A single midcycle dose of levonorgestrel similar to emergency contraceptive does not alter the expression of the L-selectin ligand or molecular markers of endometrial receptivity

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Objective: To examine the effects of a single-dose of 1.5 mg of levonorgestrel (commonly used as emergency contraceptive) on endometrial receptivity biomarkers through the oral or vaginal route.
Design: Prospective randomized single-blinded trial.
Setting: Affiliated Hospital and University Research Center.
Patient(s): Fertile normal women previously sterilized by tubal ligation.
Intervention(s): Levonorgestrel (1.5 mg) was administered on the day of LH surge either orally (n = 14) or vaginally (n = 13).
Main Outcome Measure(s): Molecular assessment of endometrial progesterone receptors, L-selectin ligand, glicodelin-A and $\alpha v\beta 3$ integrin by Immunohistochemistry and reverse transcriptase-polymerase chain reaction.
Result(s): Plasma progesterone concentration and endometrial dating were not different. The pattern of progesterone receptors and glycodelin-A expression was not affected during the early and midsecretory phase. Some endometrial biopsies from the group in which levonorgetrel was orally administered showed areas of glandular atrophy and stromal decidualization. However, the expression of the progesterone receptor, L-selectin ligand, $\alpha v\beta 3$ integrin, and glycodelin-A were not different between the groups.
Conclusion(s): Levonorgestrel, given as emergency contraceptive on the day of LH surge, does not disrupt either ovulation or progesterone production by the corpus luteum. The contraceptive mechanism of levonorgestrel at the time of LH surge does not include changes in the progesterone receptors or the endometrial receptivity biomarkers. (Fertil Steril® 2010;94:1589–94. ©2010 by American Society for Reproductive Medicine.)

Key Words: Levonorgestrel, midcycle administration, progesterone receptor, L-selectin ligand

Levonorgestrel is a synthetic progestin that has been used for emergency contraception (LNG-EC) with clinical success. However, its mechanism of action is not completely understood (1, 2). It has been demonstrated that LNG-EC administration during the proliferative phase effectively inhibits or delays ovulation, whereas any effect on endometrial morphology is observed when it is administered after ovulation (3, 4). However, the reproductive process that is inhibited by LNG-EC when it is administered on the day of LH surge remains unclear.

Because levonorgestrel (LNG) is a potent progestin, the high dose delivered as LNG-EC could affect the steroid receptor expression, abundance, or tissue distribution. An abnormal steroid receptor expression could, in turn, interfere with the endometrial transformation and maturation process, making the uterus unable to interact or facilitate the embryo implantation. The human implantation process occurs during a narrow period of the secretory phase during which the endometrium becomes receptive. The molecular phenotype of this critical reproductive time is characterized by epithelial progesterone receptor (PR) down-regulation and concomitant expression of endometrial receptivity biomarkers (5).

In this study, we test the hypothesis that a single 1.5-mg dose of LNG-EC delivered either by the oral or vaginal route at the time of LH surge affects the endometrial receptivity molecular phenotype. We assessed the expression of PR by examining both the total AB (PR-AB) and B (PR-B) isoforms during the early and midsecretory phases. The molecules recognized as possible uterine receptivity biomarkers, L-selectin ligand (LS-L), glycodelin-A (Gly-A), and the $av\beta 3$ integrin subunit, were evaluated in the endometrial biopsies from women treated with LNG-EC and controls.

MATERIALS AND METHODS

Fertile women who were previously sterilized by tubal ligation were invited to participate in this study. After signing an informed consent, 38 subjects were enrolled. The institutional review board of the San Borja Arriaran Clinical Hospital, Santiago de Chile approved the study protocol.

Beginning on day 10 of the menstrual cycle, ovulation were followed and confirmed by ultrasound using a 7.5-MHz transvaginal probe (Model SA 6000; Medison, Seoul, Korea) and by the detection of urinary LH surge (Clear-plan ovulation test; Unipath Limited, Betford, UK).

In a randomized single-blinded fashion, a single 1.5-mg dose of LNG was administered on the day of LH surge (day = 0) to 14 subjects by the oral route and 13 by the vaginal route; 11 participants did not receive any treatment.

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TABLE 1

Demographic, clinical, endocrine, and endometrial features and HSCORE values of the participants treated with levonorgestrel as an emergency contraceptive and controls.

Variable	Controls (n = 11)	LNGEC-O (n = 14)	LNGEC-V (n = 13)	*P* value
Age (y)	36 ± 4.6	35.2 ± 4.9	35.8 ± 3.2	NS
BMI	27.9 ± 2.4	25.9 ± 3.4	26.7 ± 2.6	NS
Cycle length before study (d)	28.6 ± 2.7	27.6 ± 2.5	28.3 ± 0	NS
Serum progesterone (ng/mL)	9.5 ± 6.3	8.1 ± 4.7	7 ± 5	NS
Cycle length during the study (d)	30 ± 3.1	27.2 ± 3.6	30.2 ± 3.6	NS
Histologic dating				
Biopsy on day LH +2 (d)	16.5 ± 1	17.6 ± 1	16.1 ± 0.8	NS
Biopsy on day LH+7 (d)	20.6 ± 0.9	21.2 ± 1	20.5 ± 1.1	NS
HSCORE				
Glycodelin-A	3 ± 0.2	2.5 ± 0.7	2.4 ± 0.9	NS
L-selectin ligand	2.7 ± 0.6	2.1 ± 0.5	2.3 ± 0.5	NS
$\alpha v\beta 3$ integrin	2.2 ± 0.6	2.12 ± 0.6	1.9 ± 0.7	NS

Note: The values are mean ± DES unless otherwise noted. NS = not significant when $P>.05$ determined by Kruskal-Wallis test; BMI = body mass index (kgm); LNGEC-O = levonorgestrel given as an emergency contraceptive by oral route; LNGEC-V = levonorgestrel given as an emergency contraceptive delivered through vaginal route; Control = nontreated subjects.

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This study is an extension of our previous published research, including the participant clinical features and methodology (6). Briefly, endometrial biopsies were scheduled 48 hours and 7 days after LH surge (LH day + 2) and (LH day + 7), respectively. Endometrial samples were obtained using a pipelle device (CCD Laboratories, France), and peripheral blood samples were obtained the same day of the endometrial biopsy. Specific RIA was used to determine the progesterone plasma concentration as previously reported (7). A portion of each endometrial sample was fixed in 4% buffered formaldehyde and embedded in a paraffin block for histology and immunohystochemistry evaluation. Another portion was quickly frozen in liquid nitrogen for further reverse transcriptase-polymerase chain reaction (RT-PCR).

Immunostaining was performed on paraffin-fixed endometrial samples for LS-L, Gly-A, and PR-AB and PR-B, whereas frozen samples were used for avβ3 integrin staining using a LAB-SA immunodetection system kit (Zymed Laboratories, Invitrogen Immunodetection, Carlsbad, CA, USA). The samples were deparaffinized in xylene and rehydrated with serial grades of ethanol. After a phosphate-buffered saline (PBS) rinse, endogenous peroxidase was quenched with a 30-minute incubation with 0.3% H_2O_2 in absolute ethanol, and the sample was incubated with blocking serum for 10 minutes at room temperature. After overnight primary antibody incubation, the sections were washed with PBS followed by treatment with blocking serum for an additional 10 minutes. Subsequently; the sections were washed with PBS and incubated with Biotin-SP-conjugated secondary antibody, which was provided with the immunodetection kit, for 30 minutes at room temperature. After rinsing with PBS, the immunoreactive antigen was visualized using an avidin–biotin–peroxidase complex and aminoethilcarbazol as a chromagen. Finally, the slides were counterstained with hematoxylin, washed in PBS and distilled water, and mounted with mounting medium.

The following primary antibodies were used: rat–antimouse PNAd carbohydrate epitope; CD62L-ligand, Clone: MECA79 (BD Pharmingen Biosciences, San Diego, CA, USA) at final concentration of 0.5 μgm/mL for L-Selectin ligand; rabbit monoclonal (EP870Y) to placental protein 14/Glycodelin A (Abcam, Cambridge, MA, USA) at 1:1,000 dilution; mouse SSA6 anti-β3 integrin subunit antibody at 1:2,000, kindly donated by Professor Bruce Lessey, REI Division Department of Obstetrics and Gynecology, Greenville Hospital system, Greenville, SC, USA; mouse antihuman PR B-form clone SAN27 (Novocastra Laboratories Ltd, Newcastle, UK) at 1:100, and mouse anti-PR-AB (PRAB-52), Santa Cruz, CA, USA, at 1:50.

Two blinded observers evaluated the resulting staining on a light microscope, Model BX 51TF (Olympus Optical Co. Ltd, Japan). The staining intensity value was assigned using a semiquantitative HSCORE as previously described (8). The tissue distribution and the abundance of both PR-AB and PR-B were evaluated and calculated into the different endometrial compartments using the Image Pro Plus system, version 6.2, Media Cybernetics Inc., Woods Hole, MA USA, as described elsewhere (9).

Total cellular RNA from endometrial samples was extracted with Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The RT-PCR was performed with the Access Quick RT-PCR system (Promega Corporation, Madison, WI, USA) on an Eppendorf Mastercycler gradient 5331 thermocycler for DNA amplification (Eppendorf, Barkhausenweg, Hamburg, Germany). The oligomers used for RT-PCR (Integrated DNA technologies IDT, IA, USA) specific to PR-AB were as follows, forward: 5′-GTCAGTGGGCAGATGCTGTA- and reverse: 5′-TGTGAGCTCGACACAACTCC-3; for Gly-A, forward: 5′-ACGGCACTCTTCCATCTGTT-3′and reverse: 5′-AAGTTGGCAGGGACCTGGCACTC-3′. Ribosomal 18S RNA was used as the internal standard, sense: 5-TAACGATCCATTGGAG-3, antisense: 5CCCTCTTAATCATGGCCTCA-3. The routine RT-PCR program was two pre-PCR cycles of 45 minutes at 48°C and 5 minutes at 94°C, and 28 cycles of PCR amplification of the target gene. The expected size of the PR-AB and Gly-A were 99 and 452 base pairs, respectively. All PCR products from a single experiment were run on a 2% agarose gel and stained with ethidium bromide and visualized on a Thyphoon variable mode imager (Amersham Biosciences, Sunnyvale, CA, USA). The relative density of transcripts normalized to 18S mRNA was calculated using ImageJ 1.41 software for windows. The averages of the ratios for each group were then compared using the appropriate test.

Statistical Analysis

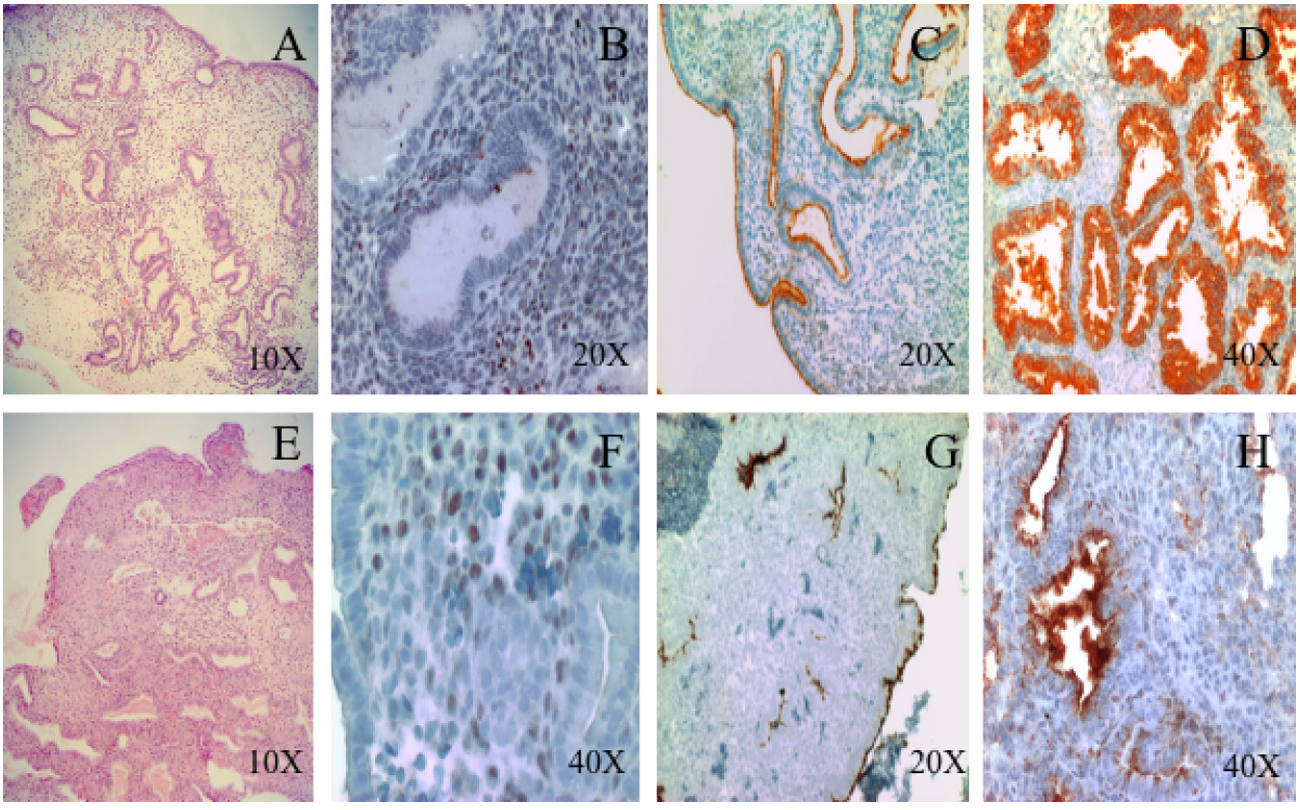
The analysis of the data was performed using Graph Pad Prism version 5.0 CA, USA. Continuous means were compared using a nonparametric Kruskal-Wallis test with posttest correction when appropriate. For a comparison of the abundance of progesterone receptors, the proportion of positive/negative stained nuclear cells were analyzed and compared with a chi-square test (positive/negative stained nucleus for PR × controls/LNG-EC by oral route/LNG-EC by vaginal route). The alpha level was 0.05.

RESULTS

The clinical and demographic characteristics of subjects, including age, body mass index, and length of menstrual cycle before and during the study were similar, and no statistical differences were found (Table 1). The following functional parameters: [1] the follicular

FIGURE 1

Hematoxylin-eosin (H&E) and immunohistochemical localization of PRB, LS-L, and Gly-A in biopsies obtained during the window of implantation from (**F**–**H**) treated women and (**A**–**D**) controls. (**E**) Note the areas of glandular atrophy and intense decidualization in the patients treated with LNG-EC by the oral route. Despite the histologic alterations, neither the down-regulation of the (**F**) epithelial PR nor the expression of (**G**) LS-L or (**H**) Gly-A was affected.



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rupture assessed by vaginal ultrasound was detected in all participants after urinary LH surge both in LNG-EC and nontreated subjects; [2] the plasma progesterone concentration determined at the time of endometrial biopsy was not different in any study group; [3] histologic dating revealed all samples within the interval of days 21–22 of an ideal 28-day cycle evaluated according to Noyes, criteria (10).

The immunostaining of LS-L was intense and restricted to the luminal and glandular compartments during the midsecretory phase in all samples. The Gly-A and avβ3 immunostaining was also intense during this period of the menstrual cycle in all biopsies. In addition, the HSCORE demonstrates no differences in the expression of LS-L, Gly-A, and $\alpha v\beta 3$ integrin between the treated and nontreated groups (Table 1).

The histologic assessment revealed small areas of glandular atrophy and intense stromal decidualization in only 3 of 12 biopsies from subjects who received LNG through the oral route. Interestingly, despite this finding, it was not associated with any significant reduction in the expression of PR or the endometrial molecular biomarkers that were examined. The corresponding images are shown in Figure 1.

Figure 2 illustrates intense Gly-A immunostaining in the glands and lumen in LH + 7 biopsies but very low or negative in LH + 2 from LNG-EC samples (Fig. 2D and C). Similar to protein, the mRNA expression of Gly-A during the window of implantation was not affected by LNG-EC as demonstrated by RT-PCR in the representative samples from treated and untreated biopsies (P=.12; Fig. 2E).

It is thought that the molecular phenotype of the endometrial transformation process from the early to midsecretory phase is characterized by the down-regulation of PR in the luminal and epithelial compartment during the midsecretory phase. This changing pattern of expression was similar in the nontreated subjects and the LNG-EC-treated group for both PR isoforms (Fig. 2A and B). Although PR down-regulation into the epithelial compartment was clearly observed, the stroma showed intense PR expression in all samples. No differences in the abundance of PR in LNG-EC-treated and nontreated endometria were observed (P=.30; Fig. 3).

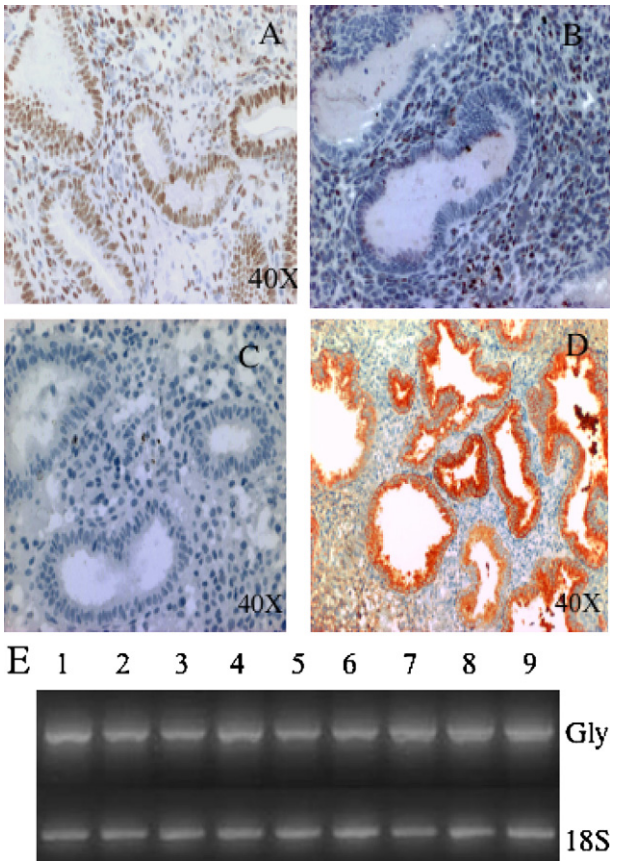
The RT-PCR assay for PR-AB mRNA in representative samples from LNG-EC and nontreated subjects were compared, corroborating that most of the PR gene was not affected (P=.21; Fig. 3D).

DISCUSSION

We designed this in vivo study to test the hypothesis if a single dose of LNG administered on the day of LH surge may alter the

FIGURE 2

Immunostaining of PR-B and Gly-A in biopsies obtained during the early (**A–C**) secretory phase LH + 2 and (**B–D**) midsecretory phase LH + 7 from women treated with LNG-EC. (**B**) Note that the epithelial PR down-regulation during the midsecretory phase is not affected. (**B** and **D**) Concomitant high expression of Gly-A and epithelial PR-B down-regulation during the midsecretory phase is preserved. (**A–C**) LNG-EC does not alter the pattern of PR expression nor does it induce the expression of Gly-A during the early secretory phase. (**E**) The RT-PCR assay for mRNA of Gly-A, normalized to the internal 18S control demonstrated no differences between the samples from nontreated patients (lanes 1–3) and patients treated with LNG-EC by either the oral or vaginal route (lanes 4–6 and 7–9, respectively).



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expression of endometrial PR, thereby affecting the endometrial maturation and the expression of molecules associated with endometrial receptivity. LNG is a potent progestin that is currently administered in different contraceptive modes, including intrauterine device (IUD)-delivering LNG, long acting subdermal implants, and combined or progestin-only oral contraceptive pills. 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 (11, 12).

In this study, despite the high dose of LNG (1.5 mg), either by the oral or vaginal route, the abundance and tissue distribution of PR did not differ between the controls the and LNG-EC-treated subjects. The effect of LNG on endometrial progesterone receptor expression may be related to the concentration of LNG observed in the different modes of administration. As we recently reported in a study on the pharmacokinetics of LNG-EC by the oral and vaginal routes (6), the serum and endometrial tissue concentration differs between the modes of administration. For example, the endometrial tissue concentration of LNG is ~100 times higher in IUD-releasing LNG than in a single dose of LNG-EC by either the oral or vaginal route. Indeed, 808 ng/g or 2,580 nmol/mg per wet tissue in IUD-LNG and 5–20 nmol/mg at 168 hours (LH + 7) post-LNG-EC were reported (6, 13). Thus, it appears that a high LNG concentration in the tissue and a persistent, long-lasting dose rather than the acute high dose may affect endometrial PR expression (14). Endometrial transformation from the proliferative to the secretory phase is driven mainly by progesterone through its cognate PR. The necessary endometrial transformation from prereceptive early secretory toward the receptive midsecretory endometrium is characterized by PR down-regulation. The differential function and dynamic endometrial expression of PR-B and PR-AB through menstrual cycle has been described (15), but it appears that an alteration in the ratio of the PR isoforms may lead to an impairment of endometrial receptivity (16). In our study, these crucial, dynamic transitions of PR, as well as the abundance of both PR isoforms, were not affected by LNG-EC.

During the midsecretory phase, progesterone signals acts directly over the epithelial surface or indirectly from the stroma inducing the expression of genes associated with endometrial receptivity (17). Among these molecules recognized as molecular markers of uterine receptivity, we selected a panel including LS-L, Gly-A, and $\alpha v\beta 3$ integrin. Recent investigations have reported that L-selectin may be involved in the implantation process (18). Trophoblast cells express L-selectin after a hatching process, whereas its L-selectin carbohydrate ligand recognized by MECA79 antibody is highly expressed only during the endometrial receptivity phase, suggesting progesterone regulation. We have not observed any difference in the expression of L-selectin ligand in this study. This finding may be in concordance with the lack of changes observed in the progesterone plasma concentration and PR.

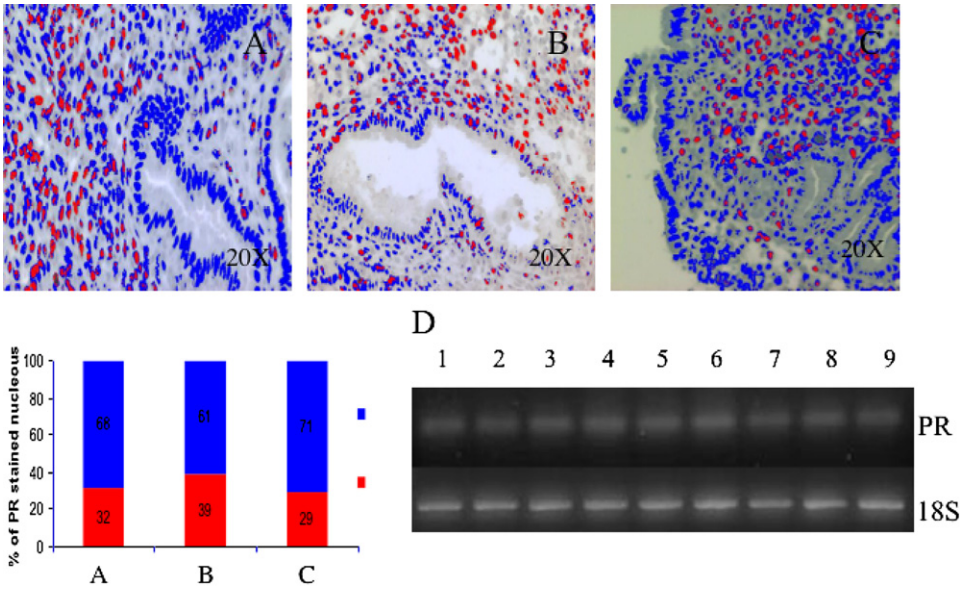
It is thought that $av\beta 3$ integrin is the best-characterized molecular biomarker associated with endometrial receptivity. It is expressed only during the period of the implantation window, and aberrant expression has been reported in conditions associated with infertility, including endometriosis, hydrosalpinx, polycystic ovary syndrome, and unexplained infertility (19, 20). Our study did not detect any differences in the expression of this remarkable protein between the treated and untreated biopsies.

Glycodelin-A (Gly-A) is the major glycoprotein produced by secretory endometrial glands. Its production rises from days 5–6 after ovulation and increases onward during pregnancy. The Gly-A is mainly produced by epithelial endometrial cells and has been postulated to have immunomodulatory properties that facilitate embryo implantation. During the midsecretory phase, high Gly-A secretion is closely associated with progesterone-B down-regulation (21). In this study, neither the pattern of PR-B down-regulation nor the Gly-A expression was affected by LNG-EC.

Paradoxically, an in vitro study has demonstrated that Gly-A is involved in the inhibition of the sperm–egg binding process. Endometrial expression of Gly-A, other than during the secretory phase, was also reported in women using IUD-releasing LNG, suggesting another contraceptive mechanism (22). An interesting investigation also demonstrated that late follicular administration of LNG, but not at the time of the LH surge, produces an early rise in serum Gly-A

FIGURE 3

Immunolocalization of PR during the midsecretory phase from (**A**) controls and women treated with LNG-EC by the (**B**) oral or (**C**) vaginal route. Note the negative stained cells (blue) in the luminal and glandular cells (PR down-regulation) and the positive-stained stromal cells (red) in all samples. The ratio of positive/negative nuclear stained cells was evaluated, and the proportions in each group were compared by the chi-square exact test ($P=.30$), demonstrating that there are no differences in the abundance of PR. Reverse transcriptase-polymerase chain reaction for PR-AB mRNA normalized to the internal 18S control in samples obtained during the midsecretory phase. (**D**) This corroborates no differences in PR expression between the nontreated patients (lines 1–3) and patients treated with LNG-EC by the oral or vaginal route (lanes 4–6 and 7–9, respectively).



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(23). In this study, very little if any Gly-A protein expression was detected in biopsies 48 hours after LNG-EC administration, a finding that is in agreement with another recent report (24). Thus, it appears that an acute, in vivo, high dose of LNG at the time of the LH surge is not enough to induce changes in endometrial PR and Gly-A expression.

In our study, the endometrial biopsies from the LNG-EC-treated subjects administered by the oral route showed areas of irregular development that were characterized by glandular atrophy and intense stromal decidualization. This morphologic change resembles the prolonged and intense effect of progestin on the endometrium. Interestingly, according to the HSCORE evaluation, neither the PR expression nor the endometrial molecular markers were affected compared with the normal endometrial tissue. Our work confirms the finding of Murray et al. (25), which demonstrates endometrial histology is not a valid method for evaluating endometrial receptivity. This finding indicates that endometrial morphology is insufficient to predict the endometrial molecular receptivity phenotype and the capability of the uterus to support embryo implantation.

It is well accepted that investigation of human implantation has limitations because of ethical constraints. An interesting in vitro model resembling the embryo–maternal environment has recently been developed to assess the effect of LNG on the human endometrium (26). In agreement with these in vitro findings, our results illustrate the in vivo effect of LNG on PR and on molecules associated with endometrial receptivity, demonstrating that LNG does not alter the molecular uterine receptivity biomarkers.

In conclusion, administration of LNG-EC on the day of the LH surge is unable to inhibit ovulation by causing ovulatory dysfunction or diminishment of the reproductive function.

The fact that endometrial maturation and receptivity are unaffected suggests that there are no direct or indirect effects of LNG on endometrial function. Thus, the mechanism of action of LNG-EC, if any, at the time of the LH surge does not include the impairment of PR or the endometrial receptivity biomarkers.

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REFERENCES

1. Croxatto HB, Ortiz ME, Müller AL. Mechanisms of action of emergency contraception. Steroids 2003;68:1095–8.
2. Marions L, Hultenby K, Lindell I, Sun X, Ståbi B, Gemzell Danielsson K. Emergency contraception with mifepristone and levonorgestrel: mechanism of action. Obstet Gynecol 2002;100: 65–71.
3. Gemzell-Danielsson K, Marions L. Mechanisms of action of mifepristone and levonorgestrel when used for emergency contraception. Hum Reprod Update 2004;10:341–8.
4. Durand M, Del Carmen Cravioto M, Raymond EG, Durán-Sánchez O, De Cruz-Hinojosa M, Castell-Rodríguez A, et al. On the mechanisms of action of short-term levonorgestrel administration in emergency contraception. Contraception 2001;64: 227–34.

5. Lessey BA, Yeh I, Castelbaum AJ, Fritz MA, Ilesanmi AO, Korzeniowski P, et al. Endometrial progesterone receptors and markers of uterine receptivity in the window of implantation. Fertil Steril 1996;65: 477–83.
6. Devoto L, Fuentes A, Palomino A, Espinoza A, Kohen P, Ranta S, et al. Pharmacokinetics and endometrial tissue levels of levonorgestrel after administration of a single 1.5-mg dose by the oral and vaginal route. Fertil Steril 2005;84:46–51.
7. Kohen P, Castro O, Palomino A, Muñoz A, Christenson LK, Sierralta W, et al. The steroidogenic response and corpus luteum expression of the steroidogenic acute regulatory protein after human chorionic gonadotropin administration at different times in the human luteal phase. J Clin Endocrinol Metab 2003;88:3421–30.
8. Palomino WA, Fuentes A, González RR, Gabler F, Boric MA, Vega M, et al. Differential expression of endometrial integrins and progesterone receptor during the window of implantation in normo-ovulatory women treated with clomiphene citrate. Fertil Steril 2005;83:587–93.
9. Angelopoulou R, Balla M, Lavranos G, Chalikias M, Kitsos C, Bakad S, et al. Evaluation of immunohistochemical markers of germ cells' proliferation in the developing rat testis. Tissue Cell 2008;40:43–50.
10. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. Fertil Steril 1950;1:3–25.
11. Guttinger A, Critchley HO. Endometrial effects of intrauterine levonorgestrel. Contraception 2007;75: S93–8.
12. Critchley HO, Bailey DA, Au CL, Affandi B, Rogers PA. Immunohistochemical sex steroid receptor distributions in endometrium from long-term subdermal levonorgestrel users and during the normal menstrual cycle. Hum Reprod 1993;8: 1632–9.
13. Pekonen F, Nyman T, Lahteenmaki P, Haukkamaa M, Rutanen EM. Intrauterine progestin induces continuous insulin-like growth factor-binding protein-1 production in the human endometrium. J Clin Endocrinol Metab 1992;75:660–4.
14. Milling Smith OP, Critchley HOD. Progestogen only contraception and endometrial break through bleeding. Angiogenesis 2005;8:117–26.
15. Mote PA, Balleine RL, McGowan EM, Clarke CL. Colocalization of progesterone receptors A and B by dual immunofluorescent histochemistry in human endometrium during the menstrual cycle. J Clin Endocrinol Metab 1999;84:2963–71.
16. Igarashi TM, Bruner-Tran KL, Yeaman GR, Lessey BA, Edwards DP, Eisenberg E, et al. Reduced expression of progesterone receptor-B in the endometrium of women with endometriosis and in cocultures of endometrial cells exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Fertil Steril 2005;84:67–74.
17. Lessey BA. Two pathways of progesterone action in the human endometrium: implications for implantation and contraception. Steroids 2003;68: 809–15.
18. Genbacev OD, Prakobphol A, Foulk RA, Krtolica AR, Ilic D, Singer MS, et al. Trophoblast L-selectin mediated adhesion at the maternal-fetal interface. Science 2003;299:405–8.
19. Donaghay M, Lessey BA. Uterine receptivity: alterations associated with benign gynecological disease. Semin Reprod Med 2007;25:461–75.
20. González RR, Palomino A, Boric A, Vega M, Devoto L. A quantitative evaluation of α1, α4, α5 and $\alpha v\beta$3 endometrial integrins of fertile and unexplained infertile women during the menstrual cycle. A flow cytometric appraisal. Hum Reprod 1999;14: 2485–92.
21. Stavreus-Evers A, Mandelin E, Koistinen R, Aghajanova L, Hovatta O, Seppälä M. Glycodelin is present in pinopodes of receptive-phase human endometrium and is associated with down-regulation of progesterone receptor B. Fertil Steril 2006;85: 1803–11.
22. Mandelin E, Koistinen H, Koistinen R, Affandi B, Seppälä M. Levonorgestrel-releasing intrauterine device-wearing women express contraceptive glycodelin-A in endometrium during midcycle: another contraceptive mechanism? Hum Reprod 1997;12:2671–5.
23. Durand M, Seppala M, Del Carmen Cravioto M, Koistinenc H, Koistinenb R, Gonzalez-Macedo J, 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 2005;71:451–7.
24. Do Nascimento J, Seppala M, Perdigao A, Espejo-Arce X, Munuce MJ, Hautala L, 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. Hum Reprod 2007;22:2190–5.
25. Murray MJ, Meyer WR, Zaino RJ, Lessey BA, Novotny DB, Ireland K, et al. A critical analysis of the accuracy, reproducibility, and clinical utility of histologic endometrial dating in fertile women. Fertil Steril 2004;81:1333–43.
26. Meng CX, Andersson KL, Bentin-Ley U, Gemzell-Danielsson K, Lalitkumar PG. Effect of levonorgestrel and mifepristone on endometrial receptivity markers in a three-dimensional human endometrial cell culture model. Fertil Steril 2009;91:256–64.