STUDY SYNOPSIS

Sponsor: ESTETRA SRL **Product:** Estetrol tablets **Active ingredient:** Estetrol	**INDIVIDUAL STUDY TABULAR FORMAT** **Referring to Part XX of the dossier** **Volume:** **Page:**	**(For National Authority Use only)**

Title of study:
A dose-finding study with active control group Yaz® to assess the contraceptive efficacy and the effect on liver function of 5 or 10 mg estetrol combined with either 3 mg drospirenone or 150 µg levonorgestrel, or 20 mg estetrol combined with 150 µg levonorgestrel, by daily oral administration to healthy female volunteers for 3 cycles of 24 days each followed by a 4-day treatment pause.

Investigator:
The principal investigator was [redacted]

Publications:

- Duijkers IJM, Klipping C, Zimmerman Y, Appels N, Jost M, Maillard C, Mawet M, Foidart JM & Coelingh Bennink HJT. Inhibition of ovulation by administration of estetrol in combination with drospirenone or levonorgestrel: Results of a phase II dose-finding pilot study. The European Journal of Contraception & Reproductive Health Care. 2015;20(6):476-489. DOI: 10.3109/13625187.2015.1074675.
- Mawet M, Maillard C, Klipping C, Zimmerman Y, Foidart JM & Coelingh Bennink HJT. Unique effects on hepatic function, lipid metabolism, bone and growth endocrine parameters of estetrol in combined oral contraceptives. The European Journal of Contraception & Reproductive Health Care. 2015;20(6):463-75. DOI: 10.3109/13625187.2015.1068934.
- Kluft C, Zimmerman Y, Mawet M, Klipping C, Duijkers IJM, Neuteboom J, Foidart JM, Coelingh Bennink H. Reduced hemostatic effects with drospirenone-based oral contraceptives containing estetrol vs. ethinyl estradiol. Contraception. 2017;95(2):140-147. DOI: 10.1016/j.contraception.2016.08.018.

Clinical phase: II

Study duration and dates:

The recruitment period was 8 months and the study period covered 13 months (First subject first visit: 02NOV2009; Last subject last visit: 30NOV2010).

First intake of study medication: 15DEC2009; Last intake of study medication: 28OCT2010.

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Objectives:

The primary objectives of the study were to investigate:

- Ovulation inhibition
- Pharmacodynamic (PD) effects on liver function.

The secondary objectives of the study were to investigate:

- Hypothalamic-pituitary-ovarian (HPO) function
- Return of fertility
- The vaginal bleeding pattern
- The effect on endometrial thickness
- The pharmacokinetics (PK) of estetrol (E4)
- Safety/tolerability
- Metabolism and excretion of E4 in urine.

Methodology:

This was an open, parallel dose-finding study with subjects assigned to 1 of 6 treatment groups (see Test Products below for details). Stratification was performed based on the day ovulation occurred in the pre-treatment cycle.

Number of subjects (total and for each treatment):

Planned: 110 subjects

Treated: 111 subjects were assigned and 109 subjects were treated; 17-20 subjects were assigned to each of the 6 treatment groups.

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Main criteria for inclusion:

Subjects were included in the study if they met all of the following inclusion criteria:

- Women aged 18-35 years (inclusive) who were willing to use a barrier method of contraception (e.g. condom) during the wash-out cycle, the pre-treatment cycle, the period of study drug administration, and the post-treatment cycle of up to 7 days after the follow-up visit.
- Women who ovulated in the pre-treatment cycle between day 9 (±1) and day 24 (±1) after start of their menses, who had a subsequent progesterone (P) concentration of ≥ 16 nmol/L (when ovulation was observed by trans-vaginal ultrasound [TVUS] and P was close to 16 nmol/L it was at the discretion of the investigator to include a subject) and whose next menstruation did not start within 6 (±1) days after ovulation.
- Body mass index (BMI) between 18 and 30 kg/m^2 (inclusive).
- Good physical and mental health as judged by the investigator based on medical history, physical examination (including breast examination), clinical laboratory and vital signs.
- Both ovaries visible by TVUS.
- Willing to give informed consent in writing.

In addition, key exclusion criteria included:

- Clinically significant abnormal results of routine hematology, serum biochemistry, urinalysis, and/or an electrocardiogram, in the opinion of the investigator, at screening.
- Women with a wash-out cycle with a duration > 42 days.
- Known or suspected pregnancy or lactation.
- Pregnancy during accurate hormonal contraceptive use in the past.
- Clinically significant abnormalities of the uterus and/or ovaries detected by examination and/or ultrasound (non-physiological ovarian mass or significant uterine pathology).
- Use in the last 6 months of depot progestin preparations, injectables, or biodegradable implants.
- Use of (hormonal) intrauterine device within 2 months before screening.
- Contraindications for contraceptive steroids.

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Test products, dose and mode of administration, batch number:

Study medication was administered orally in this study and consisted of the test product E4 combined with either drospirenone (DRSP) or levonorgestrel (LNG), and the comparator product Yaz (20 µg ethinyl estradiol [EE]/3 mg DRSP). The doses used in each of the 6 treatment groups were as follows:

Treatment groups:

- Group 1: 5 mg E4 combined with 3 mg DRSP
- Group 2: 10 mg E4 combined with 3 mg DRSP
- Group 3: 20 µg EE combined with 3 mg DRSP (Yaz)
- Group 4: 5 mg E4 combined with 150 µg LNG
- Group 5: 10 mg E4 combined with 150 µg LNG
- Group 6: 20 mg E4 combined with 150 µg LNG

Subjects in the E4 treatment groups received the study medication for a period of 3 consecutive treatment cycles of 24 days each followed by a 4-day treatment pause. Subjects in the Yaz comparator group received study medication for 3 consecutive treatment cycles of 28 days (including placebo for the last 4 days).

- E4 was supplied as 5 or 10 mg tablets, packed in blisters (5 mg tablets: lot number H0831; 10 mg tablets: lot number H0830).
- DRSP was supplied as 3 mg tablets, packed in blisters (lot number H0822).
- LNG was supplied as 150 µg tablets, packed in blisters (lot number H0844).
- Yaz was supplied as tablets, packed in the original blisters (lot number WED25Z).

Reference therapy:

Yaz was given as the active control in this study, as described above.

Duration of treatment:

E4 plus DRSP or LNG treatment groups: subjects were treated for 3 treatment cycles of 24 days each followed by a 4-day pause.

Active control treatment group: subjects were treated for 3 treatment cycles of 28 consecutive days (24 active tablets followed by 4 placebo tablets).

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Criteria for evaluation:

The study was explorative and the aim was to gather information to compare the effects of E4 versus EE and help decide which dose of E4 and type of progestin should be used in future studies. To this end, data were collected to evaluate the following variables:

Primary variables:

- Follicle development was measured in both ovaries by TVUS and the average diameter was calculated.
- Analysis of liver parameters: carrier proteins; lipids; hemostasis; liver function; bone; glucose metabolism; other liver proteins were done by taking blood samples at particular day and visit for each.

Secondary variables:

- Suppression of the HPO axis was evaluated by measuring of luteinizing hormone, follicle stimulating hormone, estradiol, progesterone, and testosterone at particular day and visit for each.
- Return to fertility was measured by monitoring follicular growth using TVUS in the post-treatment cycle until ovulation occurred.
- Vaginal bleeding was assessed by the completion of a daily diary by the subjects starting on day 1 of the pre-treatment cycle up to the follow-up visit.
- Endometrial thickness (double-layer) was measured by TVUS at the same time points as follicular growth was measured.
- PK of E4 was investigated by the collection of steady-state plasma samples in the E4 treatment groups 24 hours after study medication intake.
- Metabolism and excretion of E4 in urine was investigated by the collection of 24-hour urine at steady-state.
- Safety and tolerability was evaluated by recording blood hematology, biochemistry, urinalysis, physical, gynecological and breast examinations, blood pressure, heart rate, body weight, and objective and subjective side effects. A questionnaire was completed by the subjects about well-being during study medication use.

Statistical methods:

Efficacy analysis: No formal statistical testing was performed.

Efficacy was evaluated by measuring follicle development, liver parameters (carrier proteins, lipids and lipoproteins, hemostasis parameters, liver function parameters, bone parameters, glucose metabolism parameters, and other liver proteins), endometrial thickness, vaginal bleeding, laboratory assessments (endocrinology – steroid endocrinology and growth endocrinology), PK analysis of E4, and analysis of E4 excretion.

Safety analysis:

Safety was evaluated by analysis of the data collected on blood hematology and biochemistry, urinalysis, physical, gynecological and breast examination, blood pressure, heart rate, body weight, and objective and subjective side effects.

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Demographics and baseline characteristics:
All subjects included in this study were female. With the exception of mean weight and BMI, the demographic and baseline characteristics were generally similar across the treatment groups. The mean age was 22.9 years (range, 18 to 33 years) and the majority of subjects were white (102/111; 91.9%). Subjects had a mean height of 170.0 cm (range, 152 to 188 cm), a mean weight of 65.84 kg (range, 44.3 to 93.2 kg), and a mean BMI of 22.74 kg/m^2 (range, 18.2 to 30.0 kg/m^2). However, the mean weight was lower in the 5 mg E4 plus LNG group (61.85 kg) and 10 mg E4 plus LNG group (63.50 kg) compared to the other groups (ranging between 66.10 and 68.08 kg). Similarly, mean BMI was also lower in the 5 mg E4 plus LNG group (21.51 kg/m^2) and 10 mg E4 plus LNG group (21.78 kg/m^2) compared to the other treatment groups (22.54 to 24.28 kg/m^2).

Efficacy results:

Primary efficacy analysis

No ovulations were observed in any of the treatment groups demonstrating the efficacy of E4 combined with either 3 mg DRSP or 150 µg LNG in suppressing ovulation.

Mean maximum follicle size decreased with increasing dose of E4. This would be anticipated given the effects of estrogen on follicular development and ovulation. In both treatment cycles 1 and 3 the EE group and the 20 mg E4 plus LNG group had the smallest mean maximum follicle size. No difference was observed between the 2 progestins in the 5 and 10 mg E4 groups.

During treatment cycle 1, in all treatment groups the majority (80.0% or more) of subjects had no ovarian activity (Hoogland score 1) or potential activity (Hoogland score 2). The remaining subjects had a non-active follicle-like structure (FLS) (Hoogland score 3) or active FLS (Hoogland score 4). The number of subjects with non-active FLS or active FLS was too small to draw conclusions on differences between the dose groups. For the E4 treatment groups, the number of subjects with non-active FLS or active FLS was higher in treatment cycle 3 compared to treatment cycle 1. Still, also during treatment cycle 3 the majority (50.0% to 100%) of the subjects had no activity (Hoogland score 1) or potential activity (Hoogland score 2). The percentage of subjects with non-active FLS or active FLS was lower in the EE plus DRSP group (0%) and in the 20 mg E4 plus LNG group (12.6%) than in the other treatment groups (26.7% to 50.0%), so these two dose groups appeared most effective in suppressing ovarian activity. In treatment cycle 3, there was no discernible difference in Hoogland score distribution between the two progestins. Importantly, no luteinized unruptured follicles (Hoogland score 5) or ovulations (Hoogland score 6) were reported in any of the groups.

Of the liver function parameters, increases were observed with EE plus DRSP for the liver proteins sex hormone-binding globulin (SHBG), corticosteroid-binding globulin (CBG), activated protein C (APC) resistance, C-reactive protein and angiotensinogen, whereas the effect of E4 plus DRSP was much less or absent and in the E4 plus LNG groups, in some cases even a decrease was observed in these parameters.

Secondary efficacy analyses
No consistent dose-related trends in mean LH or FSH were evident for subjects treated with E4 plus DRSP or LNG. However, mean LH and FSH were notably lower in the 20 mg E4 plus LNG group and the 20 µg EE plus DRSP group compared to the other treatment groups. Over the entire treatment period, mean LH and FSH levels were lowest for subjects treated with EE plus DRSP compared to those treated

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with E4 plus DRSP. No notable differences in mean LH and FSH levels were observed with type of progestin received. Lower E2 levels were observed with increasing doses of E4, regardless of whether subjects were treated with E4 plus DRSP or LNG. This dose-dependent trend was observed across all 3 treatment cycles. Over the entire treatment period, mean E2 levels were lower for subjects treated with EE plus DRSP than those treated with E4 plus DRSP. Across the treatment cycles, mean E2 levels were variable; however, values tended to be lowest in the EE group across the 3 treatment cycles. Mean E2 levels over the entire treatment period and across the cycles were slightly lower for subjects treated with LNG compared to subjects treated with DRSP. Progesterone and testosterone levels were generally comparable across the entire treatment period for all subjects, with no dose-related trends with increasing dose of E4, and no difference between subjects treated with E4 plus DRSP or LNG and those treated with EE plus DRSP. Furthermore, no notable differences in mean progesterone and testosterone levels were observed with type of progestin received. Results for testosterone should be interpreted with caution as a direct comparison between groups is not truly possible as most of the testosterone values were below the lower limit of quantification.

Generally, growth endocrinology parameters remained constant over the treatment cycles; however, IGF 1 decreased in subjects treated with EE plus DRSP but remained relatively stable in subjects treated with E4 plus DRSP or LNG. IGFBP1 and GH levels increased compared to baseline across the treatment cycles in all treatment groups, with the exception of IGFBP1, which did not increase in the 5 mg E4 plus LNG group. The steroid endocrinology parameters E1 and E1S decreased from baseline at treatment cycle 3 day 24 in the EE plus DRSP treatment group, the 10 mg E4 plus DRSP group and all E4 plus LNG treatment groups. There was no evidence of a dose relationship with E4 dose for the change in either E1 or E1S from baseline.

Subjects treated with E4 plus DRSP had their first day of ovulation approximately 17 days after the last treatment, with no difference evident with 5 or 10 mg E4. The mean number of days to first ovulation was considerably longer for subjects treated with E4 plus LNG (approximately 21 days) but no difference was observed with increasing dose of E4 in this regimen.

Across the treatment cycles, no dose-related trends in endometrial thickness were observed with increasing dose of E4. No notable differences in endometrial thickness were recorded between subjects treated with E4 plus DRSP and those treated with EE plus DRSP, or between subjects treated with E4 plus DRSP and those treated with E4 plus LNG. The presence of cervical mucus when a follicle was larger than 13 mm was variable across treatment groups, with no strong trends evident.

Vaginal bleeding patterns were variable across the treatment cycles but no clear trends between the treatment groups were evident.

Plasma E4 trough levels showed a dose-dependent trend, with the highest mean levels reported at the 20 mg E4 dose level. For the 5 and 10 mg E4 treatment groups, the mean plasma E4 trough levels were lower in the treatment groups who received E4 with LNG compared with those who received E4 with DRSP.

E4 was primarily excreted in the urine as E4 glucuronide and only a small amount of E4 sulfate. Urinary excretion of unconjugated E4 was low.

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Safety results:

An overall summary of treatment-emergent AEs (TE-AEs) is presented in the table below:

Type of TE-AE	**Number (%) of subjects**					
	5 mg E4/ DRSP N=17	**10 mg E4/ DRSP N=19**	**20 µg EE/ DRSP N=20**	**5 mg E4/ LNG N=18**	**10 mg E4/ LNG N=17**	**20 mg E4/ LNG N=18**
Subjects with:						
At least 1 TE-AE	17 (100)	17 (89.5)	17 (85.0)	17 (94.4)	16 (94.1)	18 (100)
At least 1 SAE	0	0	0	0	0	0
SAEs outcome death	0	0	0	0	0	0
Drug related TE-AEs	13 (76.5)	11 (57.9)	12 (60.0)	14 (77.8)	14 (82.4)	9 (50.0)
TE-AEs of known severe intensity	3 (17.6)	6 (31.6)	4 (20.0)	3 (16.7)	0	2 (11.1)
TE-AEs as primary reason for discontinuation	2 (11.8)	1 (5.3)	0	0	2 (11.8)	0
In-treatment pregnancy	0.	0	0	0	0	0

SAE = serious adverse event; TE-AE = treatment-emergent adverse event

More than 85% of subjects per treatment group experienced at least 1 TE-AE. Events were most commonly reported in the system organ classes infections and infestations, gastrointestinal disorders, reproductive system and breast disorders, and nervous system disorders, with a higher incidence for reproductive system and breast disorders in subjects who received E4 plus DRSP compared with E4 plus LNG whereas there was a higher incidence for skin and subcutaneous tissue disorders in subjects who received E4 plus LNG compared with E4 plus DRSP. A higher proportion of subjects in the E4 plus DRSP treatment groups reported dysmenorrhea and breast enlargement compared with E4 plus LNG. Acne and diarrhea were more frequently reported for subjects who received E4 with LNG compared with subjects who received E4 plus DRSP.

Severe TE-AEs were reported for between 0% and 31.6% of subjects, with vomiting, influenza, and headache the only severe TE-AEs reported for more than 1 subject in any treatment group. TE-AEs considered drug-related were reported for at least 50% of subjects per treatment group. The most common drug-related TE-AEs were abdominal pain lower, nausea, headache, dysmenorrhea, breast enlargement, and acne. The incidence of drug-related headache was slightly higher in subjects treated with E4 compared to EE and the incidence of drug-related acne was higher in subjects treated with E4 plus LNG compared to E4 plus DRSP or EE.

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TE-AEs leading to discontinuation were reported for 5 subjects overall (2 subjects each in the 5 mg E4 plus DRSP and 10 mg E4 plus LNG groups and 1 subject who received 10 mg E4 plus DRSP). These included events of fatigue, bronchitis, affect lability, headache, mood swings, and decreased libido. Of these events, headache was the only TE-AE that resulted in the discontinuation of more than 1 subject. No deaths or serious adverse events occurred during the study. No safety concerns were raised from hematology, biochemistry or urinalysis results or for vital signs or physical examination findings.

Conclusions:

In conclusion, treatment with 5, 10 or 20 mg E4 combined with either DRSP or LNG inhibited ovulation in all cycles investigated and was highly effective in suppressing the HPO axis and ovarian follicular development with maximal effect in the 20 mg E4 plus LNG regimen based on Hoogland scores.

Analysis of the PD effects of E4 on liver function demonstrated a much lower impact than EE, especially on parameters of hemostasis and on liver proteins (SHBG, CBG, APC resistance, C-reactive protein and angiotensinogen).

E4 was very well tolerated and there was no evidence of any safety concern.

Date of final report:

02 December 2011 (Version 1.0)

Date of synopsis:

14 June 2022 (Version 1.1 for Disclosure and Transparency)