff and Trussell, "Plan B and the Politics of Doubt," 1776.

[40] Gemzell-Danielsson and Marions, "Mechanisms of Action of Mifepristone and Levonorgestrel," 342.

[41] Gemzell-Danielsson, "Emergency Contraception," 302, emphasis added.

[42] M. Durand et al., "Late Follicular Phase Administration of Levonorgestrel as an Emergency Contraceptive Changes the Secretory Pattern of Glycodelin in Serum and Endometrium during the Luteal Phase of the Menstrual Cycle," *Contraception* 71.6 (June 2005): 451–457. Hereafter, Durand et al., "Late Follicular Phase Administration," 2005.

ovulatory administration of LNG-EC, leading to impairment of sperm–egg binding and thus decreased fertilization.[43] They measured both serum and endometrial glycodelin-A levels before, during, and after the LH (luteinizing hormone) surge. The mean serum glycodelin-A "from day LH +1 to LH +7 … was significantly higher in LNG-treated cycles in the subjects of Group 1," who received two 0.75 mg doses between day LH -4 and LH -2, whereas "no differences were found between control and treatment cycles in Group 2," who received the first dose at the time of the LH rise.[44] However, while characterizing the glycodelin-A finding in group 1 as significantly higher than the control, the authors acknowledge that the finding could not support their theory that it interfered with sperm–egg binding, because "the concentration required for significant inhibition of sperm–egg binding is about 25 μg/ml, that is, several orders of magnitude higher than the levels we found in serum in this study."[45]

Durand et al., having already shown that in vivo glycodelin-A serum levels were far below those needed for inhibition of binding, also acknowledged the questionable basis for the proposed MOA, given results of earlier studies showing that glycodelin-A could be displaced by corona cells surrounding the oocyte.[46] And in their 2010 study, they cite research by Philip Chiu et al. which shows that glycodelin-A is displaced from sperm when the sperm pass through the oocyte–cumulus cell complex, resulting in *enhanced* sperm–egg binding.[47] Given their 2005 glycodelin-A finding, the lack of confirmatory data in the do Nascimento and Wilder Palomino studies, and the Chiu finding of enhanced sperm–egg binding in vivo, this proposed prefertilization MOA, the prevention of sperm-egg binding, is not realistic.[48]

---

[43] "Glycodelin-A is a major secretory progesterone-regulated glycoprotein of the human endometrium. During the normal peri-ovulatory phase, glycodelin-A is absent from the endometrium, and it becomes highly expressed during the last week of the luteal phase only. The temporal expression is significant because glycodelin-A was thought to be a potent inhibitor of sperm–zona binding. Through its inhibitory activity on the immune cells, glycodelin-A is also believed to play a role in feto-maternal defense mechanisms." Ibid., 451–452.

[44] Ibid., 452, 454.

[45] Ibid., 455.

[46] Ibid., 456, citing J. Y. Tse et al., "The Synthesis and Fate of Glycodelin in Human Ovary during Folliculogenesis," *Molecular Human Reproduction* 8.2 (February 2002): 142–148.

[47] P. C. Chiu et al., "Cumulus Oophorus-Associated Glycodelin-C Displaces Sperm-Bound Glycodelin-A and -F and Stimulates Spermatozoa–Zona Pellucida Binding," *Journal of Biological Chemistry* 282.8 (February 23, 2007): 5378–5388, cited in M. Durand et al., "Hormonal Evaluation and Midcycle Detection of Intrauterine Glycodelin in Women Treated with Levonorgestrel as in Emergency Contraception," *Contraception* 82.6 (December 2010): 532 (hereafter, Durand et al., "Hormonal Evaluation and Midcycle Detection," 2010).

[48] Do Nascimento et al. studied glycodelin-A expression, noting that the 2005 Durand study showed an effect when LNG-EC was administered before the LH surge. Do Nascimento et al. used uterine flushings, similar to Durand in 2010. They found no effect of LNG on uterine glycodelin levels. Palomino et al. found no glycodelin-A effect from LNG-EC administered at time of the LH surge. Do Nascimento et al., "In Vivo Assessment of the

Third Proposed MOA: The Ability of Levonorgestrel Emergency Contraception to Prevent or Delay Ovulation

In October 2008, the International Consortium for Emergency Contraception (ICEC) and the International Federation of Gynecology and Obstetrics (FIGO) issued an influential joint statement asserting that, when taken before ovulation, LNG-EC inhibits the LH surge, thereby retarding follicular development and thus preventing or delaying ovulation. The statement maintains that this is "the primary and possibly the only mechanism of action."[49] The statement cites seven scientific papers in support of that claim, the first six of which are original research studies.[50] The statement then goes further, concluding that "review of the evidence suggests that LNG [emergency contraceptive pills] cannot prevent implantation."[51] We now critically examine the original research source studies cited by the statement in support of its conclusion.

In undertaking this review, it is important to understand how scientists measure ovulation. Currently, the gold standard for observing ovulation is a transvaginal ultrasound (TVUS) capturing follicular rupture. Because the ovum is too tiny to be visualized on TVUS, follicular rupture is measured instead by tracking the dominant

---

Human Sperm Acrosome Reaction," 2194; and Palomino et al., "A Single Midcycle Dose of Levonorgestrel," 1592–1593.

[49] International Consortium for Emergency Contraception (ICEC) and International Federation of Gynecology and Obstetrics (FIGO), "Statement on Mechanism of Action," October 2008, 1, http://www.figo.org/files/figo-corp/International%20consortium%20for%20emergency%20contraception%20statement.pdf. The statement was subsequently updated in March 2011 and March 2012.

[50] The seven cited studies are D. Hapangama, A. F. Glasier, and D. T. Baird, "The Effects of Peri-ovulatory Administration of Levonorgestrel on the Menstrual Cycle," *Contraception* 63.3 (March 2001): 123–129; L. Marions et al., "Emergency Contraception with Mifepristone and Levonorgestrel: Mechanism of Action," *Obstetrics and Gynecology* 100.1 (July 2002): 65–71; L. Marions et al., "Effect of Emergency Contraception with Levonorgestrel or Mifepristone on Ovarian Function," *Contraception* 69.5 (May 2004): 373–377; M. Durand et al., "On the Mechanisms of Action of Short-Term Levonorgestrel Administration in Emergency Contraception," *Contraception* 64.4 (October 2001): 227–234 (hereafter, Durand et al. "On the Mechanisms of Action," 2001); H. B. Croxatto et al., "Pituitary–Ovarian Function following the Standard Levonorgestrel Emergency Contraceptive Dose or a Single 0.75-mg Dose Given on the Days Preceding Ovulation," *Contraception* 70 (2004): 442–450; I. A. Okewole et al., "Effect of Single Administration of Levonorgestrel on the Menstrual Cycle," *Contraception* 75.5 (May 2007): 372–377; and H. B. Croxatto et al., "Mechanism of Action of Hormonal Preparations used for Emergency Contraception: A Review of the Literature," *Contraception* 63.3 (March 2001): 111–121. The first six original research studies are assessed in this paper.

[51] In the 2008 ICEC/FIGO statement, the principal authority cited for the claim that LNG-EC cannot prevent implantation is N. Novikova et al., "Effectiveness of Levonorgestrel Emergency Contraception Given Before or After Ovulation: A Pilot Study," *Contraception* 75.2 (February 2007): 112–118. In the updated ICEC/FIGO statement (2012), Noé et al., "Contraceptive Efficacy" 2011, is also cited in support of this claim.

follicle and noting from one day to the next when it collapses. Instead of detecting follicular rupture, other studies have relied on measurement of serum or urine hormonal levels of LH, estrogen, and progesterone to indicate when ovulation occurs. Early efficacy studies of emergency contraception relied on women's historical menstrual cycle estimations, which were often inaccurate.[52] With this background in mind, we now turn to the original research studies cited by ICEC/FIGO.

Studies by the International Consortium for Emergency Contraception and the International Federation of Gynecology and Obstetrics

ICEC/FIGO Study 1: Hapangama 2001

In 2001, Dharani Hapangama and colleagues showed that seven out of twelve healthy women (58 percent) who were given LNG-EC in the preovulatory period ovulated normally, as determined by urinary gonadotropin levels.[53] All exhibited significantly reduced luteal LH levels and a significantly shortened luteal phase.[54] ICEC/FIGO used this study to support its conclusion that LNG-EC prevents or delays ovulation, despite the fact that only five of the twelve women in the study showed this effect.[55] The Hapangama authors themselves noted that the shortened luteal phases could be caused by reduced total LH and might be contragestive.[56] That observation recognizes that luteal dysfunction can interfere with the transformation of the endometrium necessary for implantation of the embryo.[57] In fact, lower luteal LH levels and a shortened luteal phase are clinical evidence of effects on the endometrium that may have the potential to negatively affect implantation.[58]

---

[52] R. T. Mikolajczyk and J. B. Stanford, "A New Method for Estimating the Effectiveness of Emergency Contraception That Accounts for Variation in Timing of Ovulation and Previous Cycle Length," *Fertility and Sterility* 83.6 (June 2005): 1764–1770.

[53] Hapangama et al., "Effects of Peri-ovulatory Administration of Levonorgestrel."

[54] Ibid., 128.

[55] ICEC/FIGO statement 2008, 2.

[56] "Basal levels of LH are essential for the normal secretory function of the corpus luteum. In the mid-luteal phase, LH inhibition by the administration of GnRH antagonists consistently results in luteolysis in women as well as in non-human primates. There are no direct ways of measuring whether the function of the corpus luteum is compatible with the establishment of pregnancy. Although there was no significant difference in the urinary pregnanediol levels after LNG, it is possible that the shortened luteal phase observed was a consequence of reduced total LH and may have a contragestive effect." Hapangama et al., "Effects of Peri-ovulatory Administration of Levonorgestrel" 128.

[57] E. R. Norwitz, D. J. Schust, and S. J. Fisher, "Implantation and the Survival of the Early Pregnancy," *New England Journal of Medicine* 345.19 (November 2001): 1400–1408.

[58] There are two types of luteal phase defect: The first is a shortened luteal phase, which is an almost universal finding in these studies. The second is a progesterone-deficient luteal phase. Of these two well-described variants of luteal phase defect, the short luteal phase was described first: C. A. Strott et al., "The Short Luteal Phase," *Journal of Clinical Endocrinology and Metabolism* 30.2 (February 1970): 246. This was followed by the work of Michael Soules and others, who identified the progesterone-deficient luteal phase:

Furthermore, Hapangama et al. realized that "if LNG acts as an emergency contraceptive only by interfering with ovulation, the expected efficacy should fall below 42% (5 of 12 women)."[59] But other studies had previously reported 60 and 85 percent reductions in the expected number of pregnancies with LNG use. This means that the efficacy of LNG-EC reported in the Hapangama study must rely on an alternative MOA. Hapangama et al. state, "The discrepancy noted in the estimated effectiveness of LNG and the prevalence of ovulation delay or inhibition in our study may be due to mechanisms of action other than interference with ovulation."[60]

Of note, Hapangama et al. relied on urinary gonadotropin levels to document ovulation, instead of relying on follicular rupture as determined by TVUS. It is possible that some of their subjects could have had a luteinized unruptured follicle, which would explain lower progesterone levels without a postfertilization MOA. However, the incidence of luteinized unruptured follicle in healthy, fertile women is only 4 percent, and it is more often a consideration in infertile women. Moreover, women with a luteinized unruptured follicle typically have a luteal phase of normal length, unlike those in the Hapangama study.[61]

ICEC/FIGO Studies 2 and 3: Marions 2002 and 2004

Lena Marions and colleagues, in their 2002 and 2004 studies, found that preovulatory LNG administration effectively inhibited the LH peak, delayed ovulation, or both.[62] The two studies were very small, having only six and seven subjects respectively. In the 2002 Marions study, six women were given LNG-EC before ovulation (two days before the LH surge, on day LH -2); then, after a treatment-free cycle, they were given LNG-EC after ovulation (at day LH +2). Although Marions et al. performed serial TVUS on the women, they based the timing of drug administration on urinary LH levels correlated with follicular size. A chart of mean LH levels shows no significant LH elevation in the women treated before ovulation, suggesting that the LH surge was severely blunted and delayed in these women, yet the authors report that "there was no difference at the 95% significance level between the means of LH measurements during the *entire* cycle" and that "urinary excretion of estrone

M.R. Soules et al., "Luteal Phase Deficiency: Abnormal Gonadotropin and Progesterone Secretion Patterns," *Journal of Clinical Endocrinology and Metabolism* 69.4 (October 1989): 813–820; E.A. Pritts and A.K. Atwood, "Luteal Phase Support in Infertility Treatment: A Meta-analysis of the Randomized Trials," *Human Reproduction* 17.9 (September 2002): 2287–2289; and J. Jordan et al., "Luteal Phase Defect: The Sensitivity and Specificity of the Diagnostic Methods in Common Clinical Use," *Fertility and Sterility* 62.1 (July 1994): 54–62.

[59] Hapangama et al., "Effects of Peri-ovulatory Administration of Levonorgestrel," 128.

[60] Ibid.

[61] J.F. Kerin et al., "Incidence of the Luteinized Unruptured Follicle Phenomenon in Cycling Women," *Fertility and Sterility* 40.5 (November 1983): 620–626.

[62] Marions et al., "Emergency Contraception" (2002), and Marions et al., "Effect of Emergency Contraception" (2004).

and pregnanediol glucoronide in both pre- and postovulatory treated cycles" were similar to controls.[63]

In the 2004 Marions study, the effects of LNG-EC and mifepristone on luteal function were studied in more detail.[64] "The study included one control cycle and two treatment cycles, subjects serving as their own controls." In the first treatment cycle, subjects received 10 mg mifepristone as a single dose two days prior to the expected LH peak (day -2). In the second treatment cycle, after a treatment-free cycle, they received two doses of 0.75 mg LNG on day -2, with the doses separated by twelve hours.[65]

The LNG treatment caused either delay or inhibition of the LH peak in all seven women. This time, the median LH levels were significantly lower following treatment with LNG than in controls. Luteal-phase progesterone levels were also lower than in controls, and average cycle length was over four days shorter with LNG than with controls.

It is notable that the 2004 researchers used mifepristone (an anti-progestin hormone) in the first cycle, followed by a treatment-free cycle, followed by a cycle when levonorgestrel was administered. In all the studies we reviewed, a necessary inclusion criterion was that subjects had no prior use of hormonal contraception for a defined period of time before entering the study. Here, two drugs known to affect the hypothalamic-pituitary-adrenal axis, LNG and mifepristone, were given to the same women within two months of each other.

ICEC/FIGO Study 4: Durand 2001

In 2001, Marta Durand and colleagues studied forty-five women using the older LNG-EC regimen of 0.75 mg given twice.[66] They found that twelve of fifteen women (80 percent) in group A had an anovulatory cycle after receiving LNG-EC on cycle day 10—a day that is not yet in the fertile window or is at the very beginning of it, with a very low conception probability;[67] the other three women in the group had a shortened luteal phase and lower progesterone levels. In groups B and C, subjects received LNG immediately on detection of urinary LH or forty-eight hours after LH detection, respectively. All ovulated, and no statistically significant differences in either cycle length or luteal progesterone levels were noted, although both were diminished compared with controls.

Women in group D received LNG in the late follicular phase, three days before the LH surge as determined by urinary LH detection. Follicular rupture was confirmed in all subjects in this group. In other words, LNG-EC administered in

[63] Marions et al., "Emergency Contraception," 69, emphasis added. If the LH was severely blunted in the preovulatory group, and if the rest of the luteal phase LH measurements were comparable, it seems unusual that the LH levels would be reported as "similar" between all groups. See figure 4 on page 69 of the study.

[64] Marions et al., "Effect of Emergency Contraception."

[65] Ibid., 374.

[66] Durand et al., "On the Mechanisms of Action," 2001.

[67] See Wilcox et al., "Timing of Sexual Intercourse," 1519.

the late follicular phase did *not* suppress ovulation. Most importantly, subjects in group D had significantly deficient progesterone production with a significantly shorter luteal phase. Thus, all women who received LNG-EC before the onset of the LH surge ovulated and had significant shortening of the luteal phase. As noted previously, luteal deficiency impairs normal transformation of the endometrium so that if fertilization occurs, changes in the endometrium may impair implantation.

ICEC/FIGO Study 5: Croxatto 2004

In 2004, Horacio Croxatto and colleagues carried out a randomized double-blind, placebo-controlled study.[68] A group of fifty-eight presumably healthy women with normal cycles were treated with the older LNG-EC regimen (two doses of 0.75 mg), with a single 0.75 mg dose and a placebo pill, or with two placebos. "Participants were randomly assigned to three groups: one group received the first pill when the leading follicle reached a mean diameter of 12–14 mm (n=18). In the second group, it was given when the diameter of the follicle was 15–17 mm (n=22). The third group received the first pill when the follicle reached ≥ 18 mm (n=18)."[69] Interestingly, in 36 percent of the control (placebo) cycles, no ovulation occurred within five days. Even 13 percent of controls in the group with advanced follicular size were anovulatory. It should be pointed out that a certain percentage of women may have anovulatory cycles at any given time; if this was the case in the control group, one cannot reasonably attribute the cause to LNG-EC.

Follicular rupture occurred in over half of all LNG-treated cycles (56 percent two-dose LNG and 50 percent single-dose LNG).[70] As expected, the percentage of cycles without follicular rupture was inversely proportional to the size of the leading follicle at the time of treatment. Treatment at smaller follicle size was more likely to inhibit follicular rupture than treatment at larger follicular size. In other words, the further the woman was from the LH surge when she received LNG-EC, the more likely she was not to ovulate, whereas the closer she was to the LH surge, the more likely she was to ovulate. It is also significant that 30 percent of cycles in the two-dose group and 23 percent of cycles in the single-dose group were of short duration (less than twenty-four days long), compared with 7 percent of cycles in the placebo group. As noted above, a short luteal phase is associated with suboptimal corpus luteal function and decreased progesterone levels, and could impair implantation.[71]

Croxatto et al. use the term "ovulatory dysfunction" to describe a hypothesis based on their observations. They define ovulatory function as observed "follicular rupture not preceded by an LH peak or preceded by a blunted LH peak (<21 IU/L)

---

[68] Croxatto et al., "Pituitary–Ovarian Function." This is a randomized, double-blind, placebo-controlled study, which provides one of the highest levels of evidence within evidenced-based medicine.

[69] Ibid., 443.

[70] Ibid., 444.

[71] A. J. Wilcox, D. D. Baird, and C. R. Weinberg, "Time of Implantation of the Conceptus and Loss of Pregnancy," *New England Journal of Medicine* 340.23 (June 10, 1999):1796–1799; and Norwitz et al., "Implantation and the Survival of Early Pregnancy," 1400–1405.

or not followed by elevation of serum [progesterone levels] over 12nmol/L."[72] They state, "Although follicular rupture occurred in many women, absence of an adequate LH and [follicle-stimulating hormone] peak or their lack of proximity to the day of ovulation are deviations [from] the normalcy required for the success of the reproductive process. … When a normal gonadotropin surge acts on a mature follicle, it triggers a series of coordinated local responses that eventually lead to the extrusion of a fertilizable oocyte and the formation of a fully functional corpus luteum. … The coordinated development of these responses requires a normal gonadotropin surge."[73]

Given all this, it would be more fitting to speak of *postovulatory* dysfunction,[74] since the researchers seem to be defining a deficiency of the corpus luteum. They describe events that occur as the result of preovulatory LNG administration, even though the events are going to affect postovulation outcomes. If the LNG-EC is given several days before ovulation, it will not stop ovulation in over half the cases, but it will affect subsequent events. Croxatto et al. calculate that ovulatory dysfunction (as they define it) was observed in 35 percent of standard-dose LNG cycles and 36 percent of single-dose LNG cycles, in contrast with 5 percent of placebo cycles. LH levels were significantly decreased relative to placebo cycles. "The highest [progesterone] concentration was significantly lower in LNG-treated cycles with ovulatory dysfunction than in corresponding placebo cycles."[75] The corresponding area under the curve for progesterone levels in the luteal phase was also lower than in the placebo group. Finally, the "frequency of cycles of short duration (<24 days) was significantly higher in standard and single-dose treated cycles" than in the placebo group,[76] adding to the evidence of a postfertilization effect.

Croxatto et al. advance the hypothesis that if LH is deficient after the administration of LNG-EC, then women with subsequent dysfunctional ovulation could release ova that could not be fertilized. No LNG-EC studies provide evidence of this. Croxatto et al. reference the work of Willem Verpoest et al. when discussing this theory,[77] but the Verpoest study is not an LNG study, and it involved a totally different population (i.e., infertile women). Moreover, these women were between fertility treatments, which are well known to alter the hypothalamic-pituitary-adrenal axis. These results should not be compared with findings for fertile women taking LNG-EC.

Verpoest et al. found that women with lower levels of LH had impaired fertilization of oocytes compared with women whose oocytes could become fertilized and whose LH levels were higher. Yet the Verpoest study includes no controls, and the

[72] Croxatto et al., "Pituitary-Ovarian Function," 444.

[73] Ibid., 448.

[74] Ibid., 449.

[75] Ibid., 447.

[76] Ibid. See figure 5.

[77] Ibid., 49; W. M. Verpoest et al., "Relationship between Midcycle Luteinizing Hormone Surge Quality and Oocyte Fertilization," *Fertility and Sterility* 2373.1 (January 2000): 75–77.

methodology for collecting LH varies significantly.[78] Despite these shortcomings, we looked at the subjects with unfertilizable ova and "low" peak LH median levels of 42.5 IU/L and at the healthy fertile controls of the 2004 Croxatto study, whose peak LH mean value was approximately 43 IU/L.[79] In other words, the women in the Verpoest study who had "low" LH levels associated with their unfertilizable ova had peak LH levels that were comparable to the healthy controls of Croxatto's own study.[80] Moreover, Verpoest et al. define fertilization as "the presence of two pronuclei at 24 hours and continued cleavage until [embryo transfer] at 2–3 days."[81] This definition appears to include embryos that do not continue to divide properly, thus treating young embryos as unfertilized ova. If such a process were causally associated with LNG-EC, it would constitute a postfertilization MOA best described as early embryo demise.

Finally, Verpoest data should not be compared with LNG-EC findings because the Verpoest subjects were not treated with LNG-EC. In animal studies, exogenous progesterone administered in the periovulatory period actually enhances nuclear maturation (allowing oocyte fertilization); it does not prevent this process. Croxatto et al. maintain that the lower LH alone might cause impaired "fertilizability."[82] But the addition of a powerful progestin has been shown to enhance maturation of the ovum.[83] LNG-EC is a very potent progestin. Conversely, the use of a progesterone receptor antagonist (mifepristone) has been shown to prevent oocyte meiotic resumption in vivo.[84] These findings cast doubt on the use of Verpoest findings to postulate dysfunctional ova that cannot be fertilized.

ICEC/FIGO Study 6: Okewole 2007

In 2007, Idris Okewole and colleagues administered 1.5 mg LNG in the periovulatory phase "to determine the effects on serum gonadotropins, estradiol and progesterone levels."[85] Eight women in group A took LNG at estimated day -3 (three days before the expected day of ovulation) while six women in Group B took LNG at estimated day -1.[86] Perturbations of gonadotropins were measured via serum

[78] Luteinizing hormone was measured in the Verpoest study in two ways: as LH serum levels and as LH in follicular fluid sampled several times per day. LH was measured in LNG-EC studies by once daily serum or urinary LH testing.

[79] Croxatto et al., "Pituitary–Ovarian Function," 445, figure 1.

[80] Ibid.

[81] Verpoest, "Relationship between Midcycle," 76.

[82] Croxatto et al., "Pituitary–Ovarian Function," 449.

[83] L. C. Siqueira et al., "Angiotensin II, Progesterone, and Prostaglandins Are Sequential Steps in the Pathway to Bovine Oocyte Nuclear Maturation," *Theriogenology* 77.9 (June 2012): 1779–1787.

[84] O. Haccard et al., "Naturally Occurring Steroids in Xenopus Oocyte during Meiotic Maturation: Unexpected Presence and Role of Steroid Sulfates," *Molecular and Cellular Endocrinology* 362.1–2 (October 15, 2012): 110–119.

[85] Okewole et al., "Effect of Single Administration," 372.

[86] The timing of LNG administration was determined by estimating the day of ovulation, "by subtracting 14 days from the expected date of the next period, which was determined from each woman's three previous menstrual cycles." Ibid., 373.

measurements of FSH (follicle-stimulating hormone), LH, estradiol, and progesterone. Although the women in group A had a "significant delay in … LH peak by about 96–120 [hours]" compared with their control cycles,[87] this finding was a reported mean value based on all eight subjects. Examination of table 3 in the report reveals that for two subjects, the delay in the LH surge was as small as one day (subject 7) or two days (subject 4), and two subjects had only a four-day delay (subjects 6 and 8).[88] Grouping the delay by mean reporting thus obscures the fact that for some subjects (four of eight), the delay may not have been significant enough to prevent fertilization.

Subjects in group A also had significantly lower levels of estrogen and progesterone during their follicular and luteal phases, and four of eight women had vaginal bleeding, suggestive of endometrial instability due to lower progesterone levels. Fertilization under such conditions could lead to impaired implantation because of endometrial changes.

Among the women in group B (who received LNG at the day before ovulation was expected), LNG did not interfere with ovulation but was associated with a statistically significant shortening of the mean cycle length in comparison with pretreatment cycles (20.2 vs. 25.1 days) and with a diminution of luteal mean progesterone levels. Although the lower progesterone levels were not statistically significant,[89] these findings, taken together with the shortened luteal length, indicate that LNG impaired the corpus luteum. The authors confirm this: "This shows that LNG administration at late follicular phase (Group B) did not interfere with the estradiol-mediated midcycle gonadotrophin surge and probably ovulation*, but did alter progesterone production by the corpus luteum. It suggests that LNG might have caused premature degeneration of the corpus luteum*."[90] The authors recognize that lower progesterone and shortened luteal phase follow the administration of preovulatory LNG-EC as likely consequences of inadequate luteinization, a postfertilization effect.

Limitations of this study include its small sample size and the method used to determine ovulation. Studies using actual measurements of fertile-window indicators (i.e., ultrasound or serum levels of LH) have a much stronger methodology.

ICEC/FIGO Study 7: Novikova 2007

The ICEC/FIGO claim that LNG-EC cannot prevent implantation relies heavily on a 2007 study by Natalia Novikova and colleagues, which concludes that LNG-EC "has little or no effect on postovulation events but is highly effective when taken

[87] Ibid., 375.

[88] Ibid., 374, table 3.

[89] It should be noted that Okewole et al. calculated the "mean of log of progesterone level" for the mid-luteal phase. Other studies have used area under the curve (AUC) or integrated progesterone levels as a more standardized way of comparing luteal phase insufficiency. As noted earlier, Croxatto et al. used serum "mean progesterone" for the entire luteal phase, and Palomino et al. and Devoto et al. used one-day "plasma progesterone concentration" obtained at the day of endometrial biopsy. The variance in how progesterone levels are measured makes meaningful comparison between studies elusive.

[90] Okewole et al., "Effect of Single Administration," 375, emphasis added.

before ovulation."[91] The authors state, "Among 17 women who had intercourse in the fertile period of the cycle and took [LNG-EC] after ovulation"—on days +1 to +2, specifically—there was no difference between expected and observed pregnancies, showing that LNG-EC had no effect when administered after ovulation. However, "among thirty-four women who had intercourse on Days -5 to -2 … and took [LNG-EC] before or on the day of ovulation, four pregnancies could have been expected, but none were observed."[92] In this study, the serum levels of progesterone, estradiol, and LH were measured at the time of LNG-EC ingestion in order to provide a more reliable estimate of the time at which unprotected intercourse occurred in relation to ovulation.[93]

Like previous studies, the Novikova study shows that LNG-EC prevents pregnancy only in the preovulatory period. This conclusion, however, does not exclude postovulatory effects, which the study was not designed to measure. The study's limitations are that (1) only a one-time assessment of serum gonadotropins was made, when the women first presented to the clinic for emergency contraception; (2) no serial measurements of gonadotropins were made, and no TVUS examinations or endometrial biopsies were performed; and (3) no information about cycle length or episodes of post-LNG vaginal bleeding was reported. Any assertion about the effects LNG-EC on postovulation events would require serial measurements of gonadotropins and reports on luteal phase length and vaginal bleeding. In short, the data reported by Novikova et al. do not provide an adequate basis for claims about a lack of postfertilization events following administration of LNG-EC. The researchers can fairly report only that LNG-EC was efficacious in the preovulatory period and was not efficacious in the postovulatory period.[94]

These studies represent the main data that ICEC/FIGO used to support its assertion that the dominant MOA of LNG-EC is prevention of ovulation and not impairment of implantation. Yet the two largest studies, Durand 2001 and Croxatto, show that ovulation occurred in the majority of women who received LNG-EC in the late follicular phase of their cycles.[95] The Hapangama findings and those in Okewole's group B also show that the majority of subjects ovulated despite preovulatory administration of LNG-EC. In the other studies, either the methodology or the findings were questionable with regard to conclusions about postovulatory effects of LNG-EC.

---

[91] Novikova et al., "Effectiveness of Levonorgestrel," 116. Although the Novikova study does not assess delay or prevention of ovulation as an MOA, it assumes as much and so is included in this section on ICEC/FIGO source authorities.

[92] Ibid., 112.

[93] Ibid., 113. In fact, the researchers found that the women's self-reported menstrual data were unreliable and did not correlate well with the endocrine data (115–116).

[94] P. Ventura-Junca, M. Besio, and M. Santos, letters to the editor, *Contraception* 77.6 (June 2008): 463–464.

[95] Although the Novikova study was also relatively large, the methodology did not allow for assessment of ovulation after LNG-EC intake.

Other Studies

Four larger studies were published after ICEC/FIGO issued its statement. One of these, the 2010 Durand study, is discussed here with a related Durand study from 2005. The five studies provide convincing evidence that the efficacy of LNG-EC is not achieved solely by preovulatory MOAs. The studies also provide evidence for a postfertilization MOA.

Study 1: Tirelli 2008

In 2008, Alessandra Tirelli and colleagues studied the effects of LNG-EC on the bleeding pattern and the pituitary-ovarian function of sixty-nine women who were given LNG in the follicular phase (n=26), periovulatory phase (n=14), or luteal phase (n=29) of their cycles.[96] The data indicate that LNG-EC given before the LH surge significantly shortens cycle length, virtually eliminating the luteal phase and rendering implantation impossible.[97]

Tirelli et al. also examined serum gonadotropin levels and TVUS findings in eight subjects who were in the late follicular, or fertile, phase (cycle days 11 to 13). Seven of the eight did not have follicular rupture, meaning that ovulation had not yet occurred. However, the mean diameter of the largest leading follicle in these women was only 8 mm, which is very small, consistent with the size of a follicle in an earlier phase, before the fertile period.[98] Other LNG-EC studies that utilize TVUS exclude women from the fertile window if the leading follicle measures less than 12 mm.[99] This means that the lack of follicular rupture in the Tirelli subjects was irrelevant, because LNG-EC was administered before the women entered the fertile window, when intercourse would not lead to pregnancy.

Study 2: Noé 2010

Noé and colleagues reported the results of a large clinical trial in two sequential studies.[100] Their data carry greater weight than data from previous studies because of their methodology and the relatively large number of women studied.[101]

---

[96] Tirelli et al., "Levonorgestrel Administration in Emergency Contraception," 328.

[97] Tirelli et al. demonstrated luteal phase shortening of 10.9 days. Thus, the implantation window between days 20 and 24 was lost. See Norwitz et al., "Implantation and the Survival of the Early Pregnancy," 1400.

[98] Severi et al. observe that a follicle size of 8 mm corresponds to day -7 (where day 0 = ovulation) and thus is outside the fertile window. F.M. Severi et al., "Transvaginal Ultrasonography in Women Receiving Emergency Contraception," *Fertility and Sterility* 79.5 (May 2003): 1075.

[99] Croxatto et al., "Pituitary-Ovarian Function," 444; and Noé et al., "Contraceptive Efficacy" 2010, 415.

[100] Noé et al., "Contraceptive Efficacy" 2010 and 2011.

[101] Although the combined Noé et al. study was the largest undertaken on LNG-EC methodology, it should be noted that there were no controls reported in the 2010 trial. In the 2011 study, the characteristics of regular ovulating cycles from healthy women attending the same study center were obtained from more than one hundred cycles serving as a control

In 2010, Noé et al. studied the efficacy of preovulatory and postovulatory administration of LNG-EC in women who requested emergency contraception at a family planning clinic. For all subjects they recorded menstrual history and time of intercourse, took blood samples for LH, estrogen, and progesterone, and used TVUS to determine follicular rupture. Three hundred and eighty-eight women requested emergency contraception, and one hundred and twenty-two had had intercourse during their fertile window, as established by ultrasound and serum gonadotropin results. Eighty-seven subjects took LNG-EC between preovulation days -5 to -1, and thirty-five took it in day 0 or beyond. Thirteen pregnancies were expected in the first group and seven in the second. Actual pregnancies were zero and six, respectively. The authors concluded that LNG-EC prevents pregnancy only when taken "before fertilization of the ovum has occurred."[102] LNG-EC had no efficacy when taken at or after ovulation.

Noé et al. report, "In the 87 women treated before ovulation, [follicular rupture] was confirmed in 62 by means of TVU and elevated [progesterone] level (n=39), or TVU only (n=18), or by luteal phase values of [progesterone] only (n=5)."[103] Because fifteen of the eighty-seven women (17 percent) did not attend follow-up visits, they were subtracted for percentage calculations. Thus, 86 percent (sixty-two of seventy-two) of the women had confirmed follicular rupture using the Noé definition above, despite receiving preovulatory LNG-EC.

Noé and colleagues acknowledge that ovulation in such a significant majority "suggests that other mechanism than suppression of ovulation prevents pregnancy in these women." They propose "that increased cervical mucus viscosity caused by LNG impedes the migration of the sperm from their reservoir in cervical crypts to the Fallopian tubes."[104] No mention is made of recent studies showing that LNG-EC has *no* effects on cervical mucus or sperm migration,[105] nor do Noé et al. acknowledge studies showing that sperm can be retrieved from the fallopian tubes within minutes of insemination.[106]

It is surprising that the researchers do not mention a possible postfertilization MOA when the data clearly show that ovulation is not inhibited in most women

group. The Croxatto study in 2004, although smaller, was a randomized, double-blind, placebo-controlled study.

[102] Noé et al., "Contraceptive Efficacy" 2010, 414.

[103] Ibid., 417.

[104] Ibid., 419–420.

[105] As noted above, the studies showing no LNG-EC effect on sperm at doses found in vivo are do Nascimento et al., "In Vivo Assessment of the Human Sperm Acrosome Reaction"; Yeung et al., "Effects of Levonorgestrel"; and Hermanny et al., "In Vitro Assessment of Some Sperm Function."

[106] As noted above, the following studies confirm that sperm reach the fallopian tubes in minutes to hours: Kesserü et al., "Hormonal and Peripheral Effects"; Kunz et al., "Dynamics of Rapid Sperm Transport"; Ortiz and Croxatto, "Copper-T Intrauterine Device"; Settlage et al., "Sperm Transport from the External Cervical Os"; and Ahlgren, "Sperm Transport."

taking LNG-EC and when FDA-mandated labeling states that it could act by a postfertilization MOA.[107]

Study 3: Noé 2011

In 2011, Noé et al. reported the completion of their previous study, with data from a total of 450 women.[108] One hundred and three women had intercourse during their fertile days and took LNG-EC before ovulation, between day -5 and day -1; another forty-five women took LNG-EC on day 0 or later. Sixteen pregnancies were expected in the preovulatory group, but none occurred. In the postovulatory group, 8.7 pregnancies were expected and 8 occurred. More importantly, "eighty-two of the 103 women treated before ovulation completed the five days of follow up, and in sixty-three (80%) of them, [follicular rupture, indicating ovulation] was detected. In the group that had intercourse on day -2, [*follicular rupture*] *was detected in 22* (*92%*) *of the 24 women who completed the follow-up days*."[109]

As in 2010, Noé et al. acknowledge that the absence of pregnancies in the women who received LNG-EC during the preovulatory fertile window indicates an MOA other than suppression of ovulation. They again propose that increased cervical mucus viscosity may interfere with sperm migration, suggesting that it is "transient and more pronounced in the 12 [hours] following LNG intake but vanishes after 24 [hours]." Their theory of a transient effect on sperm migration is an attempt to reconcile (1) the 1974 Kesserü findings of impaired cervical mucus and a reduced number of sperm between three and ten hours after intake of d-norgestrel with (2) the contrary 2007 do Nascimento finding that an adequate number of viable and motile sperm were recovered from the cervix and uterine cavity twenty-four to forty-eight hours after intake. But this attempt fails for at least two reasons. First, sperm that arrive in the fallopian tube five minutes after intercourse can be maintained in a fertile state by interacting with the oviductal epithelium and can become capacitated and hyperactivated by the ovulation process.[110] Yet pre-ovulatory LNG-EC resulted in no pregnancies. A ten-hour MOA cannot account for the 100 percent preovulatory efficacy of the drug in this scenario. Second, their proposed robust "transient" MOA accounting for such a profound effect on sperm migration would prevent pregnancy in some of the women who had intercourse on day -1 and took LNG-EC shortly thereafter on the day of ovulation. Fourteen women had intercourse on day -1, the day on which the likelihood of conception is highest, and took LNG-EC on or after ovulation. In these women, a transient sperm MOA should have prevented three to four pregnancies, yet no pregnancies were prevented.[111]

Noé et al. also postulate dysfunctional ovulation as a possible explanation for the 100 percent efficacy of LNG-EC when given between days -5 to -1, but as we

[107] See C. Lopez-del Burgo, R. T. Mikolajzyk, and J. Stanford, "Emergency Contraception: An Unresolved Issue," *Contraception* 83.2 (February 2011): 187.

[108] Noé et al., "Contraceptive Efficacy" 2011, 488.

[109] Ibid., 490, emphasis added.

[110] Suarez and Pacey, "Sperm Transport in the Female Reproductive Tract," 23.

[111] Ibid., fig 3B, 4.

show above, no empirical evidence supports this theory other than extrapolations made from infertile women.

The 2010 and 2011 Noé studies, and to some extent those of Novikova before them, have shown that preovulatory use of LNG-EC results in *no* clinical pregnancies. Earlier studies compared LNG-EC with the older Yuzpe regimen and reported varying figures of effectiveness, from 49 to 85 percent.[112] But Noé et al. show with methodological rigor that preovulatory LNG-EC (taken on days -5 to -1) is 100 percent efficacious,[113] which demonstrates beyond doubt that the suppression of ovulation does not explain its efficacy.

Studies 4 and 5: Durand 2005 and 2010

In 2001, Marta Durand and colleagues investigated the ability of LNG-EC to suppress ovulation, disrupt luteal function, and impair endometrial receptivity.[114] That study, cited by ICEC/FIGO and critically examined above (under the heading "ICEC/FIGO Study 4: Durand 2001"), provided data showing that LNG-EC intake during the late follicular phase did not suppress ovulation and did result in a significantly shorter luteal phase with significantly lower progesterone levels.

In their studies from 2005 and 2010, Durand et al. examined serum glycodelin-A concentrations and endometrial expression during the luteal phase after LNG-EC intake at different cycle stages.[115] Here we look at their data related to ovulation.

Durand 2005. In 2005, Durand et al. reanalyzed the 2001 data from thirty women who ovulated. Subjects were divided into three treatment groups according to the timing of standard two-dose 0.75 mg LNG intake. Subjects in group 1 were treated three to four days before the LH surge, group 2 at time of the LH surge, and group 3 forty-eight hours after the LH surge was detected. A control cycle enabled the estimation of the LH surge and comparison of glycodelin-A expression, luteal phase duration, and progesterone levels between groups and baseline. In women treated before the LH surge (group 1), the mean length of the luteal phase and the serum progesterone levels were significantly lower than in controls. In addition, glycodelin-A immunostaining in biopsy specimens obtained at day LH +9 was less

[112] J. B. Stanford, "Emergency Contraception: Overestimated Effectiveness and Questionable Expectations," *Clinical Pharmacology and Therapeutics* 83.1 (January 2008): 20.

[113] Even if theory of a transient effect on sperm could explain a small percentage of efficacy during this window (and as we have shown above under the heading "First Proposed MOA: Cervical Mucus, Sperm Transport and Sperm Capacitance," all recent evidence suggests otherwise), it would not explain how LNG-EC works during the remainder of the fertile window.

[114] Durand et al., "On the Mechanisms of Action," 2001.

[115] Durand et al., "Late Follicular Phase Administration," 2005; and Durand et al., "Hormonal Evaluation and Midcycle Detection," 2010. See the discussion above under the heading "Second Proposed MOA: Prevention of Sperm–Egg Binding."

intense in group 1, indicating weaker endometrial expression, than in subjects treated at or after the LH rise (groups 2 and 3).[116]

The shortened luteal phase and lower progesterone levels suggest a postfertilization MOA following pre-LH intake. The study also shows that a progesterone-dependent endometrial protein (glycodelin) is diminished following such use. When taken three to four days before the LH surge, LNG-EC caused a profound luteal-phase progesterone deficiency and significantly weaker glycodelin-A immunostaining at day LH +9. The glycodelin endometrial staining provides an accurate confirmatory correlation—a kind of bioassay—for insufficient luteal phase defect, not only demonstrating progesterone inadequacy but providing excellent tissue-level proof of it. Additionally, the weaker glycodelin-A staining, independent of progesterone-mediated effects, suggests a possible mechanism of embryocidal activity in its own right, since glycodelin-A is thought to be another mediator needed by the developing embryo in its efforts to implant through appropriate immunosuppressive activity. Durand et al. note this possibility.[117]

Durand 2010. In 2010, Durand et al. examined LNG-EC effects on the cycles of thirty sterilized ovulating women whose untreated cycles served as controls.[118] The primary purpose was "to assess the presence of glycodelin-A in uterine flushing at the midcycle of ovulatory women treated with LNG during the preovulatory phase of their menstrual cycle."[119] All thirty subjects received LNG-EC two days before the LH surge. Midcycle glycodelin-A levels were measured both in serum and uterine flushings to test the researchers' previous hypothesis that glycodelin-A levels in utero would be found at levels thought to impede sperm–egg binding. Relevant hormonal markers were also measured to assess the effects on parameters of ovulation and luteal function.

Ovulation occurred in two-thirds of the women, with short luteal phases, marked reductions in LH, and perturbations of all other hormones evaluated. Glycodelin-A levels in both serum and endometrium were also increased in the periovulatory phase, but at lower levels than needed to interfere with fertilization. Durand et al. note that the greatly diminished LH levels suggest "a defective ovulatory process with oocytes carrying impaired fertilizable activity"—that is, the dysfunctional ovulation and unfertilizable ovum theory of Croxatto and Verpoest.[120]

---

[116] Durand et al., "Late Follicular Phase Administration," 2005, 451. Endometrial glycodelin-A is a necessary implantational molecule that is progesterone-dependent and essential to embryo survival. In the study we see that as the progesterone levels decreased, so too did the endometrial glycodelin-A expression, thus triggering another potential postfertilization mechanism. The reader is asked to consider how many other possible progesterone-dependent molecules may be similarly affected by lower progesterone levels.

[117] Ibid., 456.

[118] Durand et al., "Hormonal Evaluation and Midcycle Detection," 2010.

[119] Ibid., 527.

[120] Durand et al., "Hormonal Evaluation and Midcycle Detection," 2010, 531. See the discussion under "ICEC/FIGO Study 5: Croxatto 2004" above.

Despite finding significantly shortened cycle lengths in subjects who took LNG and significantly shortened luteal phases,[121] Durand et al. attributed no causal significance to the finding. In light of the previous Durand work and that of Hapangama, Okewole, and Croxatto (showing shortened luteal phase, lower luteal progesterone, and lower endometrial glycodelin), one would have liked to see the authors explore a post-fertilization MOA of LNG-induced short luteal phase. Instead they conclude that "apparently normal" luteal phase estrogen and progesterone "suggested a normal luteinization and corpus luteum function in [ovulation-after-LNG] cycles, which agree with the lack of deleterious effects on the endometrium."[122]

Despite the shortened luteal phase, which should indicate early decline of progesterone, Durand et al. reported lower but not statistically significant integrated luteal-phase progesterone levels. But a primary concern arises because the subjects who ovulated reportedly received their LNG-EC two days before the LH surge, yet their mean follicular size at intake (18.4) correlates with a more advanced cycle day.[123] The LH surge should occur thirty-seven to thirty-nine hours before ovulation.[124] Filiberto Severi et al. observed that a follicle diameter of 18 to 19 mm correlates with a dominant follicle on day -2 (relative to ovulation).[125] Since the women who ovulated in this study were treated with LNG when their follicle size was over 18 mm, the timing corresponds (according to Severi measurements) to two days before *ovulation*, not two days before the LH surge.[126] Therefore, these women could have been given the drug between days LH -1 and LH 0, which is at or about the time of the LH surge, rendering analysis questionable for pre-LH surge intake. This group would then be similar to group B in the 2001 Durand study (with LNG intake at the time of LH surge), which had lower integrated progesterone levels, though also not statistically significant.

---

[121] Ibid., table 2, 528.

[122] Ibid., 532.

[123] Severi et al., "Transvaginal Ultrasonography," 1075; Croxatto et al., "Pituitary–Ovarian Function," 444; Noé et al., "Contraceptive Efficacy" 2011, 489; and Noé et al., "Contraceptive Efficacy" 2010, 418. In both Noé studies, the women presenting with follicular diameters of 18 mm or more were placed in the most advanced follicular size group, closest to ovulation.

[124] "The start of the LH surge . . . occurs 37–39 hours before ovulation." J. Testart et al., "Plasma and Intrafollicular Hormonal Profiles in the Late Preovulatory Phase, 1: Spontaneous Cycles" [in French], *Journal de Gynécologie, Obstétrique et Biologie de la Reproduction* 122 (1983): 119–127, English abstract available at PubMed, http://www.ncbi.nlm.nih.gov/pubmed. Marc Fritz et al. established a mean time interval between surge onset and follicle rupture of 37.6 hours. M. A. Fritz et al., "Onset and Characteristics of the Midcycle Surge in Bioactive and Immunoactive Luteinizing Hormone Secretion in Normal Women: Influence of Physiological Variations in Periovulatory Ovarian Steroid Hormone Formation," *Journal of Clinical Endocrinology and Metabolism* 75.2 (August 1992): 489–493.

[125] Severi et al., "Transvaginal Ultrasonography," 1075.

[126] "The larger the leading follicle at the time of treatment, the more likely EC is given during or after the LH surge." Croxatto et al., "Pituitary–Ovarian Function," 448.

If Durand et al. in 2010 wished to replicate their 2001 findings, they should have administered LNG-EC three days before the LH peak, as this drug timing definitively showed statistically significant lower progesterone levels in 2001. Since the primary target of this study was midcycle glycodelin-A analysis and not progesterone levels, it is conceivable that the researchers were more interested in the window during which they postulated LNG interference with sperm–egg binding via effects on glycodelin-A.

Finally, there are many problems with measurement of serum progesterone in the luteal phase, and these have been noted by several authors.[127] Even when comparing the different ways that various LNG-EC researchers have reported luteal progesterone levels, we see that Okewole et al. used "mean log of progesterone" for the mid-luteal phase, Croxatto et al. used serum "mean progesterone" for the entire luteal phase, and Palomino et al. and Luigi Devoto et al. used one-day "plasma progesterone concentration" obtained at the day of endometrial biopsy.[128] Because there is no way to compare significant findings across studies,[129] the usefulness of these indicators is limited. What remains constant through most of the studies, however, is the shortened luteal phase. Because of this, we believe that it should be given added consideration.

In summary, we found that in the majority of studies LNG-EC did not show a consistent or strong ability to impair ovulation when administered in the preovulatory fertile window, and thus any claims of moral or scientific certitude regarding this MOA should be reconsidered.

---

[127] M. Filicori, J.P. Butler, and W.F. Crowley Jr., "Neuroendocrine Regulation of the Corpus Luteum in the Human: Evidence for Pulsatile Progesterone Secretion," *Journal of Clinical Investigation* 73.6 (June 1984): 1638–1647; M.R. Soules et al., "Progesterone Modulation of Pulsatile Luteinizing Hormone Secretion in Normal Women," *Journal of Clinical Endocrinology and Metabolism* 58.2 (February 1984): 378–383; U. Schweiger et al., "Decreased Follicular Phase Gonadotropin Secretion Is Associated with Impaired Estradiol and Progesterone Secretion during the Follicular and Luteal Phases in Normally Menstruating Women," *Journal of Clinical Endocrinology and Metabolism* 68.5 (May 1989): 888–892; and M.J. McNeely and M.R. Soules, "The Diagnosis of Luteal Phase Deficiency: A Critical Review," *Fertility and Sterility* 50.1 (July 1988): 1–15.

[128] Okewole et al., "Effect of Single Administration"; Croxatto et al., "Pituitary–Ovarian Function"; Palomino et al. "A Single Midcycle Dose of Levonorgestrel"; and L. Devoto et al., "Pharmokinetics and Endometrial Tissue Levels of Levonorgestrel after Administration of a Single 1.5 mg Dose by the Oral and Vaginal Route," *Fertility and Sterility* 84.1 (July 2005): 46–51.

[129] Researchers have tried to develop meaningful indicators of luteal phase defect. See J. Jordan et al., "Luteal Phase Defect: The Sensitivity and Specificity of the Diagnostic Methods in Common Clinical Use," *Fertility and Sterility* 62.1 (July 1994): 54–62. These researchers state that integrated luteal progesterone levels from area-under-the-curve determinations that are less than 80 ng /ml a day are unequivocally diagnostic of luteal phase defect because the cutoff of 80 is so strict. The Durand preovulatory group had a luteal progesterone AUC of 78 ng/ml.

Fourth Proposed MOA: Effects on the Corpus Luteum

We have thus far reviewed studies which claim a prefertilization MOA for LNG-EC. We now present data that indicate that preovulatory administration of LNG-EC disrupts the postovulatory function of the corpus luteum.

In a normal cycle, the FSH-induced appearance of LH receptors on preovulatory granulosa cells allows LH to take over the functions of FSH in the terminal stages of follicular maturation. "These receptors also enable the granulosa cells to become competent to respond to the LH surge that initiates the resumption of meiosis, ovulation, and subsequent luteinization of the granulosa and theca cells."[130] After ovulation, the ruptured follicle is reorganized into the corpus luteum. "The process of luteinization and formation of a corpus luteum is associated with significant alterations in gene expression, encompassing hundreds of different genes in the granulosa cells alone."[131] The corpus luteum produces progesterone, which is known to play a pivotal role in maintaining pregnancy.[132] "The well-known function of [progesterone] during early pregnancy is to regulate (i) uterine receptivity for blastocyst attachment, (ii) progressive phases of embryo-uterine interactions, and (iii) differentiation of the endometrial stroma that maintains an environment conducive for the growth and development of the implanting embryo. The cellular actions of [progesterone] are mediated through intracellular progesterone receptors …, which are well-studied gene regulators."[133]

Throughout the luteal phase of normal ovulatory cycles, the corpus luteum depends on the support of the pituitary gonadotropins.[134] The "slowing down of the gonadotropin releasing hormone (GnRH) pulse generator along with diminished luteinizing hormone (LH) pulse amplitude is responsible for the demise of the corpus luteum."[135] And during the luteal phase "any defect in the pattern of luteal

[130] Jerome F. Strauss and Robert L. Barbieri, *Yen and Jaffe's Reproductive Endocrinology: Physiology, Pathophysiology, and Clinical Management*, 6th ed. (Philadelphia: Saunders, 2009), 179.

[131] Ibid., 174.

[132] N. F. Hacker and J. G. Moore, *Essentials of Obstetrics and Gynecology* (Philadelphia: Saunders, 1998), 59–71.

[133] I. C. Bagchi et al., "Progesterone Receptor Regulated Gene Networks in Implantation," *Frontiers in Bioscience*, landmark 8 (September 1, 2003): s852–s861.

[134] J. S. Hutchinson and A. J. Zeleznik, "The Rhesus Monkey Corpus Luteum Is Dependent on Pituitary Gonadotropin Secretion throughout the Luteal Phase of the Menstrual Cycle," *Endocrinology* 115.5 (November 1984): 1780; and M. R. Soules et al., "Progesterone Modulation of Pulsatile Luteinizing Hormone Secretion in Normal Women," *Journal of Clinical Endocrinology and Metabolism* 58.2 (February 1984): 378–383.

[135] N. G. Beckers et al., "The Early Luteal Phase Administration of Estrogen and Progesterone Does Not Induce Premature Luteolysis in Normo-ovulatory Women," *European Journal of Endocrinology* 155.2 (August 2006): 355.

gonadotropin secretion could have a deleterious effect on the functioning of the corpus luteum."[136]

It is reasonable to posit that LNG-EC interference with the hypothalamic-pituitary-adrenal axis (pituitary feedback system), an altered luteinization process, altered LH and/or progesterone, and a shortened luteal phase may change this complex and sensitive environment such that normal implantation is impaired or thwarted.

In most studies examined thus far, the recurrent assumptions are that (1) preovulatory efficacy of the drug implies that it works exclusively prior to fertilization in preventing pregnancy and (2) postovulatory non-efficacy of the drug implies the absence of embryocidal effects. But there is another plausible explanation. When LNG-EC is given in the late follicular phase of the fertile window (before ovulation), it can disrupt normal pituitary-ovarian feedback mechanisms, alter LH secretion, and hinder luteinization of the follicle and its supporting network of cells (the corpus luteum), leaving the embryo unsupported and resulting in its early death.

What evidence exists to support this explanation? Most of the twelve studies reviewed above under the heading "Third Proposed MOA: The Ability of Levonorgestrel Emergency Contraception to Prevent or Delay Ovulation" directly or indirectly indicate an altered corpus luteum.[137] For example, Hapangama et al. showed a shortened luteal phase and decreased luteal LH levels. In the 2001 Durand study, the subjects in group A who ovulated (who had been given LNG-EC on cycle day 10) showed decreased luteal phases and decreased progesterone levels, and the subjects in group D (who received LNG in the late follicular phase) showed deficient progesterone production and significantly shorter luteal phase lengths. The Croxatto study showed significantly shorter cycles in 30 percent of the subjects who received two doses of LNG-EC and in 23 percent of those who received a single dose of LNG-EC; the study also showed decreased progesterone concentrations in the LNG-treated cycles. In the Okewole study, the subjects who took LNG at estimated day -3 (group A, of whom only four of eight had significant delay) showed significantly lower progesterone levels and some vaginal bleeding, and those who took LNG at estimated day -1 (group B) showed shortened luteal phases and lower mean progesterone levels. Tirelli et al. showed that LNG-EC given before the LH surge significantly shortened cycle length by almost eleven days. In 2005 and 2010, Durand et al. showed that, consistent with their 2001 findings, LNG taken before the LH surge had pronounced deleterious effects on key luteal function parameters needed for implantation of embryos; these effects included markedly reduced progesterone levels (2005), shortened luteal phases (2010), severely blunted LH levels

[136] M. R. Soules et al., "Luteal Phase Deficiency: Abnormal Gonadotropin and Progesterone Secretion Patterns," *Journal of Clinical Endocrinology and Metabolism* 69.4 (October 1989): 814.

[137] These findings pertain solely to the women who received preovulatory LNG-EC and went on to ovulate.

(2010), and impaired endometrial expression of progesterone-dependent glycodelin-A in the luteal phrase (2005 and 2010).[138]

We reviewed four additional studies regarding luteal function. Linan Cheng and colleagues reviewed all evidence from randomized controlled trials and controlled clinical trials relating to the effectiveness of different methods of emergency contraception and examined various outcome measures, including menstrual bleeding and early return of menses.[139] They found that LNG-EC was associated with an earlier return of menses than mifepristone or ulipristal and with a higher risk of bleeding than low-dose mifepristone. These findings suggest indirect effects on the corpus luteum.

In a case series study, Elizabeth Raymond and colleagues showed that LNG-EC use by one hundred twenty women during the first three weeks of the menstrual cycle "significantly shortened that cycle compared to the usual cycle length and to the cycle duration in a comparison group."[140] In addition, the incidence of intermenstrual bleeding in either the first or the second cycle was higher in the LNG-EC group. These findings are consistent with those of other studies of emergency contraception that also show higher rates of bleeding.[141]

Erin Gainer and colleagues' prospective observational study found that, of 232 women who took LNG-EC, there were "thirty-four cases (14.7%) of incident intermenstrual bleeding within seven days of [taking the drug], which is similar to the rates of bleeding not related to menses seen in previous large-scale studies: 16% in a WHO study of a total of 1,978 LNG-EC users, and 16% in a Nigerian study of 544 LNG-EC users."[142] Moreover, emergency contraception taken "early in the menstrual cycle (two or more days before expected ovulation) was associated with a shortened cycle length and incident intermenstrual bleeding."[143]

The fourth study, by Kesserü et al., has already been discussed with respect to the effects of levonorgestrel on sperm.[144] The d-isomer of levonorgestrel was used in this study in a dose different from current formulations (i.e., 400 mcg tablets), and subjects were given different amounts of the drug during the follicular phase. Plasma LH was decreased with doses as low as one to three tablets (400 to 1200 mcg) per cycle. The midcycle peaks, however, were usually blunted but not abolished. More

---

[138] Hapangama et al., "Effects of Peri-ovulatory Administration of Levonorgestrel"; Durand et al., "On the Mechanisms of Action," 2001; Croxatto et al., "Pituitary–Ovarian Function"; Okewole et al., "Effect of Single Administration": Tirelli et al., "Levonorgestrel Administration in Emergency Contraception"; Durand et al., "Late Follicular Phase Administration," 2005; and Durand et al., "Hormonal Evaluation and Midcycle Detection," 2010.

[139] Cheng et al., "Interventions for Emergency Contraception."

[140] E. G. Raymond et al., "Bleeding Patterns after Use of Levonorgestrel Emergency Contraceptive Pills," *Contraception* 73.4 (April 2006): 380.

[141] Ibid., table 1, 376.

[142] E. Gainer et al., "Menstrual Bleeding Patterns following Levonorgestrel Emergency Contraception," *Contraception* 74.2 (August 2006): 118–124.

[143] Ibid., 121.

[144] Kesserü et al., "Hormonal and Peripheral Effects of d-Norgestrel."

importantly, the urinary pregnanediol (progesterone) values were significantly lower with doses of four and seven tablets (1600 and 2800 mcg) per cycle.[145]

In summary, when administered in the late follicular phase before the LH surge, LNG-EC has been shown to impair various aspects of luteal function. These luteal phase alterations, coupled with doubtful evidence supporting prefertilization MOAs, make it necessary to reconsider the interceptive nature of LNG-EC.

Fifth Proposed MOA: Effects on Endometrial Receptivity and Embryo Implantation

We now examine studies of the effects of LNG-EC on endometrial receptivity and embryo implantation. Such studies present special limitations, because the implantation of the human embryo is an extremely complex and poorly understood process. It has been described as "a three-stage process … involving synchronized crosstalk between a receptive endometrium and a functional blastocyst."[146] The embryo can implant in the endometrium only during "a self-limited period spanning between days 20 and 24 of a regular menstrual cycle (day LH +7 to LH +11). Throughout this … window of implantation, the human endometrium is primed for blastocyst attachment, given that it has acquired an accurate morphological and functional state initiated by ovarian steroid hormones."[147] The embryo is not an inactive bystander in this process, but interacts with the endometrium through a variety of molecular mediators, many of which are progesterone-dependent. "The cellular actions of [progesterone] are mediated through intracellular progesterone receptors (PRs), which are well-studied gene regulators."[148] Bruce Lessey points out that "as more is known about the gene products of the endometrium, it appears that many of the secreted products of the glandular epithelium function to support the nascent embryo" and are critical for continuing this early pregnancy.[149]

We can measure endometrial effects by the analysis of histological or endometrial biomarkers. Histological evaluation, while still important, is now being supplemented by gene and biochemical analysis.[150] A number of studies have

---

[145] Ibid., 417. Recall that the dose of levonorgestrel for LNG-EC is 1.5 mg (or 1500 mcg), a dose practically identical to the dose that Kesserü found to affect urinary pregnanediol (progesterone) levels.

[146] H. Achache and A. Revel, "Endometrial Receptivity Markers, the Journey to Successful Embryo Implantation," *Human Reproduction Update* 12.6 (November–December 2006): 731.

[147] Ibid.

[148] Bagchi et al., "Progesterone Receptor Regulated Gene Networks," 852.

[149] B. A. Lessey, "The Role of the Endometrium during Embryo Implantation," *Human Reproduction* 15.6 suppl (December 2000): 39.

[150] Gemzell-Danielsson notes, "The features of uterine receptivity include histological changes in which the endometrium becomes more vascular and edematous, [and] the endometrial glands display enhanced secretory activity. … In addition, multiple signals synchronize development of the blastocyst and the preparation of the uterus." Gemzell-

evaluated whether LNG-EC produces changes in the histological, electron microscopic, and biochemical characteristics of the endometrium.[151]

Studies of Endometrial Analysis

What does the evidence show? "A common side effect reported in long-acting implants and IUD-LNG is unscheduled vaginal bleeding, which is associated with glandular atrophy, increased decidualization, and aberrant endometrial steroid receptor expression."[152] Endometrial exposure to LNG is associated with down-regulation of sex steroid receptors in all cellular components. As a consequence of this down-regulation, "there is perturbation of progesterone-regulated locally acting mediators, and the integrity of blood vessel walls is disturbed."[153] This is why vaginal bleeding is reported in several of the studies in which LNG-EC was administered in the preovulatory phase.[154] Bleeding is a clinical sign of a disrupted luteal phase due to hormonal alterations.

The Yuzpe regimen, the most common emergency contraceptive regimen prior to LNG-EC, also produced histological changes and reduction in some endometrial receptors and receptivity genes, although it did not affect glycodelin.[155] Britt-Marie Landgren and colleagues studied the proliferative activity of the endometrium after it had been exposed to large doses of LNG at different stages of the cycle.[156] They found that many subjects exhibited insufficient luteal function. This study's main limitation is that LNG was given multiple times throughout the cycle, unlike the typical one-time dose given at a particular day in the cycle.

Danielsson "Emergency Contraception," 303. B. A. Lessey observes that traditional histologic dating of the endometrium, as proposed by scientists such as R. W. Noyes and others, "has remained the gold standard for nearly 50 years. Although the use of marker proteins provides additional information and may reflect endometrial function or receptivity, such markers cannot yet replace traditional methods of endometrial assessment." Lessey, "Use of Integrins to Date the Endometrium," *Fertility and Sterility* 73.4 (April 2000): 779.

[151] We have already examined the following studies: Marions et al., "Emergency Contraception"; Marions et al., "Effect of Emergency Contraception"; Durand et al., "On the Mechanisms of Action," 2001; Durand et al., "Late Follicular Phase Administration," 2005; do Nascimento et al., "In Vivo Assessment of the Human Sperm Acrosome Reaction"; Durand et al., "Hormonal Evaluation and Midcycle Detection," 2010; and Palomino et al., "A Single Midcycle Dose of Levonorgestrel."

[152] Palomino et al., "A Single Midcycle Dose of Levonorgestrel," 1592.

[153] A. Guttinger and H. O. Critchley, "Endometrial Effects of Intrauterine Levonorgestrel," *Contraception* 75.6 suppl. (June 2007): s93–s98.

[154] Raymond et al., "Bleeding Patterns," 376–381.

[155] E. G. Raymond et al., "Effect of the Yuzpe Regimen of Emergency Contraception on Markers of Endometrial Receptivity," *Human Reproduction* 15.11 (November 2000): 2351–2355.

[156] B. M. Landgren et al., "The Effect of Levonorgestrel Administered in Large Doses at Different Stages of the Cycle on Ovarian Function and Endometrial Morphology," *Contraception* 39.3 (March 1989): 275–289.

G. Ugocsai and colleagues studied endometrial surface changes by scanning electron microscope in three women who took high doses of levonorgestrel (about four to six times the standard emergency contraceptive dose).[157] They found that in comparison to controls, specimens displayed "marked restructuralization of the endometrium" and disappearance of pinopods (necessary implantational structures). They concluded that the contraceptive effect of LNG-EC was accomplished by alteration of the endometrial surface and, therefore, receptivity. Although these findings support LNG-EC changes of the endometrium, the paper's main limitation is the women's use of LNG-EC at higher than recommended doses. Nonetheless, Ugocsai notes that, although the surface alterations observed at higher doses may not be observed following normal emergency contraception use, "the underlying molecular changes, caused by levonorgestrel, may correspond to the contraceptive effect," which is described as "the 'phasing-out' of the endometrium . . . accomplishing effective endometrial contraception."[158]

In another study on the relationship between pinopods (which serve as implantational markers) and progesterone levels, Anneli Stavreus-Evers and colleagues found that "pinopod formation and regression were closely associated with increases and decreases, respectively, in serum progesterone concentration."[159] Pinopods, which are "bleb-like protrusions found on the apical surface of the endometrial epithelium," are preferred sites of embryo-endometrial interactions.[160] This study provides just one example of how structures that are necessary for implantation, like pinopods, are dependent on adequate serum progesterone levels. It is likely that other structures and molecules are adversely affected when a supra-physiological dose of a sex steroid like LNG-EC is administered during the fertile window.

Palomino and colleagues showed that a single midcycle dose of LNG-EC did not alter the expression of the L-selectin ligand (progesterone receptor) or molecular markers of endometrial receptivity.[161] When administered by means of IUD-releasing systems, LNG had been shown to alter glycodelin, endometrial progesterone-receptor expression, and histologic features of the endometrium,[162] but Palomino et al. speculate that a single oral dose of LNG-EC is not enough to affect measurement of the endometrial receptivity markers. We note that LNG-EC intake occurred on the day of the LH surge, which may have been too late to show a measurable effect on the LH-progesterone-mediated functions governing endometrial receptivity biomarkers. Indeed, endometrial biopsies from the treated subjects *did* show areas of irregular

[157] G. Ugocsai, M. Ròzsa, and P. Ugocsai, "Scanning Electron Microscopic (SEM) Changes of the Endometrium in Women Taking High Doses of Levonorgestrel as Emergency Postcoital Contraception," *Contraception* 66.6 (December 2002): 433.

[158] Ibid., 436.

[159] A. Stavreus-Evers et al., "Formation of Pinopodes in Human Endometrium Is Associated with the Concentrations of Progesterone and Progesterone Receptors," *Fertility and Sterility* 76.4 (October 2001): 782.

[160] Achache and Revel, "Endometrial Receptivity Markers," 732.

[161] Palomino et al., "A Single Midcycle Dose of Levonorgestrel," 1589–1594.

[162] Ibid., 1592.

development characterized by glandular atrophy and intense stromal decidualization.[163] Since implantational loss during natural cycles can approach 30 percent,[164] it would seem logical that any alterations in histologic features would further increase natural rates of embryonic loss. Nevertheless, Palomino et al. dismissed endometrial histology as a valid method for evaluating endometrial receptivity.[165]

In 2010, Chun-Xia Meng and colleagues studied the effects of four repeated oral doses of 0.75 mg LNG-EC administered at twenty-four-hour intervals on days LH +1 to LH +4, compared with a single vaginal dose of 1.5 mg LNG-EC on LH +2, to determine if these regimens affect the endometrium.[166] Oral LNG-EC was given to eight women, and endometrial biopsies were performed on days LH +6 to LH +8. Although the expression of two endometrial receptivity markers (the progesterone receptor and leukemia inhibitory factor) were significantly altered, the authors doubted that "these changes would be enough to prevent implantation."[167] The limitations of the study were the small number of women included and the timing of drug administration, which occurred on and after ovulation when it is known not to have efficacy.

In 2012, Macarena Vargas studied the effects of LNG-EC on the endometrium administered on day 1 of the luteal phase, one day after ovulation, when it has no clinical efficacy.[168] Endometrial biopsy was done on day LH +7 or LH +8, and various genes involved in the implantational process were examined. Not surprisingly, except for the level of one gene, all changes remained within the ranges observed in untreated controls.

Several studies discussed above under the heading "Third Proposed MOA: The Ability of Levonorgestrel Emergency Contraception to Prevent or Delay Ovulation" reported on endometrial histology and receptivity markers.[169] Durand 2001 examined endometrial samples from their subjects, although only twenty-four of the thirty-three

---

[163] Ibid., 1591.

[164] Norwitz et al. "Implantation and the Survival of Early Pregnancy," 1400, 1405; A. Revel, "Defective Endometrial Receptivity," *Fertility and Sterility* 97.5 (May 2012): 1028–1032; and A. J. Wilcox et al., "Time of Implantation," 1797–1798.

[165] Palomino et al., "A Single Midcycle Dose of Levonorgestrel," 1593. Histological dating has been the gold standard for over fifty years. Lessey, "Role of the Endometrium," 39–50.

[166] C. X. Meng, L. Marions, B. Bystrom, K. Gemzell-Danielsson, "Effects of Oral and Vaginal Administration of Levonorgestrel Emergency Contraception on Markers of Endometrial Receptivity," *Human Reproduction* 25.4 (April 2010): 874–883.

[167] Ibid., 881.

[168] M. F. Vargas et al., "Effect of Single Post-ovulatory Administration of Levonorgestrel on Gene Expression Profile during the Receptive Period of the Human Endometrium," *Journal of Molecular Endocrinology* 48.1 (January 2012): 25–36.

[169] Marions, "Emergency Contraception With Mifepristone," 69; Durand, "Late Follicular Phase"; Durand et al., "Hormonal Evaluation and Midcycle Detection," 2010; Durand et al., "On the Mechanisms of Action," 2001. Although Marions' small studies were inconclusive regarding a postfertilization MOA for LNG-EC, we do have information on

biopsies could actually be studied, and these came almost entirely from subjects who took LNG-EC at or after ovulation.[170] Yet on the basis of just three endometrial samples from subjects who took LNG-EC in the late follicular phase (day LH -3), the authors conclude that there were no histological endometrial effects. However, Durand's team reexamined the data on ovulating women in 2005 and noted diminished glycodelin-A levels in the group that received the LNG-EC three to four days before the LH surge (group 1). Importantly, glycodelin-A levels were significantly lower, despite the previous determination in 2001 that endometrial histology was normal. The authors note, "The low staining score for endometrial glycodelin-A in Group 1 indicates that intake of LNG before the LH surge has endometrial effects that are not identified by normal histology."[171] Moreover, the 2005 study clearly showed the progesterone-mediated deficiency of endometrial glycodelin-A. The latter is possibly an additional interceptive MOA in its own right, exerting its effects through impaired immunosuppressive activity affecting the implanting embryo.[172]

Studies on Embryo Implantation

We conclude with a discussion of the "implantational" studies, which have their own ethical and technical limitations.

P.G.L. Lalitkumar and colleagues used an artificial endometrial construct cultured with LNG-EC to assess impairment of embryo attachment.[173] Endometrial biopsies were performed on women who did not receive LNG-EC in the critical late follicular period. In fact, they were *never* exposed to LNG-EC in vivo.[174] Endometrial cells were removed at day LH +4, which is during the luteal phase before the "implantation window,"[175] and only after removal were exposed to LNG-EC in vitro. Not surprisingly, LNG-EC did not significantly impair the ratio of embryo attachment when compared with non-LNG-exposed endometrial culture. The artificial

endometrial biopsies taken from six of the women who were given LNG-EC at day LH -2. Of the findings for six biopsies, only three were reported as "normal."

[170] B. Mozzanega and E. Cosmi, "How Do Levonorgestrel-Only Emergency Contraceptive Pills Prevent Pregnancy? Some Considerations," *Gynecological Endocrinology* 27.6 (June 2011): 439–442.

[171] Durand et al., "Late Follicular Phase Administration," 2005, 456.

[172] M. Kämäräinen et al. "Normal Human Ovary and Ovarian Tumors Express Glycodelin, a Glycoprotein with Immunosuppressive and Contraceptive Properties," *American Journal of Pathology* 148.5 (May 1996): 1435–1443.

[173] P.G.L. Lalitkumar et al., "Mifepristone, but Not Levonorgestrel, Inhibits Human Blastocyst Attachment to an In Vitro Endometrial Three-Dimensional Cell Culture Model," *Human Reproduction* 22.11 (November, 2007): 3031–3037.

[174] See Davis, "Plan B Agonistics," 741–772, note 18, and in his letter in the same issue of the *National Catholic Bioethics Quarterly* (Winter 2010): 641.

[175] The implantation window, the limited period when the human endometrium is receptive to the embryo and allows implantation, begins on approximately day 6 after the LH peak (LH +6) and completes by LH +10 (or days 20 to 24 of an idealized twenty-eight-day cycle. See Meng et al., "Effects of Oral and Vaginal Administration," 875; and Achache and Revel, "Endometrial Receptivity Markers," 731.

environment the researchers created and the lack of preovulatory exposure made it impossible to ascertain the effects of the LNG-EC. The research also raises other issues (e.g., in the use of frozen or aged embryos), which are beyond the scope of this paper. We concur with the objections raised by Marie Hilliard, who has also pointed out that the study's methodology is not designed to demonstrate whether or not LNG-EC in vivo has anti-implantational endometrial effects.[176]

Meng and colleagues carried out a similar study.[177] They obtained cells by endometrial biopsy at days LH +4 to LH +5 and, using a three-dimensional stromal model (in vitro), examined the effect of LNG-EC on the expression of endometrial receptivity factors. Treatment with LNG-EC given several days after ovulation did not affect endometrial receptivity factors. Again, the women were not given LNG-EC during their fertile window before the biopsy samples were obtained. Endometrial changes will not be apparent if a woman is not exposed to LNG-EC during the critical preovulatory time period.

In conclusion, some data show that LNG-EC can lead to histological and endometrial changes that could impair embryo implantation. Moreover, there are serious questions about the existing studies that claim it has no effect on implantation.

A Moral Evaluation of Emergency Contraception

The human embryo is a nascent human being from the time the ovum is fertilized by a sperm.[178] This new human individual is genetically the same being as the adult who develops over many years. The destruction of a human embryo is contrary to the dignity of the nascent human being, and is therefore gravely wrong. In *Evangelium vitae*, Blessed John Paul II affirmed that, regardless of debates over the moment of ensoulment, the embryo must be treated as a person: "The human being is to be respected and treated as a person from the moment of conception; and therefore from that same moment his rights as a person must be recognized, among which in the first place is the inviolable right of every innocent human being to life."[179]

It has been accepted practice in Catholic medical ethics for a woman who has been raped to take certain acts by which she seeks to prevent conception, including

[176] Marie T. Hilliard, "Plan B's Abortifacient Effect," letter, *National Catholic Bioethics Quarterly* 8.1 (Spring 2008): 9–11.

[177] C. X. Meng et al., "Effect of Levonorgestrel and Mifepristone on Endometrial Receptivity Markers in a Three-Dimensional Human Endometrial Cell Culture Model," *Fertility and Sterility* 91.1 (January 2009): 256–264.

[178] N. López-Moratalla, E. Santiago, and G. Herranz Rodríguez, "Inicio de la Vida de Cada Ser Humano: Qué Hace Humano el Cuerpo del Hombre?" *Cuadernos di Bioética* 22.75 (May–August 2011): 283–308, accessed at http://arvo.net/uploads/file/ACRE/15%20BIOETICA%2075–5.pdf.

[179] John Paul II, *Evangelium vitae* (March 25, 1995), n. 60, quoting Congregation for the Doctrine of the Faith, *Donum vitae* (February 22, 1987), I.1.

douching to remove semen.[180] In such circumstances, the victim seeks to impede the effects of the sexual attack, which may include an unwanted pregnancy charged with psychological pain and social difficulties. Based on the notion of self-defense from an aggressor, it is argued that a drug that can prevent fertilization would justly prevent an unwanted pregnancy after rape. This concept has been expressed as directive 36 of the *Ethical and Religious Directives for Catholic Health Care Services*, which states, "If, after appropriate testing, there is no evidence that conception has occurred already, she [a victim of sexual assault] may be treated with medications that would prevent ovulation, sperm capacitation, or fertilization. It is not permissible, however, to initiate or to recommend treatments that have as their purpose or direct effect the removal, destruction, or interference with the implantation of a fertilized ovum."[181] In this context, we examine the morality of the use of LNG-EC after rape. Our conclusion is that LNG-EC cannot be used in rape treatment protocols. However, until very recently the scientific data concerning its MOA left the moral analysis of this question in doubt. That is no longer the case.

The data available from the 2010 and 2011 Noé studies indicate that in as many as 80 percent of the women treated with LNG-EC, ovulation was not prevented and yet no pregnancies resulted, thus pointing to a postovulatory effect of the drug. (Without such an effect, a number of women who ovulated would have been likely to conceive.) The absence of confirmed pregnancies strongly suggests that in some cases embryos were unable to implant.[182]

The data available from the studies analyzed above (under the heading "Effects on the Corpus Luteum") suggest that LNG-EC produces impairment of the LH surge, leading to inadequate corpus luteal support that is manifested in luteal phase shortening and altered luteal hormone levels, all of which have the effect of interfering with the implantation of embryos. If LNG-EC is to be used in Catholic hospitals, it is necessary to establish moral certitude that its MOA is not abortifacient (interceptive). These findings establish sufficient doubt to preclude the necessary moral certitude. It is not possible to provide, for a woman or a health care provider treating her, the necessary moral certainty that taking or administering LNG-EC poses no significant risk to the present or future well-being of a human embryo that has already been conceived or will be conceived in the immediate future.

Thomas V. Berg and colleagues have used the principle of double effect to analyze the use of LNG-EC in Catholic health care.[183] The principle applies moral analysis to an action that has both a good and a bad effect. It has been used in cases

[180] William E. May, *Catholic Bioethics and the Gift of Human Life* (Huntington, Indiana: Our Sunday Visitor, 2000), 154–155.

[181] US Conference of Catholic Bishops, *Ethical and Religious Directives for Catholic Health Care Services*, 5th ed. (Washington, DC: USCCB, 2009).

[182] Noé et al., "Contraceptive Efficacy" 2010, 414–420; Noé et al., "Contraceptive Efficacy" 2011, 486–492.

[183] Thomas V. Berg, Marie T. Hilliard, and Mark F. Stegman, "Emergency Contraceptives and Catholic Healthcare: A New Look at the Science and the Moral Question," *Westchester Institute White Paper Series* 2.1 (June 2011): 20.

of lethal-force self-defense, treatment of ectopic pregnancy and gravid uterus, use of opiates in gravely ill patients, and tactical bombing. In each of these scenarios, there are always two effects, a good one and an evil one. For instance, in lethal-force self-defense, the good effect is the preservation of one's life and integrity and the bad effect is the injury or death of the assailant. If the effects of a moral action are *either* good or bad, and not both, however, then the morality of an action can be decided without recourse to the principle of double effect.

The criteria traditionally applied in double-effect reasoning are that "(1) the act considered independently of its evil effect is not in itself wrong; (2) the agent intends the good and does not intend the evil either as an end or as a means; and (3) the agent has proportionately grave reasons for acting, addressing his relevant obligations, comparing the consequences, and, considering the necessity of the evil, exercising due care to eliminate or mitigate it."[184] In his monograph on double effect, Cavanaugh notes that the order in which the criteria are applied is important: "The first condition excludes the application of the remaining conditions to acts impermissible in kind."[185] In other words, acts that are morally evil in themselves can never be justified by the principle of double effect.

LNG-EC acts in various ways when administered in the preovulatory fertile window, which is its only possible efficacious use. Less than 20 percent of the time, its use may inhibit ovulation. More than 80 percent of the time, ovulation occurs, so another MOA must account for its efficacy. As we have shown, some supposed prefertilization effects related to sperm or ovulation are doubtful and some are precluded. It is equally or more likely that LNG-EC impedes implantation of the blastocyst. But since any anti-implantation effect presumes prior fertilization, it is not possible for a prefertilization effect and the prevention of implantation to occur simultaneously. Accordingly, resort to the principle of double effect cannot be justified.

Berg holds that the principle of double effect can be applied to this analysis and that in some cases all the criteria for the licit use of LNG-EC can be met: "This is particularly true in the case of a victim presenting with a negative LH test and with a history supporting the likelihood that the victim is not peri-ovulatory, even if there is the possibility that she might ovulate despite the administration of LNG. If the object and intent are to prevent ovulation and if circumstances to achieve the object and intent are documented, then the principle of double effect can be invoked

[184] Thomas A. Cavanaugh, "Double-Effect Reasoning, Craniotomy, and Vital Conflicts: A Case of Contemporary Catholic Casuistry," *National Catholic Bioethics Quarterly* 11.3 (Autumn 2011): 454. Rev. Joseph Mangan describes the same but states it in four conditions instead of three: "All four conditions in this principle must be present at one and the same time, namely, the ultimate end of the author must be good, the cause of the effects must be good or at least indifferent, the evil effect must not be the means to the good effect, and there must be a proportionately serious reason for actuating the cause." Joseph T. Mangan, "An Historical Analysis of the Principle of Double Effect," *Theological Studies* 10 (1949): 60.

[185] Thomas A. Cavanaugh, *Double Effect Reasoning: Doing Good and Avoiding Evil* (Oxford, UK: Oxford University Press, 2006), 32.

in the administration of LNG to a sexual assault victim."[186] However, the necessary precondition for use of the principle is that the action at issue have two effects, a good one *and* an evil one, not just one effect (e.g., a good one like preventing ovulation *or* a bad one like impeding implantation).[187] Moreover, the first criterion for the use of the principle—that the action is not in itself wrong—cannot be met in cases involving the use of LNG-EC.

Nonetheless, assuming for the sake of argument that the precondition and first criterion for acting licitly can be met; the third criterion—that the agent has proportionately grave reasons for acting—still cannot be met. Although an abortive effect can be considered unforeseen, because it is not likely to occur every time LNG-EC is used, the moral agent should be informed that an abortive effect is not impossible, another MOA is doubtful or not likely, and in a significant number of women implantation is likely to be impeded.[188] Thus, it is not clear that a rape victim or those providing her health care have proportionally grave reasons for using LNG-EC when they cannot preclude to a moral certitude the substantial risk it poses to embryonic life.

For some years, various Catholic health care facilities have attempted to provide a moral justification for the use of LNG-EC in cases of rape. Some hospitals have developed protocols designed to detect the LH surge in women who were raped, for the purpose of administering LNG-EC to those who had not ovulated. The reasoning behind such protocols is straightforward: if ovulation has occurred, fertilization may follow, and LNG-EC could harm the embryo. The so-called ovulation approach, or Peoria Protocol, seeks to detect imminent ovulation by testing urine or serum LH

[186] Ibid. Without entering into a detailed discussion about the moral object, we make the following observation: The only time when LNG-EC administration is efficacious is during the fertile window before ovulation (the LH surge). When administered within that window, ovulation will occur in the vast majority of cases. Suppression of ovulation can only be confidently predicted if LNG-EC is administered during the earliest phase of that window, when the likelihood of pregnancy is already low. Therefore, the true object of LNG-EC is not the suppression of ovulation but the prevention of pregnancy by whatever means is operative, and that is likely to be by a postfertilization MOA in most cases. This view of the proper object shows that the act of taking or providing LNG-EC is morally evil.

[187] German Grisez explains that the traditional analysis of PDE requires that the good and evil effects be realized simultaneously. *The Way of the Lord Jesus,* vol. 1, *Christian Moral Principles* (Quincy, Illinois: Franciscan Press, 1983), 307. Furthermore, the good effect should not be produced by means of the evil effect. For further analysis of the PDE, see Davis, "Plan B Agonistics," 766–767.

[188] Some think that, when an unforeseen effect is minor, it can be tolerated, but if the unforeseen effect is something gravely wrong, the agent does not have proportionately grave reasons for acting. Although Monsignor William Smith mistakenly held that the ovulation test could be sufficient, he expressed the moral teaching that *probabilism* cannnot be applied when a third party is in danger. See William B. Smith, "Questions Answered," *Homiletic and Pastoral Review* 104.6 (March 2004): 68–70, reprinted in *Modern Moral Problems: Trustworthy Answers to Your Tough Questions*, ed. Donald Haggerty (San Francisco: Ignatius Press, 2012), 150.

and then, if the results are positive, testing for serum progesterone levels. A modified Peoria Protocol uses a simple over-the-counter LH test.[189]

But it is pointless to test for the LH surge. A negative result does not preclude pre-LH surge fertile window timing when prefertilization MOA may be operative. Neither is there any rationale for rape protocols to identify a supposed safe period prior to ovulation for administration of LNG-EC because it is now clear that at such a time administration of LNG-EC is likely to have post-ovulation effect rather than impede ovulation or it would be outside the fertile window and meaningless to prevention of pregnancy.

The question of justice and informed consent is also very important. It has received some attention but not enough.[190] Respect for patients and their autonomy calls for true informed consent. Even a small but realistic possibility of an abortifacient MOA obliges health care providers to disclose this information to patients. However, when scientific evidence points to an even higher likelihood of postfertilization effect on embryos, women have an even greater right in justice to be provided with this information so that they can reach an informed decision. Dr. James Trussell, a contraceptive expert and advocate of emergency contraception, has acknowledged that administration of LNG-EC during the periovulatory phase may have a postfertilization effect, and that women have a right to know about this MOA.[191]

---

[189] The Peoria Protocol administers a test for preexisting pregnancy along with a urine LH test for ovulation. If the urine LH test results are positive, indicating that the hormonal shift is under way, a serum progesterone test is also administered. A serum progesterone level greater than 1.5 ng/mL is an indicator that ovulation has occurred. A modified Peoria Protocol does not require a serum progesterone test because this test is not as readily available. Marie Hilliard, writing for The National Catholic Bioethics Center, discusses different protocols for administering emergency contraception to victims of sexual assault. (Marie T. Hilliard, "*Dignitas personae* on Caring for Victims of Sexual Assault: A Commentary on *Dignitas personae*, Part Two, n. 23," http://www.ncbcenter.org/page.aspx?pid=1314.) She says that in all cases, "The National Catholic Bioethics Center holds that administration of EC must be consistent with [directive] n. 36—to 'prevent ovulation, sperm capacitation, or fertilization.' Current research indicates that the impact of EC on sperm capacitation is not fast enough to prevent fertilization. Therefore, *the only reason for which EC morally can be given is to prevent ovulation*" (emphasis added). Since the 2010 and 2011 Noé data show that prevention of ovulation is highly unlikely when LNG-EC is taken during the fertile window, before the LH surge, it is clear that the NCBC standard for licit use of LNG-EC cannot be met on the basis of LH testing, since a negative test result cannot preclude ovulation and subsequent interception. The new data from Noé et al. show the need for formal review and revision of the NCBC position on LH testing.

[190] This question received substantial attention from members of the FDA Advisory Committees on December 16, 2003, at a meeting that drove the subsequent FDA scientific review that resulted in the current labeling information about a possible interceptive MOA. See Davis, "Plan B Agonistics," 757.

[191] "To make an informed choice, women must know that [emergency contraceptive pills]—like all regular hormonal contraceptives …—may prevent pregnancy by delaying or inhibiting ovulation, inhibiting fertilization, or inhibiting implantation of a fertilized

A Duty to Reexamine the Evidence

Recent scientific evidence shows that LNG-EC does *not* work by preventing ovulation as its sole or dominant mechanism.[192] In the largest study on the MOA of LNG-EC, inhibition of ovulation has been shown in only 20 percent of the women who receive it in the late follicular phase. The majority of other studies also indicate only a small suppression of ovulation when LNG-EC is taken during the critical fertile window, and negligible effects on cervical mucus or sperm function. The efficacy of LNG-EC cannot be explained by MOAs that have only preovulatory effects. Often, contrary to authors' conclusions, many of the studies provide compelling evidence for postfertilization MOA. When LNG-EC is given in the late follicular phase it may cause the following: (1) altered LH peak and duration, (2) inadequate luteinization of the follicle, (3) diminished luteal LH or progesterone levels, (4) shorter luteal phase, and (5) endometrial changes that are likely to interfere with implantation of the embryo. We are thus persuaded that preovulatory administration of LNG-EC often has postfertilization or interceptive effects.

Physicians and health care institutions, especially Catholic ones, have a duty to reexamine the available scientific information on LNG-EC. They have an obligation to offer the Holy See and episcopal conferences accurate information regarding this subject to guide their statements. The use of LNG-EC and associated rape protocols should be abandoned, because there is no safe period to give LNC-EC during a woman's cycle when it may be efficacious to prevent pregnancy without significant likelihood that it will have an abortifacient effect.

egg in the endometrium." J. Trussell and B. Jordan, "Mechanism of Action of Emergency Contraceptive Pills," editorial, *Contraception* 74.2 (August 2006): 88.

[192] While this article was being prepared for publication, Vivian Brache and colleagues, including Croxatto, published an important study comparing levonorgestrel and ulipristal acetate emergency contraception: V. Brache et al., "Ulipristal Acetate Prevents Ovulation More Effectively Than Levonorgestrel: Analysis of Pooled Data from Three Randomized Trials of Emergency Contraception Regimens," *Contraception* 88.5 (November 2013): 611–618. They compared LNG-EC to ulipristal, to LNG-EC plus meloxicam, and to placebo, and found that ulipristal was the most effective in delaying ovulation. LNG-EC was found to be no more effective than placebo in preventing ovulation when given in the late follicular phase. The authors admit that it is not known whether ovulatory dysfunction actually exists as a possible MOA for LNG-EC: "Whether the abnormal blunted or absent LH peak preceding follicular rupture in the LNG-treated cycles in which rupture occurs contributes to the alteration of the ovulatory process and has any clinical consequence is unknown but is biologically plausible" (617).