In vivo assessment of the human sperm acrosome reaction and the expression of glycodelin-A in human endometrium after levonorgestrel-emergency contraceptive pill administration

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BACKGROUND: The objectives were firstly to assess acrosome reaction (AR) status of spermatozoa following uterine flushing, secondly to measure levonorgestrel (LNG) levels in serum and in uterine flushing fluid and finally to measure endometrial glycodelin-A expression after administration of LNG as a form of emergency contraception (EC).

METHODS: Forty-eight experiments were conducted on 15 regularly menstruating women. Four groups were formed based on different intercourse to treatment interval and treatment to recovery of spermatozoa and the biopsies. RESULTS: Twenty-four and forty-eight hours after treatment, there were 14.5 $\pm$ 3.9 $\times 10^6$ and 17.3 $\pm$ 6.8 $\times 10^6$ sperm recovered from the uterus, respectively. There were no differences between the AR rate and the endometrial glycodelin-A staining intensity in an LNG or placebo treated cycles. The LNG in uterine flushing medium represented 1.38% of the values observed in serum 24 h after the LNG intake. CONCLUSIONS: Twenty-four and forty-eight hours after administration of EC, neither the proportion of AR sperm, nor the glycodelin-A level was influenced by 1.5 mg of LNG. LNG did not impair the cervical mucus either because viable spermatozoa were found in the genital tract 36–60 h after coitus and 24–48 h after LNG intake. The mechanism of action of LNG as EC remains unknown.

**Keywords:** emergency contraception; levonorgestrel; human spermatozoa; acrosome reaction; glycodelin-A

**Introduction**

Levonorgestrel (LNG) is widely used as an emergency contraceptive (EC). It is usually administered in two oral doses of 0.75 mg, given 12 h apart, or in a single dose of 1.5 mg (WHO, 1998; von Hertzen et al., 2002; Devoto et al., 2005; Cheng et al., 2004; Gainer et al., 2006). The mechanism of action of LNG as an EC is still poorly understood (WHO, 1998; Croxatto et al., 2003; Müller et al., 2003; Gemzell-Danielsson and Marions, 2004). The postulated mechanisms of action include an effect on the luteinizing hormone (LH) surge and ovulation, follicular or corpus luteum development, thickening of the cervical mucus affecting sperm penetration and transport and interference with fertilization (Swahn et al., 1996; Durand et al., 2001; Hapangama et al., 2001; Croxatto et al., 2001; Marions et al., 2002).

Another proposed mechanism is that LNG interferes with endometrial function or with endometrial protein expression. One of such protein is glycodelin-A, a secretory progesterone (P)-regulated glycoprotein (Seppala et al., 2002). Inappropriate expression of glycodelin-A by sustained delivery of LNG has been observed in women using LNG-releasing intrauterine system (LNG-IUS) and subdermal contraceptive implants (Mandelin et al., 1997, 2001). Additionally, the administration of LNG for EC prior to the LH surge alters the luteal phase expression pattern of glycodelin-A in serum and endometrium (Durand et al., 2005).

Because it has been reported that the local application of LNG into the tail of the epididymis of rats impairs the in vivo fertilizing potential, suggesting that the drug has a direct effect on spermatozoa (Nikkanen et al., 2000), our
group has been working on the hypothesis that LNG as EC could interfere with the fertilizing capacity of spermatozoa (Bahamondes et al., 2003; Brito et al., 2005; Munuce et al., 2005). However, we have seen no effects of in vitro exposures to LNG.

The duration of the fertilizing capacity of human spermatozoa in the woman is unknown (Gould et al., 1984). Previous studies based on the recovery of spermatozoa from the cervical mucus, uterus, oviducts and peritoneal fluid reported a maximal fertile life of 24–48 h, with a motile life from 48 h to 7 days (Perloff and Steinberger, 1964; Gould et al., 1984; Zinaman et al., 1989; Bielfeld et al., 1992; Williams et al., 1993). In humans, reliable estimates of the duration of spermatozoa fertility are not available due to the ethical and logistic constraints, and to the technical difficulties associated with retrieving the contents of the uterus (Williams et al., 1993). Spermatozoa deposited in the vagina during intercourse may remain in the endocervix for many hours or even days before ascending to the Fallopian tubes, and only these cells have the ability to fertilize (Croatto, 1996). Consequently, spermatozoa deposited in the vagina of women who receive LNG for EC could be exposed to unknown concentrations of LNG for hours or days, and this may influence their fertilizing capacity.

Since the mechanism of action of EC is still poorly understood, the objectives of this study were to assess the acrosome reaction (AR) status of spermatozoa recovered in vivo following flushing of the human uterine cavity after sexual intercourse or artificial insemination, to measure the LNG concentrations in serum and uterine flushings in respect of the endometrial glycolipid-A expression after the administration of LNG as EC, or placebo.

Subjects and Methods

The study was conducted at the Human Reproduction Unit, Department of Obstetrics and Gynaecology, School of Medicine, Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil. Women aged 30–41 years old were invited to participate in the study. The admission criteria included surgical sterilization, regular menstrual periods (25–35 day intervals), negative screening for Chlamydia and gonorrhea, no use of any hormone therapy, no breast feeding or pregnancy in the 3 months preceding the study and a partner with a normal semen analysis performed 2 weeks before the experiments, assessed according to the World Health Organization Manual (WHO, 1999). All the couples gave their written informed consent and the study protocol was approved by the Institutional Ethical Committee.

Study design

This was a double blind, placebo-controlled study. The participants were randomly assigned to four groups (see below) and the time-elapsed to spermatozoa recovery from the uterine cavity was addressed. All the groups were studied during two consecutive cycles, a control cycle (placebo administration) and the treatment cycle with 1.5 mg of LNG (Postinor-2, Ache, Sao Paulo, Brazil). Placebo pills were provided by a specialized pharmaceutical company, which also prepared the vials. For randomization, sealed envelopes were used and the pills were prepared in opaque vials by a person not involved in the study. The coded envelopes were kept outside of the institution.

Forty-eight experiments were conducted, six with placebo and six with LNG in each group. In group I, the women took LNG or placebo 12 h after coitus and the uterine flushing was performed 24 h after pill intake. Group II was given LNG or placebo 12 h after coitus and the uterine flushing was performed 48 h after the pill administration. Group III received the LNG or placebo treatment 36 h after coitus and the uterine flushing was performed 24 h after the pill administration. Group IV received the treatment (LNG or placebo) 24 h after artificial vaginal insemination and the uterine flushing was performed 24 h after the pill administration.

Experiments

In all women, follicular development was monitored daily by ultrasound using a 5.0 MHz vaginal probe (Justavision 400, Toshiba, Toshigi-Ken, Japan), according to the characteristics of the cervical mucus as defined in the WHO Manual (WHO, 1999) and by serum progesterone. The couples were asked to abstain from sexual intercourse during the 5 days preceding the experiment, a behaviour confirmed by absence of spermatozoa in daily assessment of the cervical mucus. The volunteers were instructed to have sexual intercourse on the evening of the day when the greatest follicular diameter showed positive correlation with cervical mucus i.e. spinnbarkheit > 10 cm and crystallization > 2+ (WHO, 1999). In group IV, they were asked to have an artificial insemination the next morning. On the following morning, the cervical mucus sample was taken in order to carry out a post-coital test (PCT) and the experiment was performed only if the PCT was adequate according to the WHO definition (WHO, 1999).

At 12 or 36 h after sexual intercourse or 24 h after insemination, LNG or placebo was administered at the clinic. Uterine flushing was performed 24 or 48 h after pill intake. All experiments were performed in the middle of the menstrual cycle before ovulation. To confirm this event, a daily blood sample was collected and the serum was separated. Presence of the follicular phase of the cycle was confirmed by daily serum progesterone levels below 3 ng/ml (Israel et al., 1972). The samples of serum were stored at −20°C until the measurement of LNG.

Uterine flushing was performed using the following technique. To minimize discomfort, one tablet of 5 mg of midazolam (Dormonid®, Roche, Sao Paulo, Brazil) was administered 20 min before the procedure. Next, the cervix was exposed using a speculum, and the cervical mucus was gently removed using a syringe and placed in a sterile tube with 3 ml of human tubal fluid medium (HTF; GIBCO, BRL, Life Technologies, Inc., Grand Island, NY, USA). The cervix was cleaned with saline solution and then its diameter was estimated. A cervical adaptor (Wisap, Munich, Germany) of appropriate size was fixed to the cervix by vacuum. The uterus was then flushed by gently introducing 5 ml of HTF medium (GIBCO) and recovering it by aspiration with a syringe. The “dead space” in the cannula was estimated to be about 2.5 ml. This procedure was repeated five times using the same fluid, to ensure removal of a maximal number of spermatozoa. The pressure applied to the vacuum syringe and to the aspiration syringe of the cervix adaptor used for flushing was moderate to prevent bleeding, which could have contaminated the material.

Sonographic monitoring of uterine flushing was performed using a 3.5 Mhz abdominal probe (Justavision 400, Toshiba) to assure that the fluid had gone through the uterine cavity and that it had been totally removed. Whenever we observed retained fluid in the uterus, a neonatal feeding tubes number 6 (Embramed, Sao Paulo, Brazil) were gently inserted through the cervix to remove the fluid. The recovered fluid was placed in a sterile tube. The tubes containing
the cervical mucus and fluid recovered after flushing were placed at 37°C under CO₂ for 1 h to stabilize the temperature of the HTF medium and to allow the migration of spermatozoa into the medium (Gould et al., 1984; Zinaman et al., 1989). Following this procedure, two centrifugation cycles were performed using phosphate-buffered saline (PBS) (Dulbecco’s, Gibco) and the pellet was diluted in 1 ml of the PBS. Ten μl were placed into a Makler counting chamber (Sefi Medical Instruments, Haifa, Israel) to assess the sperm concentration. When the material was very thick, due to the condition of the mucus, it was passed several times through a syringe with a fine needle to break the mucus filaments before centrifugation and no enzymatic treatment was used to solubilize the cervical mucus (WHO, 1999).

Endometrial biopsy
After uterine flushing, endometrial specimens were obtained by biopsy (Pipelle de Cornier®, Prodimed, Neually-en-Thelle, France), trying to obtain a representative specimen from the uterine cavity. The specimens were fixed in 10% buffered neutral formalin, dehydrated, embedded in paraffin and cut into 4 μm blocks, deparaffinized, rehydrated, stained with hematoxylin and eosin according to standard protocols and submitted to histology evaluation and immunohistochemical analysis.

Glycodelin-A immunohistochemical staining
Paraffin-embedded tissue sections were deparaffinized and hydrated in graded ethanol. Then, processed as described, including microwave heat treatment (Kamaraen et al., 1996). Endometrial expression of glycodelin-A was evaluated in 5 μm sections of endometrial tissue. Rabbit anti-glycodelin IgG was used as the first antibody, and biotinylated swine anti-rabbit IgG (Dako, Glostrup, Denmark) and normal rabbit serum was used as the second and the control antibody, respectively. Another negative control was added using the first antibody, but immunoabsorbed with purified glycodelin-A (16 μg/ml). Endogenous peroxidase activity was blocked by treatment with 0.6% perhydrol in methanol. The staining was carried out using EnVision® + System-HRP (DakoCytomation, Carpinteria, CA, USA) and rabbit anti-glycodelin IgG (1.2 μg/ml, 20 h, +4°C). Preimmune IgG from the same rabbit was also used as a negative control. The tissue sections were counterstained with haematoxylin (blue). Staining intensity was recorded using a semi quantitative scale of 0, 1, 2 or 3 (none, weak, moderate or strong, respectively).

Assessment of AR status
After the sperm concentration had been evaluated, the fluorescent probe fluorescein isothiocyanate-labelled Pisum sativum lectin (FITC-PSA) was used to evaluate the AR status. Two slides were prepared from the sperm suspension, air-dried at room temperature and protected from light. After drying, they were immersed in cool absolute methanol for 30 s. After that, the slides were stained by immersing them in FITC-PSA for 30 min at a concentration of 40 μg/ml in PBS and protected from light at room temperature. After incubation, the slides were washed in PBS and stored in the dark until evaluation for AR and vitality. Evaluation was done using a fluorescent microscope (Zeiss, Axiosplan II, Jena, Germany) equipped with a specific filter for the FITC-PSA method (with 494-blue excitation, 520 emission, 510–514 barrier). Two hundred cells were evaluated in the fields chosen at random. The only spermatozoa that were considered acrosome-reacted were those with the following patterns: (i) patchy fluorescence of the acrosomal region (partially acrosome reacted) and (ii) fluorescence of the equatorial band only (acrosome reacted) (Cross et al., 1988).

LNG and progesterone assay
The concentration of serum and uterine LNG was determined by using a validated method of high performance liquid chromatography coupled to a tandem mass spectrometry. All procedures were carried out in compliance with Good Laboratory Practices approved by the Brazilian National Authority on Sanitary Surveillance. The standards of LNG used in the experiments were USP (Rockville, MD, USA). Biological specificity of the method was checked by processing independent plasma samples and blank samples obtained from women not using any kind of hormones and with the use of pure HTF medium.

Briefly, the bioanalytical assay for quantification of LNG was developed using an online SPE method (Spark Holland model Symbiosis Generic, Emmen, Netherlands). The mass spectrometer (Sciex/Applied Biosystems, model API5000, Toronto, Canada) was equipped with a photoionization source (APPI) running in positive ion mode was set up in Multiple Reaction Monitoring for the transition m/z 313.3 → 245.1 for LNG. Toluene was used as solvent at the flow rate of 0.15 ml/min. The lowest limit of quantification of the method was 20 pg/ml and it was linear over the range of 20–5000 pg/ml. The run time was 5.5 min and the retention time of the LNG was 3.8 (+0.3) min (Theron et al., 2004).

The determination of progesterone was performed in duplicate using a commercial electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany) with a measurement range of 0.030–60.0 ng/ml and an inter-assay coefficient of variation (CV) of 2.4% and an intra-assay CV of 2.7%.

Statistical analysis
The total number of spermatozoa recovered from the uterine cavity and from the cervical mucus was compared using ANOVA and the Tukey–Kramer test for paired samples. The level of significance was established at P < 0.05. All values are shown as mean ± SEM. As this was an experimental (pilot) study we did not estimate the sample size before conducting the study. However, we calculated a retrospective power analysis to identify if the changes on AR rate or glycodelin-A expression had value. The analysis showed that the study was able to detect differences from 58 to 67% of the SEM.

Results
Spermatozoa recovery from uterine cavity
A total of 33 women were invited and enrolled in the study; however, 18 women were discontinued due to problems during the experiments (see below). Fifteen women contributed with the cycles referred to in this study. All of them contributed at least 2 cycles (n = 30 experiments) and the other 18 experiments were performed in three out of the 15 women. The women had a mean (±SEM) age of 35.1 ± 3.2 years (ranged 30–41) and parity of 2.6 ± 0.7 and 13.0 ± 0.7 mm for the experiments performed in groups I, II, III and IV, respectively.
The number of recovered spermatozoa was $14.5 \pm 3.9 \times 10^6$ cells/ml (range: $0.2–104.9 \times 10^6$ motile spermatozoa/ml) and $17.3 \pm 6.8 \times 10^6$ cells/ml (ranged $0.1–66.4 \times 10^6$ motile spermatozoa/ml) in the groups in which uterine flushing was performed either 24 or 48 h after the pill, respectively, without differences between the cycles treated with LNG or placebo. The characteristics of cervical mucus after the LNG or placebo administration were not changed significantly.

**AR status**

The AR status was evaluated in the spermatozoa separately in the samples recovered from the cervical mucus and the uterine cavity after uterine flushing. The values in each treatment group are shown in Table 1. The AR ranged 9.3–11.0% in those spermatozoa obtained from cervical mucus in the placebo groups and 8.0–12.5% in the LNG treated groups. The spermatozoa AR percentage obtained in uterine flushing ranged 6.2–10.3% and 7.8–13.0% in placebo- and LNG-treated groups, respectively. There were no statistical significant differences between the AR rates in LNG or placebo treated cycles at different times of LNG exposure after sexual intercourse, or after artificial insemination.

### Endometrial biopsies and glycodelin-A staining

All the biopsies showed a proliferative pattern. Glandular and stromal elements were considered separately and given equal importance. Treatment with LNG did not change the expression pattern of glycodelin-A. There were no differences between the glycodelin-A staining intensity on single dose of 1.5 mg of LNG- or placebo-treated cycles when the biopsies were obtained at 48 h after pill intake, the results were taken at 24 or 48 h after treatment. However, when the biopsies were obtained at 48 h after pill intake, the results were affected by the small number of cases available for the analysis (Fig. 1).

### Table 1: AR status of spermatozoa recovered from cervical mucus and uterine flushing, in the different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Spermatozoa from</th>
<th>AR (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Placebo</td>
<td>Mucus</td>
<td>11.0 ± 2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flushing</td>
<td>10.3 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>LNG</td>
<td>Mucus</td>
<td>11.0 ± 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flushing</td>
<td>12.7 ± 2.6</td>
</tr>
<tr>
<td>II</td>
<td>Placebo</td>
<td>Mucus</td>
<td>10.2 ± 2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flushing</td>
<td>6.2 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>LNG</td>
<td>Mucus</td>
<td>10.0 ± 1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flushing</td>
<td>13.0 ± 3.0</td>
</tr>
<tr>
<td>III</td>
<td>Placebo</td>
<td>Mucus</td>
<td>9.8 ± 3.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flushing</td>
<td>7.0 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>LNG</td>
<td>Mucus</td>
<td>12.5 ± 2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flushing</td>
<td>7.8 ± 1.1</td>
</tr>
<tr>
<td>IV</td>
<td>Placebo</td>
<td>Mucus</td>
<td>9.3 ± 3.3</td>
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<td></td>
<td></td>
<td>Flushing</td>
<td>7.3 ± 2.3</td>
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<tr>
<td></td>
<td>LNG</td>
<td>Mucus</td>
<td>8.0 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flushing</td>
<td>10.0 ± 1.5</td>
</tr>
</tbody>
</table>

### Table 2: LNG concentrations in serum and uterine flushing at different time points after 1.5 mg of LNG intake

<table>
<thead>
<tr>
<th></th>
<th>Serum ($n = 18$)</th>
<th>Uterus flushing ($n = 8$)</th>
<th>Serum ($n = 6$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h after LNG administration</td>
<td>3462.9 pg/ml</td>
<td>47.9 pg/ml</td>
<td>1458.8 pg/ml</td>
</tr>
<tr>
<td>48 h after LNG administration</td>
<td>275.5</td>
<td>10.6</td>
<td>88.6</td>
</tr>
<tr>
<td>Range</td>
<td>(1790–5495)</td>
<td>(9.4–12.1)</td>
<td>(1123–1760)</td>
</tr>
</tbody>
</table>

### LNG concentrations

The LNG concentration was measured only on the day of the experiment. The LNG concentration in the uterine flushing medium after 24 h of LNG administration was 1.38% of the total value observed in serum (47.9 versus 3463 pg/ml). At 48 h after the LNG intake the hormone was no longer detected in uterine flushings (<20 pg/ml). In serum, the value at 48 h after LNG intake was almost the 50% of those observed at 24 h after the LNG intake (Table 2).

### Discussion

LNG is a progestin that has been widely used for EC although its mechanism of action is still unclear. In a randomized clinical trial comparing LNG with the Yuzpe regimen, LNG was shown to prevent significantly more pregnancies than the Yuzpe regimen and its effectiveness increased the closer the drug was administered to the time of coitus (WHO, 1998). Some studies have shown that LNG affects the preovulatory events (Swahn et al., 1996; Durand et al., 2001; Hapangama et al., 2001; Croxatto et al., 2001; Marions et al., 2002), and it was postulated that probably it affects also ovulation, thickening of the cervical mucus, sperm migration, penetration or transport, fertilization and endometrial function (Swahn et al., 1996; Trussell and Raymond, 1999; Croxatto et al., 2001; Durand et al., 2001; Hapangama et al., 2001; Marions et al., 2002; Gemzell-Danielsson and Marions, 2004).

In order to contribute to the understanding of the mechanisms of action involved in the contraceptive effect of LNG as an EC, our primary objective was to explore if the drug...
could modify the in vivo status of spermatozoa retained in the endocervix and in the uterine cavity after sexual intercourse or artificial insemination. The results showed that a single dose of 1.5 mg of LNG given before the follicular rupture did not influence the AR status of spermatozoa after different time spans between coitus and the pill intake (12, 24 or 36 h). In addition, the AR rate was similar when it was evaluated at different times between LNG or placebo administration and the recovery of spermatozoa (24 or 48 h).

Although the LNG concentration had reached 3463 ± 276 and 48 ± 11 pg/ml in serum and uterine flushing, respectively, after 24 h of LNG intake, no change in the AR status was detected in the recovered spermatozoa. AR was evaluated previously in vivo in spermatozoa recovered from cervical mucus without any treatment (Zinaman et al., 1989). Additionally, our finding in AR rate was similar to previous results with human spermatozoa recovered from the cervix at 72 h after coitus without any drug treatment (Bielfeld et al., 1992) and a little higher than the findings from Zinaman et al. (1989) after artificial insemination. The present results are in agreement with results from the previous in vitro experiments showing that LNG at 1 ng/ml was unable to induce the AR (Brito et al., 2005; Munuce et al., 2005).

The speculation that LNG as EC could affect the cervical mucus and sperm penetration was based on a well documented effect of progestin and the main mechanism of action of progestin-only contraceptive pill (Moghissi et al., 1973). It was also based on a previous study by Kesseru et al. (1974) showing that, after a single dose of LNG intake as an EC, there is a decrease of spermatozoa recovered from the uterus. This has been observed at 3 h after coitus and more significantly at 7 h. Immobilization of spermatozoa due to alkalinization of the uterine fluid and increased viscosity of the cervical mucus after 9 h of coitus may account for this change. Those mechanisms may have contributed to the lack of spermatozoa penetration.

Our findings contradict those results because we observed that a single 1.5 mg dose of LNG did not impair the quality of cervical mucus and spermatozoa penetration. It was possible to recover an adequate number of viable and motile human spermatozoa both from the cervix and the uterine cavity at 36, 48 or 60 h after coitus. The differences between our results and those of Kesseru and his coworkers (1974), could result from the different doses of LNG used, the times between coitus and evaluation, or even the methodology of spermatozoa recovery that was used > 30 years ago. Therefore, the initial hypothesis that LNG could interfere with sperm function and penetration and could contribute to the mechanism of action in EC was not confirmed in our in vivo and in vitro studies (Brito et al., 2005; Munuce et al., 2005).

Additionally, LNG given before follicular rupture did not influence the expression of glycodelin-A in endometrial biopsies taken at 24 or 48 h after pill intake. The administration of LNG for EC prior to the LH surge does not appear to affect endometrial histology or chronological dating of endometrial maturation (Durand et al., 2001). A second study from the same group (Durand et al., 2005) evaluated only ovulatory women and observed an early rise in serum glycodelin-A concentration and its expression in the endometrium when the drug was administered before the LH peak. They observed that a maximum glycodelin-A endometrial expression was significantly lower when LNG was administered at the time of the LH peak compared to drug intake before the LH surge.

We studied the pattern of glycodelin-A expression due to its anti-fertility activity and because the study from Durand et al. (2005) showed an effect when LNG was administered before the LH surge. However, our results did not show any effect. It may be relevant that, in the Durand study, endometrial biopsies were taken under native conditions, whereas in this study, the biopsies were taken after endometrial flushing that obviously decreases the amount of secreted glycodelin in the uterine cavity. The effect of endometrial flushing on immunolocalization of glycodelin in endometrial tissue has not been determined. Nevertheless, for the purposes of the present study, the effects of prior endometrial flushing were not likely to cause any bias because comparison was made from the biopsies taken after flushing in the same way from women who had taken either LNG or placebo. Another explanation for the difference is that, although LNG was administered before the follicular rupture, the drug could have been administered close to the LH peak. Finally, because the biopsies were taken 24 or 48 h after LNG intake, the exposure time could have been too short for any significant effect to be observed in the glycodelin synthesis.

The previous results on glycodelin-A expression in the endometrium of users of an LNG-IUS (Mandelin et al., 1997) or LNG-releasing contraceptive implants (Mandelin et al., 2001) cannot be extrapolated to users of LNG as EC because the length of LNG exposure was different. The lack of an association between AR and glycodelin-A expression in the endometrium was not surprising because, unlike glycodelin-F from follicular fluid, glycodelin-A does not interfere with the AR (Yeung et al., 2000), and the spermatozoa recovered from uterine flushings are not likely to have been in contact with the inhibitory glycodelin isof orm F.

In conclusion, LNG administered as a form of EC at a single dose of 1.5 mg, 24 or 48 h after sexual intercourse or artificial insemination, did not influence the AR status or endometrial expression of glycodelin-A, suggesting that they were probably not part of the mechanism of action of this kind of EC. Additionally, the administration of the drug had no effect on the quality of cervical mucus or in the penetration of spermatozoa to the uterine cavity. Therefore, the mechanism of action of LNG as an EC remains unknown. However, the clinical significance of AR rate or glycodelin-A expression for health providers probably is small important, since the effectiveness is more important than the real mechanism of action.

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