UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO

FACULTAD DE MEDICINA
DIVISION DE ESTUDIOS DE POSGRADO E INVESTIGACION

On the Mechanisms of Action of Short-Term Levonorgestrel Administration in Emergency Contraception

TESIS DE POSGRADO

PARA OBTENER EL DIPLOMA DE ESPECIALISTA EN

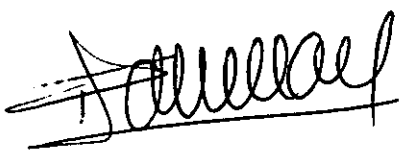
BIOLOGIA DE LA REPRODUCCION HUMANA

PRESENTA:

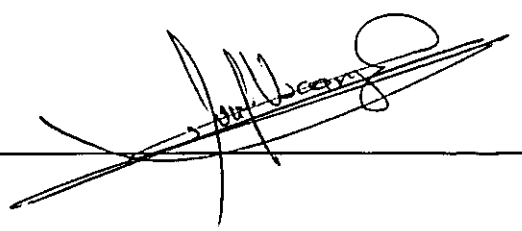
DRA. MARTA DURAND CARBAJAL

México, D.F. Mayo del 2001.

Dr. Fernando Larrea
Profesor Titular del Curso de Biología de la Reproducción Humana
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
México, D.F.



Dr. Luis Federico Uscanga Rodríguez
Director de Enseñanza
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
México, D.F.



TUTORES DE TESIS

Dr. Fernando Larrea
Profesor Titular del Curso de Biología de la Reproducción Humana
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
México, D.F.

Dra. Ma. Del Carmen Cravioto
Profesor Adjunto del Curso de Biología de la Reproducción Humana
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
México, D.F.

On the Mechanisms of Action of Short-Term Levonorgestrel Administration in Emergency Contraception

Marta Durand[1], Ma. del Carmen Cravioto[1], Elizabeth G. Raymond[2], Ofelia Durán-Sánchez[1], Ma. De la Luz Cruz-Hinojosa[1], Andrés Castell-Rodríguez[3], Raffaela Schiavon[4], and Fernando Larrea[1]

[1] Department of Reproductive Biology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City; [2] Family Health International, Research Triangle Park, North Carolina, USA; [3] Department of Cellular Biology, School of Medicine, Universidad Nacional Autónoma de México, Mexico City; and [4] Reproductive Health Service, Instituto Nacional de Pediatría, Mexico City.

Short running head: Mechanisms of action of LNG in EC

ABSTRACT

The effects of short-term administration of levonorgestrel (LNG) at different stages of the ovarian cycle upon the pituitary-ovarian axis, corpus luteum function and endometrium were investigated. Forty-five surgically sterilized women were studied during two menstrual cycles. In the second cycle each women received two doses of 0.75 mg of LNG taken 12 hours apart on day 10 of the cycle (Group A), at the time of serum luteinizing hormone (LH) surge (Group B), 48 hours after positive detection of urinary LH (Group C), and late follicular phase (Group D). In both cycles, transvaginal ultrasound and serum LH were performed since the detection of urinary LH until ovulation. Serum estradiol (E_2) and progesterone (P_4) were measured during the complete luteal phase. In addition, an endometrial biopsy was taken at day LH+9. Eighty percent of subjects in Group A were anovulatory, the remaining (3 subjects), presented significantly shortness of the luteal phase with notably lower luteal P_4 serum concentrations. In Groups B and C, no significant differences on neither cycle length nor luteal P_4 and E_2 serum concentrations were observed between the untreated and treated cycles. Subjects in Group D had normal cycle length but significantly lower luteal P_4 serum concentrations. Endometrial histology was normal in all ovulatory treated cycles. It is suggested that interference of LNG with the mechanisms installing the LH preovulatory surge depends on the stage of follicle development. Thus, anovulation results from disrupting the normal development and/or the hormonal activity of the growing follicle only when LNG is given preovulatory. In addition, peri- and post-ovulatory administration of LNG did not impair corpus luteum function.

Key Words: Corpus luteum / Emergency contraception / Levonorgestrel / Mechanism of action / Ovulation / Progestins

INTRODUCTION

Levonorgestrel (LNG) is a synthetic 13β-ethyl substituted 19-nor steroid[1,2] with potent progestational activity and widely used in contraceptive formulations. LNG represents the active isomer of norgestrel and is administered orally or delivered either via an intrauterine device or from subdermal implants.[3] LNG alone or in combination with estrogenic compounds has also been used successfully for postcoital contraception.[4] In this regard, postcoital administration of steroids is a well recognized effective mean for preventing pregnancy, currently representing a widely accepted way of emergency contraception (EC). Although efficacy is generally not as high as with other contraceptive methods, EC significantly reduces the risk of an unwanted pregnancy. Indeed, when administered within 72 hours of unprotected intercourse, LNG prevented about 85% of pregnancies compared with the expected number without treatment.[5]

All emergency methods in use act before implantation through mechanisms probably involving interference with sperm penetration, transport and/or fertilization, follicular growth and corpus luteum development, and /or by a direct action on endometrium.[6-9] LNG could work by altering any of these mechanisms depending upon the day of the cycle at which the contraceptive is given. There are few studies designed to look at the mechanisms of action of LNG as a postcoital contraceptive, in particular those considering the fact that women may require the method at different times during the menstrual cycle.[10-14] In this study, we have investigated in normal menstruating women the effects of LNG upon the pituitary-ovarian axis, corpus luteum function and endometrium when given orally at two 0.75 mg dose taken 12 hours apart, during the follicular (day 10), periovulatory (luteinizing hormone surge) and postovulatory (48 hours after urinary LH detection) phases of the cycle. This study was designed taking into consideration the expected variability of the menstrual cycle among women and therefore the need to reassign,

the initially allocated subjects, into study groups by normalizing, within the cycle, the time of administration of LNG according to the onset of luteinizing hormone (LH) surge in serum. The rationale for the timed treatment schedule was also based on the probabilities of conception by cycle day as reported by Wilcox et al.[15]

MATERIAL AND METHODS

Subjects

The study was approved by the Human Ethical and Scientific Committee of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán and all subjects signed an informed consent form. Forty five healthy surgically sterilized women, aged 29 to 35 years old (mean age 31 years), with regular menstrual cycles (cycle lengths between 25 to 32 days) were recruited for the purposes of this study. None had used hormonal contraception or any other medication within 6 months prior to the study. Subjects were in good health as determined by medical history, physical examination and routine screening laboratory tests, including Papanicolaou smear. Body weight, height and blood pressure of each subject were registered by one investigator. Subjects were issued with a menstrual calendar on which they recorded details of all bleeding episodes throughout the study.

Study design

The study was conducted in two consecutive cycles. Cycle was defined as the time elapsed from the first day of an spontaneous menstrual bleeding until the day preceding the next menses. All subjects were admitted during the first 10 days of their menstrual cycle. Before the control cycle, women were randomly allocated into three different groups as follows: Group A) women who received two doses of 0.75 mg LNG (Postinor®, Gideon Richter) taken 12 hours apart on the morning of day 10 of the menstrual cycle; Group B) women who received the same dose of LNG immediately after positive LH detection in urine, and Group C) those women who received the same dose of LNG but 48 hours after positive detection of urinary LH.

During both cycles (control and treated), all women were asked to monitor every morning for urinary LH, starting on the 11^{th} day of the menstrual cycle until the presence of LH was

detected. At this time, transvaginal ultrasound was daily performed until FR was observed. This was established by the presence of at least three of the following findings: acute decrease in mean diameter or disappearance of the follicle, presence of thickened irregular borders, increase echogenecity within the follicle, and presence of free intraperitoneal fluid.[16] At each attendance, ultrasound was undertaken by the same observer using an SSD-2000 ultrasonographic equipment (Aloka Co., LTD). Women who did not present positive LH in urine and FR during the control cycle were excluded from the study.

Daily blood samples were obtained from the positive LH detection in urine until the day menses began. All samples were centrifuged, and serum stored at –20°C until assayed. Serum progesterone (P_4) and estradiol (E_2) concentrations were determined in all samples, whereas serum LH was quantified only from the day of detected urinary LH until P_4 serum concentrations reached at least 3 ng/mL. The main purpose for measuring serum LH was to better precise the actual time at which LNG was administered during the menstrual cycle, rather than based only on urinary LH detection. Follicular phase was considered from the first day of bleeding until the day of maximum serum LH concentrations and the luteal phase from the next day of serum LH surge until the day before menses began.

In addition, endometrial biopsies were taken from all subjects during both control and treated cycles on day LH+9. This day lies within the implantation window, the time during which the endometrium has optimal receptivity to implantation.[17,18] Endometrial tissues were obtained with a Novak curette from the anterior wall of the uterine cavity without dilatation of the cervix or local anesthesia. Biopsy specimens were immediately fixed in formalin solution and used for light microscopic examination after embedding in paraffin and staining with hematoxylin and eosin. Biopsy specimens were read blindly to the examiner for histologic dating.

Hormone assays

LH in urine was monitored by a commercially available kit (Ovuquick, Corne SA de CV, Mexico), which positive predictive value for follicular collapse, within 24 to 48 hours after positive urinary LH testing, has been estimated in 73% and 92%, respectively.[19] Serum concentrations of LH, E_2 and P_4 were measured in duplicate by specific immunoradiometric assay for LH and specific radioimmunoassays for E_2 and P_4 using commercial reagents (Diagnostic Products Corporation, USA) and protocols provided by the World Health Organization Matched Reagent Programme (Geneva, Switzerland) as previously described.[20,21] The inter-assay coefficient of variation (CV) was less than 10% in all hormones, and intra-assay CV were 4.9%, 1.63% and 1.33% for LH, E_2 and P_4, respectively. These CV, were calculated from pools of standard sera at the average hormone concentrations of 16.1 mIU/mL, 50 pg/mL and 1.5 ng/mL for these hormones, respectively. To avoid inter-assay variations all samples corresponding to the same individual were measured within the same assay.

Morphological analysis

Endometrial morphology was assessed by correlating the chronological date (day after LH surge) with the morphological endometrial characteristics of specimens, as an indicator of hormone action. The dating of the endometrium was related to the serum LH surge, FR, and luteal concentrations of E_2 and P_4 rather than to the "ideal" 28-days cycle, as previously described.[22] The parameters examined were number of glands, stromal edema, and predecidual changes as evaluated by the presence of prominent spiral arteries. The readings of specimens were made blindly by the same morphologist, who had no knowledge of either the day of the cycle at which biopsy was taken nor the treatment instituted. Histologic dating was performed using criteria described by Noyes et al.,[23] and by Hendrickson and Kempson.[24] Glandular and

stromal elements were dated separately and given equal importance, as described by Lessey, et al.[25] A specimen in which glandular maturation was delayed by three or more days from the day calculated from the date of LH surge was defined as to be "out of phase".[26]

Statistical analysis

The analysis compared the differences of integrated luteal E_2 (ILE_2), integrated luteal P_4 (ILP_4), cycle characteristics, and endometrial morphology between the control and treated cycles. ILE_2 and ILP_4 were analyzed calculating the area under the curve (AUC) for both hormones during the luteal phase of the control and treated cycles. The AUC was calculated for nine days after serum LH surge by the trapezoid method with the aid of a computer program and expressed in arbitrary units, depending on the hormone. A log-normal distribution was assumed[27] and a unpaired-sample *t*-test was used to evaluate the significance of differences ($p<0.05$) between each subject's ILP_4 and ILE_2, daily hormone concentrations, cycle characteristics, and endometrial morphology, in the control and treated cycles. A one-sided test was planned because the change in markers in treated cycles is expected to occur in only one direction.[28] An analysis of variance was used to report clinical characteristics.

RESULTS

Clinical characteristics

The characteristics of the study population are given in Table 1. As depicted, examination of the distribution of the different variables revealed no significant differences among all women who were randomly allocated into the three treatment groups. Accordingly, all forty five control cycles were combined as only one reference group. All subjects had ovulatory control cycles between 21 and 34 days with luteal phase lengths from 9 to 15 days, with evidence of FR within 11 and 21 days from the first day of the cycle. Maximal serum luteal P_4 concentrations were observed at day 21 ± 3 days and mean ILP_4-AUC was 90.3 ± 41.1 ng/mL for nine days after serum LH surge (Table 2 and Figure 1).

Groups reassignment

Timing comparisons between serum with urinary LH demonstrated inconsistencies in twelve out of ninety studied cycles (13.3%). In 4 cases corresponding to control cycles (8.8%) and 8 to treated cycles (17.7%), urinary LH did not correlate with the day of maximum concentrations of serum LH. In these cases, serum LH, along with E_2 and P_4 concentrations were used rather than urinary LH for cycle dating. Thus, eight subjects during the treated cycle were identified as not corresponding to the original assigned groups. Therefore, in four subjects originally included in Group B and four in Group C the administration of LNG took place 3 ± 1 day prior the serum LH surge was installed and were reassigned into a new Group (Group D). This new group received LNG during the late follicular phase, a few days prior the occurrence of LH surge. Then, the groups studied consisted finally of 15 subjects in Group A, 11 in Groups B and C, respectively and 8 in Group D.

Cycle characteristics

In twelve subjects in Group A, LNG significantly ($p<0.05$) shortened the mean length of the cycle as compared to that of control (15 ± 2 *vs* 26 ± 3 days) and had not luteal phase and were therefore excluded from the rest of the analysis. In the three remaining subjects in Group A, LNG administration did not modify significantly the length of the cycle (28 ± 6 days) as it is shown in Table 2. In Groups B, C and D no modifications were noted on cycle length (27 ± 2, 26 ± 1 and 24 ± 5 days, respectively). Follicular phase length was significantly longer only in the three ovulatory subjects of Group A (19 ± 2 *vs* 15 ± 3 days, $p<0.05$). In the remaining groups no differences were observed in follicular phase length between the treated and control cycles (Table 2). In all subjects in Groups B and C there were no differences in luteal phase length between the treated and control cycles. The three LNG treated subjects with a normal cycle length in Group A and all the treated subjects in Group D had a significant shortening (9 ± 4 and 10 ± 4 days, respectively) of the luteal phase (Table 2).

Effects on ovulation

As it was mentioned, twelve subjects of Group A had anovulatory cycles following LNG, as evidenced by the absence of urinary LH and ultrasonographic findings of FR, and the occurrence of endometrial bleeding within 6.3 ± 1.9 days (range 4 to 11 days) after treatment. The three ovulatory subjects in Group A had a delayed positive test for LH in urine which was further corroborated by measurements of serum LH and FR (Table 2). All subjects in Groups B, C, and D had ultrasonographic findings of FR and LNG administration did not modify the day of the cycle at which FR occurred (16 ± 2, 16 ± 2, and 15 ± 2 days *vs* 15 ± 2 days, respectively) (Table 2).

Effects on E_2 and P_4 during the luteal phase

Figures 1 and 2 depict daily serum concentrations of P_4 and E_2 as well as ILP_4/ILE_2-AUC, respectively from treated and control cycles. As previously mentioned, only three subjects of Group A ovulated since presented urinary/serum LH and ultrasonographic evidence of FR. Although daily serum P_4 concentrations and ILP_4-AUC values, in these subjects, did not reach statistical difference between the LNG-treated and control cycle, the mean serum ILP_4-AUC obtained (44.7 ± 26.7 ng/mL/9 days) was notably lower than that observed in control cycles (90.3 ± 41.1 ng/mL/9 days). In addition, no changes were observed in serum ILE_2-AUC during the luteal phase between treated (1083 ± 744 pg/mL/9 days) and control cycles (989 ± 385 pg/mL/9 days).

As already mentioned, all subjects in Groups B, C and D ovulated. With the exception of Group D, the mean serum ILP_4-AUC, in all remaining groups, was similar when compared with the control cycle (Figures 1 and 2). In Group D, subjects presented a significantly lower daily serum P_4 concentrations and ILP_4-AUC when compared with those in control cycles (15.9 ± 10.6 ng/mL/9 days *vs.* 90.3 ± 41.1 ng/mL/9 days, respectively). No significant changes in ILE_2-AUC were observed in all groups.

Endometrial effects

In both control and treated cycles, neither inflammatory, reactive nor other abnormal features in tissue specimens were observed. As shown in Table 3, with exception of four endometrial specimens taken during the control cycle, endometrial morphology corresponded, according to both LH surge and FR, to the expected day (LH+ 8.6±1.3 days) at which the biopsy was obtained. The results were highly consistent with the chronological date of sampling, since differences longer than three days between the histological diagnosis and the day of the cycle

were not observed. A total of 24 out of 33 biopsies from treated cycles with ovulatory features were studied. The rest were excluded due to an insufficient tissue sample (one from Group B and D, respectively) or because sampling did not correlate with the cycle day (three from Group A and four from Group D). Table 3 summarizes the morphological findings in Groups B, C and D. As depicted, no significant changes were observed between treated and control specimens in any of the studied parameters. No significant differences among groups were also observed. Of particular importance was the finding that the predecidual changes as evaluated by the presence of prominent spiral arteries, which are considered crucial for implantation, were unchanged by LNG. [24]

DISCUSSION

Our study investigated the effects of short term administration of LNG on ovulation and luteal phase function in ovulatory women with tubal ligation. Results are consistent with other studies showing that preovulatory administration of LNG alone or in combination with estrogens suppresses ovulation in most, but not all cases.[5,29] We could not, otherwise, demonstrate significant alterations in P_4 and E_2 during the luteal phase when LNG was administered at the time of LH surge or the day after the occurrence of FR. These observations strongly suggest that effects of LNG on the hypothalamic pituitary ovarian axis depend on the stage of the menstrual cycle at which the progestin is administered.

A highlight in this study was the possibility to identify the exact cycle day of LNG administration, particularly relative to the midcycle serum gonadotropin surge. Serum LH measurements helped us to assess the day of ovulation, adding a new dimension to data interpretation of our study. Urinary LH proved to be a poor guide to determine the day of ovulation, yielding 13.3% false positives (true LH surge in serum occurred later in the cycle). This observation may explain inconsistencies found in a variety of studies on the effects of acute and timed administration of steroids upon the menstrual cycle based only on urinary LH. In addition, this study also provides information on the effects of LNG upon endometrial morphology during the implantation window.

Postcoital steroid administration is a well recognized safe, and effective mean of preventing pregnancy.[4-14] Limited information, however, exists on the mechanisms by which postcoital LNG achieves its contraceptive effect. The few studies on the contraceptive mechanisms of LNG suggest that in EC it may have, depending on the time of administration during the cycle, a wide spectrum of actions, affecting steps from follicular growth and development, midcycle gonadotropin surge and ovulation to corpus luteum and endometrial

function.[10,12,13,30-32] Interestingly, in previous studies, LNG administered during the luteal phase revealed no alterations on neither cycle length nor endometrial morphology.[12,13]

In the present study, ovulation was suppressed in 80% of subjects receiving LNG during the follicular phase (Group A). On the contrary, ovulation occurred in all those women treated immediately before the LH preovulatory surge (Group D); however, in these subjects deficient P_4 production with a significantly shorter luteal phase length were observed. Findings in Group A are consistent with an impaired follicular maturation leading to deficient E_2 and P_4 production during the follicular and luteal phase, respectively. In this regard, P_4 has been involved in follicle atresia by a mechanism leading, in part, to the accumulation of follicular fluid low-molecular-mass insulin-like growth factor binding proteins (IGFBPs) with a concomitant reduction in the content and bioavailability of insulin-like growth factor I (IGF-I).[33] Indeed, the IGF system has actually been considered as a positive regulator of follicular development as it enhances both proliferation and differentiation through amplifying the action of gonadotropins on follicular cells.[34-36] Furthermore, there is also evidence supporting the suppressive effects of progestins on FSH-stimulated E_2 production by cultures of granulosa cells,[37,38] including the *in vivo* inhibitory effects of P_4 on follicular development even in the presence of elevated serum levels of FSH.[39] In addition, LNG administration to cycling cynomolgus monkeys[40] significantly decreased the serum levels of androstenedione, implying that estrogen precursors synthesis is also a target of LNG action at the ovarian level. These observations indicate that the preovulatory effects of LNG on the hypothalamic pituitary unit are mediated, at least partially, by the progestin direct action on the growth, development and steroidogenic capacity of the ovary to produce adequate E_2 concentrations in serum as the primary signal triggering the LH surge. The finding of ovulatory cycles in three subjects belonging to Group A is unexplained but, variations in absorption and

clearance, as well as, differences in ovarian sensitivity after LNG administration should be considered among the causes of method failure.

On the other hand, the occurrence of ovulation in study subjects receiving LNG within three days before the onset of LH peak (Group D) may represent either a null-effect or an amplifying P_4-like effect of LNG on the hypothalamic pituitary unit. Under physiological conditions, a small but significant rise in P_4 has been considered as the ultimate ovarian signal to trigger gonadotropin preovulatory surge.[41-43] The finding of delayed ovulation following the administration of RU 486 just before midcycle LH surge,[44] strongly supports these observations. It remains, however, unclear whether LNG by itself could induce the positive feed-back discharge of pituitary LH at this time of the cycle. In addition, P_4 administration during follicular phase results in increased amplitude and decreased frequency of LH pulses, consistent with the pattern observed during the luteal phase of the cycle.[45] These changes may partially reflect alterations in hypothalamic GnRH secretion that without apparently affecting preovulatory surge of LH and FR could be involved in deficient P_4 production observed during the luteal phase. Evidence that LNG might affect corpus luteum function through changes in LH pulsatile pattern prior to the triggering of LH surge in addition to or rather than through a direct action upon P_4 production can be encountered in other studies.[46]

In this study, LNG administration to subjects at the time of the time (Group B) or 48 hours after (Group C) LH surge did not affect the overall P_4 production or the length of the luteal phase. This finding agreed with the well known raise in P_4 serum levels at the time and after the onset of the LH surge.[43] It is therefore possible to speculate that LNG, acting as a P_4-like factor, should not disturb the process of follicular rupture and the installment of a normal corpus luteum. Indeed, in mice lacking P_4 receptors there is no evidence of ovulation and corpus luteum formation despite the presence of LH exposed mature preovulatory follicles,[47] indicating the

important role of P_4 in the process leading to follicular rupture and ovulation. These results also correlate with the presence of normal histopathological features in endometrial biopsies taken during the implantation window in women form Groups B, C, and D. It is important to mention that existence of predecidual endometrial changes, as evaluated by the presence of prominent spiral arteries in this study, strongly suggest the apparent preservation for implantation capabilities in these LNG exposed tissues.

It is therefore possible to conclude that interference of LNG with the mechanisms involved in installing the LH preovulatory surge depends on the stage of follicular development. Thus, anovulation results from disrupting both normal development and hormonal activity of a growing follicle. In addition, the finding that LNG administration at late follicular phase did not interfere with E_2-mediated midcycle gonadotropin surge and ovulation but, otherwise, did alter P_4 production by the corpus luteum requires further investigation, particularly in those mechanisms involved on LNG actions at both the ovarian and hypothalamic pituitary unit, including the interference with preovulatory signals for adequate development and hormonal function of the human corpus luteum.

It is important to mention that additional targets, besides those described, should also be considered and further investigated for the contraceptive effects of LNG. One recent study, for example, reported that clustering of observed pregnancies around predicted ovulation markedly differed when compared with the expected number in untreated cycles regardless of timing at which coitus took place.[5] On the other hand, contraceptive efficacy is inversely related to the time interval between coitus and EC administration, regardless of the cycle day.[48] Additionally, statistical analysis of contraceptive effectiveness of Yuzpe EC regimen suggests that mechanisms other than interference with ovulation might also be involved.[49]

Our results may offer a plausible explanation for the contraceptive effects of LNG given postcoitally prior to or during the mechanism involved in triggering the LH surge and corpus luteum development. We hope to help dismantle with such experimental evidence the erroneously idea that EC typical acts as an abortifacient.

ACKNOWLEDGEMENTS

This study was supported by grants from Family Health International (North Carolina, USA) and El Consejo Nacional de Ciencia y Tecnología (Mexico). We thanks to Dr. Ma. Eloisa Dickinson who kindly helped with volunteers enrollment, Dr. Enrique Sampedro-Carrillo for endometrial evaluations, Mr. Porfirio Robles for his technical assistance in data analysis, and to Gedeon Richter, Ltd. (Budapest, Hungary) for donation of the hormonal preparation. The authors gratefully acknowledge to Dr. Charlotte Ellertson from the Population Council (Mexico) for helpful discussions during the preparation of this manuscript.

To whom correspondence should be addressed at: Fernando Larrea, M.D. Department of Reproductive Biology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. Vasco de Quiroga 15, Tlalpan 14000, Mexico City, Mexico.

Fax: (525) 655.98.59. E-mail: larrea@conacyt.mx

REFERENCES

1. Stanczyk FZ, Roy S. Metabolism of levonorgestrel, norethindrone, and structurally related contraceptive steroids. Contraception 1990; 42: 67-96.
2. Edgren RA, Smith H, Hughes GA, et al. Biological effects of racemic and resolved 13β-ethyl-4gonen-3-ones. Steroids 1963; 2: 731.
3. Coukell AJ, Balfour JA. Levonorgestrel subdermal implants. A review of contraceptive efficacy and acceptability. Drugs 1998; 55: 861-87.
4. Glassier A. Emergency postcoital contraception. N Engl J Med 1997; 337: 1058-64.
5. World Health Organization: Task Force on Postovulatory Methods of Fertility Regulation. Randomized controlled trial of levonorgestrel versus the Yuzpe regimen of combined contraceptives for emergency contraception. Lancet 1998; 352: 428-33.
6. Ling WY, Robichaud A, Zayid Y, Wrixon W, MacLeod SC. Mode of action of dl-norgestrel and ethinylestradiol combination in postcoital contraception. Fertil Steril 1979;32:297-302.
7. Ling WY, Wrixon W, Zayid Y, Acorn T, Popat R, Wilson E. Mode of action of dl-norgestrel and ethinylestradiol combination in postcoital contraception II. Effects of postovulatory administration on ovarian function and endometrium. Fertil Steril 1983; 39:292-7.
8. Ling WY, Wrixon W, Acorn T, Wilson E, Collins J. Mode of action of dl-norgestrel and ethinylestradiol combination in postcoital contraception III. Effects of preovulatory administration following the luteinizing hormone surge on ovarian steroidogenesis. Fertil Steril 1983; 40:631-6.
9. Swahn ML, Westlund P, Johannisson E, Bygdeman. Effect of post-coital contraceptive methods on the endometrium and the menstrual cycle. Acta Obstet Gynecol Scand 1996;75:738-44.

10. Kesserü E, Garmendia F, Westphal N, Parada J. The hormonal and peripheral effects of d-norgestrel in postcoital contraception. Contraception 1974;10:411-24.
11. Ugocsai G, Resch B, Traub A, Sas M. Biological, microscopic and scanning electron microscopic investigations of the effects of Postinor/d-norgestrel in rabbits. Contraception 1984;30:153-9.
12. Landgren BM, Johannisson E, Aedo AR, Kumar A, Yong-en S. The effects of levonorgestrel administered in large doses at different stages of the cycle on ovarian function and endometrial morphology. Contraception 1989;39:275-89.
13. Spona J, Matt K, Schneider WHF. Study on the action of d-norgestrel as a postcoital contraceptive agent. Contraception 1975;11:31-43.
14. Garmendia F, Kesserü E, Urdanivia E, Valencia M. Luteinizing hormone and progesterone in women under postcoital contraception with d-norgestrel. Fertil Steril 1976;27:1250-5.
15. Wilcox AJ, Weinberg CR, Baird DD. Timing the sexual intercourse in relation to ovulation. N Engl J Med 1995; 333: 1517-21.
16. Batzer FR. Ultrasonic indices of ovulation. J Reprod Med 1986;31(Suppl):764-9.
17. Harper MJK. The implantation window. Baillieres Clin Obstet Gynaecol 1992;6:351-71.
18. Yaron Y, Botchan A, Amit A, Peyser MR, David MP, Lessing JB. Endometrial receptivity in the light of modern assisted reproductive technologies. Fertil Steril 1994; 62:225-32.
19. Miller PB, Soules MR. The usefulness of a urinary LH kit for ovulation prediction during menstrual cycles of normal women. Obstet Gynecol 1996;87:13-7.
20. Larrea F, Sandoval JL, Salinas E, Franco-Rodríguez A, Méndez I, Ulloa-Aguirre A. Evidence for an altered luteinizing hormone sensitivity to naloxone in pathological hyperprolactinaemia. Clin Endocrinol 1995;43:591-600.

21. Hernández I, Parra A, Méndez I, et al. Hypothalamic dopaminergic tone and prolactin bioactivity in women with polycystic ovary syndrome. Arch Med Res 2000;31:216-222.

22. Johannisson E, Landgren BM, Rohr HP, Diczfalusy E. Endometrial morphology and peripheral hormone levels in women with regular menstrual cycles. Fertil Steril 1987;48:401-8.

23. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. Fertil Steril 1950;1:3-25.

24. Hendrickson MR, Kempson RL. Normal histology of the uterus and fallopian tubes. In: Sternberg SS, editor. Histology for pathologist. Philadelphia-New York: Lippincott-Raven, 1997. p. 879-927.

25. Lessey BA, Castelbaum AJ, Wolf L, et al. Use of integrins to date the endometrium. Fertil Steril 2000;73:779-87.

26. Peters AJ, Wentz AC. Endometrial receptivity and the luteal phase. Curr Opin Obstet Gynecol 1992;4:736-42.

27. Gaddum J. Lognormal distributions. Nature 1945;156:463-6.

28. Bolton S. Statistical inference: estimation and hypothesis testing. In: Swarbrick J, editor. Pharmaceutical Statistics: Practical and Clinical Applications. New York: Marcel Dekker Inc., 1990. p. 146-57.

29. Ho PC, Kwan MSW. A prospective randomized comparison of levonorgestrel with the Yuzpe regimen in post-coital contraception. Hum Reprod 1993;8:389-92.

30. Moggia A, Beauquis A, Ferrari F, et al. The use of progestogens as poscoital contraceptives. J Reprod Med 1974;13:58-61.

31. Kesserü E, Camacho-Ortega P, Laudahn G, Schopflin G. In vitro actions of progestogens on sperm migration in human cervical mucus. Fertil Steril 1975;26:57-61.

32. Seregély G. Results of a multicentric trial of Postinor. Ther Hung 1982;30:72-8.

33. Manikkam M, Rajamahendran R. Progesterone-induced atresia of the proestrous dominant follicle in the bovine ovary: changes in diameter, insulin-like growth factor system, aromatase activity, steroid hormones, and apoptotic index. Biol Reprod 1997;57:580-7.

34. Monget P, Monniaux D. Growth factors and the control of folliculogenesis. J Reprod Fertil Suppl 1995;49:321-33.

35. Willis DS, Mason HD, Watson H, Frank S. Developmentally regulated responses of human granulosa cells to insulin-like growth factors (IGFs): IGF-I and IGF-II actions mediated via the type-I IGF receptor. J Clin Endocrinol Metab 1998;83:1256-9.

36. Louhio H, Hovatta O, Sjoberg J, Tuuri T. The effects of insulin-like growth factor I and II on human ovarian follicles in long term culture. Mol Hum Reprod 2000;6;694-8.

37. Schreiber J, Nakamura K, Erickson G. Progestins inhibit FSH-stimulated steroidogenesis in cultured rat granulosa cells. Mol Cell Endocrinol 1980;19:165-73.

38. Fortune J, Vincent S. Progestrone inhibits the induction of aromatase activity in rat granulosa cells *in vitro*. Biol Reprod 1983;28:1078-89.

39. Goodman A, Hodgen G. Antifolliculogenic action of progesterone despite hypersecretion of FSH in monkeys. Am J Physiol 1982;243:E387-97.

40. Wiebe H, Morris C. Effect on an oral contraceptive on adrenal and ovarian androgenic steroids. Obstet Gynecol 1984;63:12-4.

41. Odell WD, Swederdloff RS. Progesterone-induced luteinizing and follicle-stimulating hormone surge in postmenopausal women: a stimulated ovulatory peak. Proc Natl Acad Sci USA 1968;61:529-36.

42. Aono T, Miyake A, Kunigasa T, Kurachi K. Progesterone advancement of estrogen-induced luteinizing hormone release during the midfollicular phase in normal cyclic women. J Endocrinol 1976;71:451-2.

43. Hoff JD, Quigley ME, Yen SSC. Hormonal dynamics at midcycle: A revaluation. J Clin Endocrinol Metab 1983;57:792-6.

44. Batista MC, Cartledge TP, Zellmer AW, Nieman LK, Merriam GR, Loriaux DL. Evidence for a critical role of progesterone in the regulation of the midcycle gonadotropin surge and ovulation. J Clin Endocrinol Metab 1992;74:565-70.

45. Soules M, Steiner R, Clifton D, Cohen N, Aksel S, Bremner W. Progesterone modulation of pulsatile luteinizing hormone secretion in women. J Clin Endocrinol Metab 1984;58:378-83.

46. Von Hertzen H. Research on new methods of emergency contraception. J Reprod Med 1996;5:12-8.

47. Lydon JP, DeMayo FJ, Funk CR, et al. Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. Genes Dev 1995;9:2266-78.

48. Piaggio G, von Hertzen H, Grimes DA, Van Look PFA. Timing of emergency contraception with levonorgestrel or the Yuzpe regimen. Lancet 1999;353:721.

49. Trussell J, Raymond EG. Statistical evidence about the mechanism of action of the Yuzpe regimen of emergency contraception. Obstet Gynecol 1999;93:872-6.

TABLES

Table 1. Baseline clinical characteristics of study groups

	Group A (n=15)	Group B (n=15)	Group C (n=15)
Age (years)	30.7 ± 3.1	31.5 ± 3.1	31.5 ± 2.2
Body mass index (Kg/m^2)	27.7 ± 3.8	25.9 ± 4.9	26.8 ± 3.3
Parity (pregnancies)	2.7 ± 0.9	2.8 ± 1.3	2.8 ± 0.9
Time post-sterilization (years)	3.2 ± 3.9	3.9 ± 2.9	4.3 ± 3.4
Length of three previous cycles [days (range)]	28.7 ± 2.0 (25-31)	28.8 ± 1.5 (26-31)	28.9 ± 2.4 (25-32)

Results expressed as the mean ± SD

Table 2. Ovarian cycle characteristics in control and treated groups

	Control (n=45)	Group A (n=3)	Group B (n=11)	Group C (n=11)	Group D (n=8)
Cycle length (days)	26 ± 3 (21-34)	28 ± 6 (21-32)	27 ± 2 (22-29)	26 ± 1 (23-28)	24 ± 5 (17-32)
Length of follicular phase (days)	15 ± 3 (11-20)	19 ± 2* (17-21)	15 ± 2 (13-18)	15 ± 1 (13-17)	14 ± 3 (10-17)
Length of luteal phase (days)	12 ± 1 (9-15)	9 ± 4* (4-12)	11 ± 2 (9-14)	11 ± 1 (10-13)	10 ± 4* (5-16)
Follicle rupture (cycle day)	15 ± 2 (11-21)	18 ± 4* (14-22)	16 ± 2 (13-18)	16 ± 2 (13-19)	15 ± 2 (11-18)
Maximal luteal P_4 (cycle day)	21 ± 3 (16-29)	24 ± 4 (20-27)	23 ± 2 (19-25)	22 ± 2 (18-24)	24 ± 3 (19-27)
ILE_2-AUC (pg/mL)	989 ± 385 (427-2608)	1083 ± 744 (517-1926)	812 ± 225 (405-1074)	775 ± 239 (416-1265)	1160 ± 817 (161-3790)
ILP_4-AUC (ng/mL)	90.3 ± 41.1 (31-254)	44.7 ± 26.7 (15-65)	69.4 ± 22.6 (42-124)	72 ± 18 (43-114)	15.9 ± 10.6* (1.3-87)

Results expressed as the mean ± SD (range)
* $p<0.05$ *vs.* control

Table 3. Endometrial morphology in control and treated groups

	Control (n=41)	Group B (n=10)	Group C (n=11)	Group D (n=3)
Postovulatory day	8.6 ± 1.3	8.7 ± 0.6	9.0 ± 0.8	9.0 ± 0
Total area of tissue (mm^2)	1,988 ± 55	2,003 ± 45	1,984 ± 62	2,015 ± 26
Number of glands per visual field	59 ± 12	58 ± 7	55 ± 8	58 ± 1
Number of glands in $1mm^2$	30 ± 6	29 ± 4	28 ± 4	29 ± 0.8
Stromal edema (mm^2)	1,049 ± 308	1,225 ± 261	1,011 ± 209	1,142 ± 40
% of tissue with stromal edema	53 ± 15	61 ± 14	51 ± 10	57 ± 1.4
Spiral arteries per visual field	6 ± 3	4 ± 1	5 ± 2	4 ± 0.7

Results expressed as the mean ± SD

FIGURES

Figure 1. Daily and integrated luteal serum P_4 following short-term LNG administration. Mean ± SD of daily serum P_4 concentrations in 45 control cycles (○) and those following short-term LNG administration (●) at middle follicular phase (Group A), periovulatory LH surge (Group B), 24 hours after FR (Group C), and late follicular phase (Group D). The ILP_4-AUC in control and treated cycles is shown in light and dark bars, respectively. The ILP_4 is expressed as the AUC of individual serum P_4 concentrations during nine days after serum LH surge.

*$p<0.05$ *vs.* control cycle.

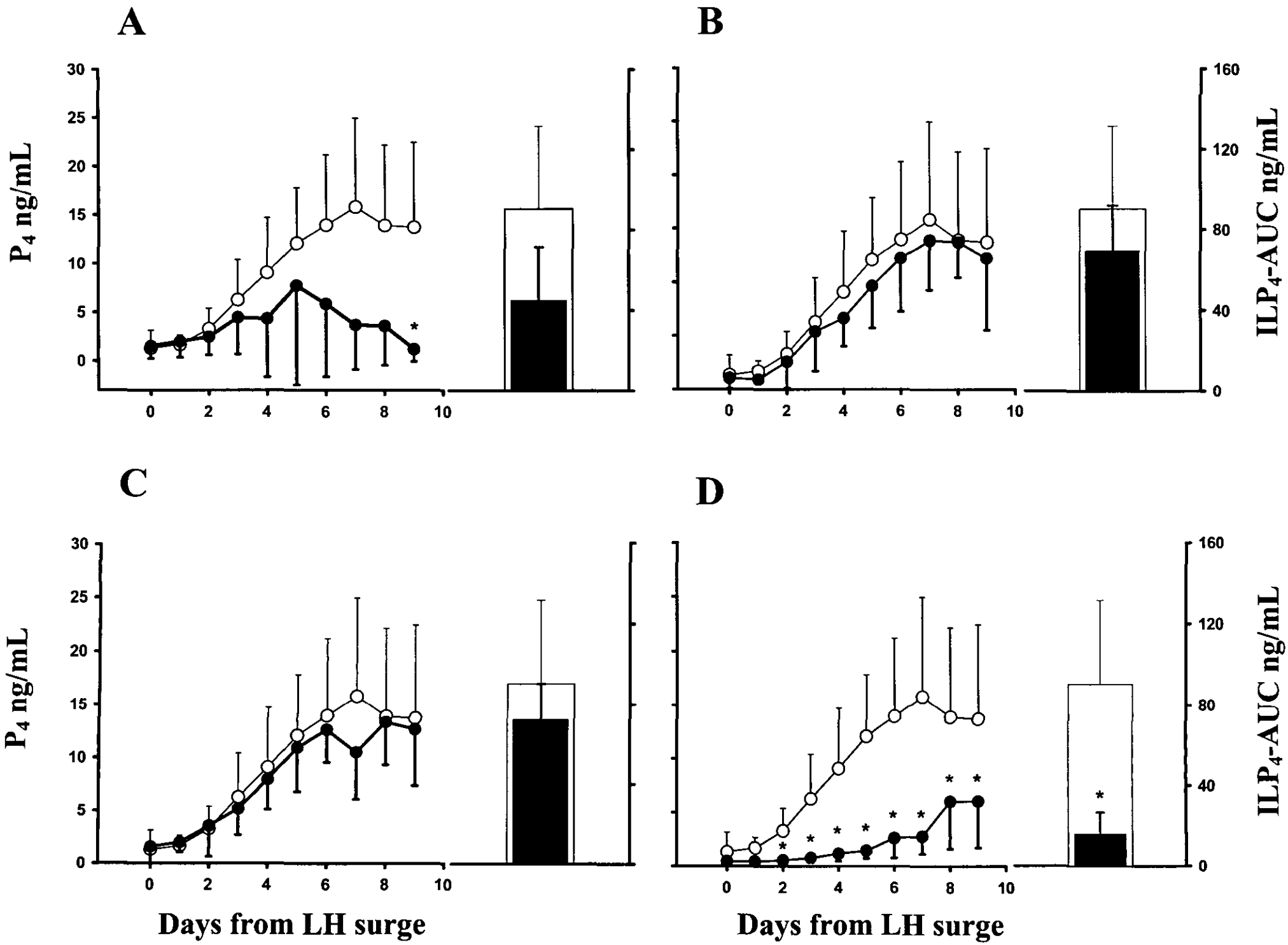
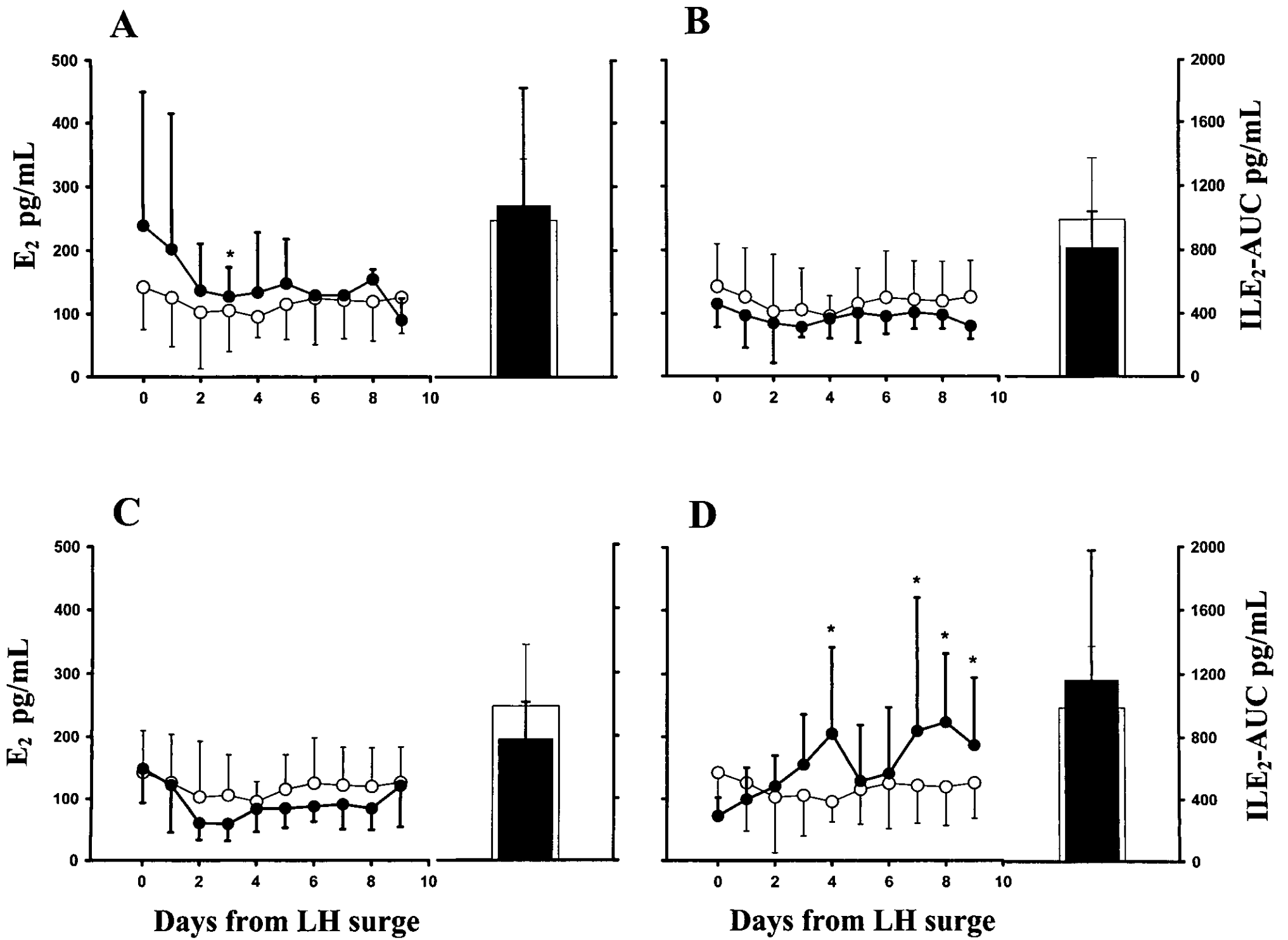

A
B
C
D
P4 ng/mL
ILP4-AUC ng/mL
Days from LH surge
Days from LH surge

Figure 2. Daily and integrated luteal serum E_2 following short-term LNG administration. Mean ± SD of daily serum E_2 concentrations in 45 control cycles (○) and those following short-term LNG administration (●) at middle follicular phase (Group A), periovulatory LH surge (Group B), twenty-four hours after FR (Group C), and late follicular phase (Group D). The ILE_2-AUC in control and treated cycles is shown in light and dark bars, respectively. The ILE_2 is expressed as the AUC of individual serum E_2 concentrations during nine days after serum LH surge.

*$p<0.05$ *vs.* control cycle.


A
B
C
D
E2 pg/mL
ILE2-AUC pg/mL
Days from LH surge
Days from LH surge
0
100
200
300
400
500
400
800
1200
1600
2000
0
2
4
6
8
10
*