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REVIEW

How do levonorgestrel-only emergency contraceptive pills prevent pregnancy? Some considerations

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Abstract
Controversial opinions exist about the possible mechanisms throughout emergency contraception prevents pregnancy. Recently, the International Federation of Gynaecology and Obstetrics and the International Consortium for Emergency Contraception released a Joint Statement declaring that 'inhibition or delay of ovulation should be their primary and possibly only mechanism of action'. They still added that 'Review of the evidence suggests that LNG-ECPs cannot prevent implantation'. Concerning levonorgestrel-only emergency contraceptive pills effects on ovulation, the Statement based on seven reference papers which considered a total of only 142 patients, divided into still different subgroups. Basing on their same references we got quite different conclusions.

Keywords: *Emergency contraception, mechanism of action, ovulation, implantation*

Introduction

In Literature the question 'How do levonorgestrel-only emergency contraceptive pills (LNG-ECPs) prevent pregnancy?' is still quite open.

Recently, ICEC and FIGO Experts affirmed [1] that LNG-ECPs 'inhibit or delay ovulation' and that 'this should be their primary and possibly only mechanism of action'. In support the Experts quoted seven papers [2–8] and concluded that evidence shows that LNG ECPs inhibit the pre-ovulatory luteinizing hormone (LH) surge.

Thereafter, basing on other papers [2,3,9–14], they added that 'Review of the evidence suggests that LNG-ECPs cannot prevent implantation'. Lastly, they looked for effects on sperm functions [15–18] but failed to reach any conclusive positions.

In this article, we will discuss the Statement's issues basing our analysis on its same references [2–21] and following the same item-order: ovulation, implantation and sperm functions.

Of course, the unprotected sex for which ECPs are suitable is that occurring in the pre-ovulatory fertile period that lasts about 4–5 days and ends around ovulation. Accordingly, ECPs use is investigated properly [2–8] only from the beginning of this period up to 48 h after ovulation.

LNG-ECPs impact on ovulation

Concerning ovulation ICEC and FIGO Experts concluded that evidence shows that 'LNG-ECPs inhibit the pre-ovulatory luteinizing-hormone surge, impeding follicular development and maturation and/or the release of the egg itself'. In support of their issue they quoted seven papers [2–8]: of them, three relied on urinary-hormones [2,4,5] while the others [3,6–8] associated also plasma-hormone assay; all the authors, but Hapanagama et al. [4], used ultrasounds to monitor follicular growth. Every study declared that enrolled women were healthy volunteers with regular cycles.

Hapanagama et al. [4] studied 12 patients: four of them assumed LNG-ECPs (0.75 mg $\times$ 2) $\geq$3 days before LH surge and their ovulations were delayed; one was treated on the day LH$-$2 and did not ovulate at all. Seven patients apparently ovulated but exhibited reduced luteal LH levels and a shortened luteal phase: most of them were treated on day LH$-$1, one on the day LH$-$0.

Marions et al. [2] administered LNG-ECPs (0.75 mg $\times$ 2) to six women twice: the first time on the day LH$-$2; the second time after ovulation, on the day LH+2. She evaluated both the urinary hormonal levels and an endometrial biopsy obtained in the day LH+[6–8], that is 6–8 days after LH surge.

LNG pre-ovulatory treatment inhibited the LH peak; however, both the cycle length and oestrone and pregnanediol-glucuronide levels were similar to those of the control cycle. The post-ovulatory treatment did not affect the cycle pattern, but the endometrium was normal only in three patients (50%), out of phase in two and insufficient in one.

Subsequently [5], she administered the same treatment to seven women on the cycle-day LH$-$2 and evaluated both the urinary hormones behaviour and the destiny of the leading-follicle: a clear rise in LH/creatinine ratios was observed in four of the seven cycles on days LH + 1 and LH + 2, with an equally delayed rise in progesterone levels. However, the leading-follicles observed at ultrasounds were either arrested ($n=3$) or growing continuously until menses ($n=4$), suggesting that ovulation did not occur.

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Durand et al. [3] studied 45 women; after a control-cycle, she administered LNG-ECPs (0.75 mg × 2) at different times of the cycle: on day 10 (15 patients, Group A), on the day LH−0 (surge) (11 patients, Group B), on the day LH+2 (11 patients, Group C) or in the late follicular phase LH−(3 ± 1) (8 patients, Group D). Transvaginal ultrasound and serum LH were performed daily since the detection of urinary LH until follicular rupture. Serum oestradiol and progesterone were measured during the complete luteal phase. An endometrial biopsy was taken at day LH+9.

Twelve patients in Group A were anovulatory (80%), the remaining three did ovulate but presented a significantly shorter luteal phase with notably lower progesterone levels. All participants in Groups B, C and D had ultrasonographic findings of follicular rupture, with no change in the cycle-day of its occurrence. In Groups B and C, no significant differences on either cycle length or luteal progesterone and oestradiol concentrations were observed, while in Group D the cycle length was normal but progesterone luteal levels were significantly lower. As to endometrial histology, the author concluded that it was normal in all the ovulatory treated-cycles. However, she reported that only 24 of the 33 biopsies from these cycles could be studied, while nine specimens were excluded: all the three from Group A and four from Group D (50%) because they were 'out of phase', one more from Group D and one from Group B because they were insufficient. Consequently, the 24 evaluated biopsies came almost entirely from the Groups B and C, the ones in which LNG had been given at or after ovulation.

Okewole et al. [7] administered a single LNG-dose (1.5 mg) to 14 women in the peri-ovulatory period. Eight women (Group A) were treated on the day LH−3, while six (Group B) on the day LH−1. LNG significantly prolonged the mean cycle length in the Group A with a delay in LH peak around 96–120 h. In the Group B, on the contrary, there was a shortening of the whole cycle length (5 days), while the LH peak was only insignificantly delayed (24 h). The author concluded that LNG administration at late follicular phase (Group B) did not interfere with oestradiol-mediated mid-cycle gonadotrophin-surge and probably ovulation, but did alter progesterone production by the corpus luteum with a significantly shorter luteal phase.

Last but not least, the work by Croxatto et al. [6]: he alternatively administered either LNG-ECPs (0.75 mg × 2), or LNG-ECPs (0.75 mg once) or placebo to 58 women presumed healthy and normally cycling. The patients were randomly assigned to three groups: one received the treatment when the leading-follicle reached a mean diameter of 12–14 mm ($n = 18$); the second when the follicle-diameter was 15–17 mm ($n = 22$); the third when the follicle reached ≥18 mm ($n = 18$). Transvaginal ultrasonography (TVU) was used to assess the mean diameter of the leading-follicle and the occurrence of follicular rupture. After the pre-assigned follicular diameter was reached, daily TVU was performed for the next 5 days and continued twice a week until menses in case of missed follicular rupture. Blood samples for hormone assay were taken daily since the treatment-day for 6 days, and then twice a week until menses.

After receiving placebo only 59% of the women ovulated normally. They were presumed healthy and normally cycling, but the spontaneous occurrence of either lack of follicular rupture or ovulatory dysfunction was rather high: up to 62% when given placebo at a follicle-diameter of 12–14 mm, 45% at 15–17 mm and 13% when the follicle-diameter already reached ≥18 mm.

After LNG-treatment, the overall proportion of cycles with either lack of follicular rupture or ovulatory dysfunction was close to 82%, while it was 41% with placebo ($p < 0.001$). If both the events were grouped together, one or the other was present in 97%, 88% and 57% of cycles when LNG was administered with a follicle of 12–14 mm, 15–17 mm or ≥18 mm, respectively.

Croxatto's data deserve more than a comment.

First, volunteers were presented as regularly cycling, but only 59% had normal ovulation in their LNG-free cycles.

Second, ovulatory dysfunction has been defined as a follicular rupture either not preceded by a LH peak, or preceded by a blunted LH peak (<21 IU/l), or not followed by elevation of serum progesterone >12 nmol/l. The experimental data quoted by Croxatto himself reported that the probability of conception in spontaneous cycles was related to the pattern of LH surge, however, it was only reduced [22] but not abolished when the LH surge was blunted or short.

Third, Croxatto himself, quoting his own data [6], reported that 'large variation is observed in the length of time it takes a given-size follicle to rupture; for instance, in a group of 24 women it took 4–10 days for follicles that were 12–14 mm to reach the day of rupture'; this means that the 18 women presenting such leading-follicles are likely not to be in their fertile period yet, or maybe that only few of them are just at its beginning.

Nevertheless, even supposing that either Croxatto's randomisation criteria were suitable and all his considerations were correct, we cannot pass over the fact that ovulation could never be excluded, even when LNG was administered at the earliest stages of follicular development, and that the larger was the leading-follicle, the higher was the probability that both ovulation occurred and fertilization could follow. This probability would appear still higher if women treated before the fertile period was carefully excluded.

Considering the aforementioned papers altogether, we observe that only Marion's series [5] (seven patients) supports the Statement directly. In all the others the evidences are strikingly contrasting, even if we imagined a high rate of unproven follicular luteinizations. Moreover, the 24 patients in which LNG really delayed or inhibited ovulation [3,4,7] were at the beginning of their fertile period (days 10 or LH−≥3) when they received ECPs and so were the 18 patients [6] treated when the leading-follicle had a mean diameter of 12–14 mm; unprotected sex in any previous day did likely occur when they were still infertile and the risk of pregnancy was likely null.

At last, the total number of patients is really too small (only 142), besides the fact that they are divided into six different studies and then into still different subgroups. In our opinion, this sample is not representative and is unsuitable to allow any conclusive remarks.

LNG ECPs impact on implantation

As reported earlier, the Experts stated that 'Review of the evidence suggests that LNG-ECPs cannot prevent implantation of a fertilized egg' [2,3,9–14] and that 'Language on implantation should not be included in LNG-ECP product labelling'.

In some of the aforementioned studies [2–4,7], a short or inadequate luteal phase did follow ovulation and in some patients who did ovulate the luteal endometrium was out of phase; these observations cannot lead straight to conclusions, but they suggest some kind of difficulty for the implanting embryo.

Durand et al. [11], some years later, added information that appears still more important and that is quoted by ICEC and FIGO Experts. Using the same material of her previous study [3] retrospectively, she evidenced a low staining-score for endometrial glycodelin-A in the patients treated by LNG on the day LH $-(3 \pm 1)$, the same patients she previously described as Group D: the ones that ovulated but had significantly lower progesterone luteal levels. This endometrial effect is not identified by normal histology and may reflect either a direct effect of LNG or an indirect effect due to an insufficient progesterone support by the corpus luteum. Considering the inhibitory effects of glycodelin-A on the natural killer cells, abundant at the implantation site, reduced endometrial glycodelin-A expression may indicate a weakened immunosuppressive microenvironment at the foeto-maternal interface at the crucial time of implantation [11].

Conversely, two studies from Sweden [10,12] demonstrated that LNG did not inhibit either the endometrial attachment of human blastocysts *in vitro*, and the expression of the progesterone-regulated endometrial receptivity-factors. In the ICEC and FIGO Joint Statement, these findings are claimed to support the absence of any anti-implantation effects for LNG-ECPs.

Let us examine both the studies.

In the first, Lalitkumar et al. [12] demonstrated that LNG did not inhibit human blastocyst attachment to an *in vitro* endometrial three-dimensional cell culture model. In their experiment, they obtained and cultured physiological secretory endometrial tissues from 22 healthy volunteers with normal menstrual cycles and proven fertility. The samples were obtained on cycle-days LH + 4 to LH + 5, which were carefully determined by the individuation of the LH peak in urine samples collected twice daily. After culturing the cells and organising the model, they evaluated the implantation rate of human blastocysts when progesterone alone or progesterone and LNG were added to the culture medium. They observed quite similar implantation rates and concluded that LNG did not inhibit implantation.

In the second study [10], the same group demonstrated that the same three-dimensional stromal and epithelial cell co-culture model did express the progesterone-regulated endometrial receptivity-factors observed in quite physiologic conditions and that the treatment with LNG did not affect their expression.

The findings from both the Swedish papers could be hardly claimed to support the hypothesis that LNG-ECPs do not inhibit embryo implantation.

In fact, the luteal endometrium in both the experiments was obtained from normal cycling women who did not use LNG-ECPs during their fertile period. Biopsies were taken during the luteal phases of normal untreated cycles, after a careful individuation of the LH peak. This model will never be representative of the endometrial tissue of cycles in which LNG-ECPs were administered in the pre- or peri-ovulatory period. The results of these studies might support only the fact that LNG-ECPs, when administered 4–5 days after fertilization, cannot inhibit the embryo implantation; however, this is clearly not the case of ECPs.

LNG ECPs impact on cervical mucus and sperm functions

Research denies any effect of LNG, at the doses used in ECPs, both on sperm functions [21] and on the quality of cervical mucus and on the penetration of spermatozoa into the uterus. In fact, viable spermatozoa were found in the genital tract 36–60 h after coitus and 24–48 h after LNG intake [18].

In conclusion, at present literature data do not seem to fully support that LNG-ECP avoid pregnancies by inhibition of ovulation.

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