OVARIAN MORPHOLOGY DURING AND AFTER LYNESTRENOL TREATMENT AROUND MENOPAUSE

L. DE BAENE*, L. J. VAN BOGAERT**, E. DE MUYLDER* and K. THOMAS***.

* Clinique St-Jean, Service de Gynécologie
Rue du Marais, 114
B – 1000 BRUSSELS , BELGIUM

** Clinique St-Jean, Anatomie Pathologique
Rue du Marais, 114
B – 1000 BRUSSELS

*** Physiology of Human Reproduction Research Unit
Department of Obstetrics and Gynecology
University of Louvain, 5330
Avenue Em. Mounier 53
B – 1200 BRUSSELS

ABSTRACT

In this histological study, the inhibition of a continuous oral administration of lynestrenol 5 mg/day on the ovarian follicle and corpus luteum development was found to be extremely pronounced. Known estrogen - producing structures such as antral, mature follicles and corpora lutea were completely absent during treatment. Structures devoid of cystic function however, as persistent and cystic follicles and follicular cysts, seemed not to be influenced in their histological appearance and frequency. As the recovery of the cyclic ovarian function after cessation of the medication compares favorably with the spontaneous cycle, a continuous lynestrenol treatment of 5 mg/day can be regarded as a safe endocrinological regimen.

INTRODUCTION

The effect of contraceptive hormones, either classically combined or single high or low-dosed progestatives, on hypothalamo-pituitary-ovarian axis and on the female genital tract can be studied either hormonally or morphologically. Both approaches are needed to clarify the physiological mechanisms of their action and the endocrinological pathways.

Submitted for publication June 1, 1981
Accepted for publication January 22, 1982

Progestative agents are known depressors of the follicular growth stimulators FSH and LH (1). Besides their central effect, progestatives can inhibit the ovarian steroidogenesis directly as suggested by *in vitro* animal studies (2).

The drug lynestrenol studied here is an estrenol derivative of 19-nor-testosterone (17-ethinyl-3-desoxynortestosterone). Lynestrenol appears to be metabolised through norethisterone, although the latter steroid may not be an obligatory intermediate in lynestrenol metabolism (3). The synthetic steroid has distinct progestative characteristics (4). Continuous administration of high doses (5 mg/day) gives rise to hypo-oestrogenic amenorrhea with infertility (5).

In this study, lynestrenol (5 mg/day), as reported earlier (6), has been used to treat endometrial-hyperplasia, fibromatosis, endometriosis, as well as dysmenorrhea.

The aims of the present investigation were two-fold. Firstly, in order to elucidate the anti-oestrogenicity of the drug, the study was intended to evaluate its influence on known oestrogen-producing ovarian functional structures. Secondly, attention was stressed to the safety of the therapeutic regimen on the recovery of the cyclic ovarian function after cessation of the medication.

PATIENTS AND METHODS

1. PATIENTS

All the patients under study were subjected to total radical hysterectomy, performed for various benign pathologic conditions considered oestrogen-dependent (cfr. introduction) or for a cervical neoplasm. The 55 subjects were divided into three groups :

GROUP A served as control since none of the 18 patients had ever used lynestrenol. Their mean age was 46.9 years (range 41 – 53 years). They all were still menstruating at the time of operation.

GROUP B consisted of 14 women who were operated upon while under lynestrenol therapy (5 mg/day). Their mean age was 45.7 years (range 38 – 52) and the mean duration of continuous therapy was 12.8 months (range 1 month – 6 years).

GROUP C was composed of 23 subjects hysterectomised within 24 days after cessation of the same lynestrenol therapy (range 4 – 24 days). The mean age of this group was 45.4 years (range 33 – 51 years) and the mean duration of continuous treatment was 12 months (range 1 month – 11 years).

2. METHODS

The uterus and both ovaries of each patient were fixed in neutral 10% formalin. The ovaries were bisected along their main axis. The uteri were cut perpendicularly to the endometrial surface. After embedding in paraffin, 5 micron-thick sections were cut and stained with haematoxylin and eosin. The whole histological study was carried out by the same examiner (L. J. v. B.). The follicles and derived structures were counted on a whole section of both ovaries, and classified according to the criteria reported in TABLE I, based on data from various authors (7, 8, 9, 10, 11). The distinction between persistent follicles (PF), cystic follicles (CF) and follicle cysts (FC) was not based on the diameter of the structure, but rather on the thickness of the granulosa-cell layer. These follicle-derived structures may be considered to lack cyclic functional activity (i.e. mainly oestrogenic). This is in contrast with the maturing follicles, which are assumed to have a cyclic oestrogenic-producing activity.

RESULTS

I. Controls (GROUP A and TABLE II)

Cyclic structures were present in 11 out of the 18 control cases, an occurence of 61.1%; non-cyclic structures showed the same incidence. Five of the eight subjects operated upon during the presumed secretory phase of the menstrual cycle showed a corpus luteum. Four women out of the ten who were in the proliferative phase ($\leqslant$ D 13) exhibited cyclic structures in their ovaries.

II. Subjects under lynestrenol therapy (GROUP B and TABLE III)

None of the ovaries resected during treatment elicited cyclic follicular oestrogen-producing structures (F II, MF, CL). However, non-cyclic structures (PF, CF, FC) were found in half of the cases. In the others none of either structures could be evidenced. This might partly

TABLE I. Morphological features of follicles and their derivatives

	Abreviation	Granulosa	Theca Interna	Theca Externa
CYCLIC STRUCTURES				
Antral follicle	FII	multilayered	–	–
Mature follicle	MF	multilayered	spindle-shaped or polyhedral cells with fine lipid droplets	fusiform cells without endocrine mophology
Corpus Luteum	CL	more than 10 large polygonal luteinized cells	luteinized, small polygonal cells	epithelioid appearance
NON–CYCLIC STRUCTURES				
Persisting follicle	PF	multilayered, mitoses present	epithelioid appearance occasional mitoses, no luteinization	idem
Cyclic follicle	CF	single layer or less than 3 cells thick	varying degree of luteinization	idem
Follicle cyst	FC	flattened or absent	spindle-shaped with scanty lutein cells	idem

TABLE II. Radical hysterectomy performed on menstruating patients (Group A)

Age	Day	FII	MF	CL	PF	CF	FC	Endometrium Proliferative (Thickness in mm)
45	3	.	.	.	xxx	xx	.	3
45	6	.	.	.	.	xxxx	.	3
53	7	.	.	.	x	xx	.	3
41	9	x	.	.	.	x	.	1
52	10	x	.	.	.	.	.	4
46	11	x	.	.	.	.	.	1.5
48	12	.	.	.	xx	.	.	3
47	13	.	.	.	.	x	x	5.5
50	13	.	.	.	.	x	.	2
46	13	.	x	.	.	.	.	4
44	14	xx	x	.	x	xxxx	xx	3
43	14	.	.	x	xx	.	.	secretory
44	14	.	x	x	.	xx	.	secretory
47	16	.	.	x	.	.	.	secretory
48	20	.	.	x	.	.	.	atypical secretory
50	24	.	.	.	.	.	.	5.5
47	27	.	.	x	.	.	.	atypical secretory
49	27	.	.	x	.	xxx	x	secretory

Classification of patients following number of days since last menstrual period

Age	Age of patients
Day	Number of days since last menstrual period
X	Presence of one of the defined structures

FII, MF, CL, PF, CF, FC : see TABLE I.

TABLE III. Radical hysterectomy performed on patients under lynestrenol therapy (Group B)

Age	Dur	FII	MF	CL	PF	CF	FC	Endometrium Proliferative (Thickness in mm)
52	1 M	.	.	.	.	.	.	1
42	2 M	.	.	.	.	xx	.	1
51	2 M 1/2	.	.	.	.	.	.	1
45	3 M	.	.	.	x	.	.	1
41	3 M 1/2	.	.	.	.	xx	.	1
51	3 M 1/2	.	.	.	.	.	.	1.5
51	5 M	.	.	.	.	.	.	1.5
50	7 M	.	.	.	.	.	x	1
41	8 M	.	.	.	.	.	.	1.5
46	1 Y	.	.	.	xx	.	.	1
38	1 Y	.	.	.	xxxxxx	.	.	1
42	2 Y	.	.	.	.	.	.	2
42	2 Y	.	.	.	x	.	.	1
48	6 Y	.	.	.	.	.	.	1

Classification of patients following duration of lynestrenol therapy

Age	Age of patients
Dur	Duration of lynestrenol therapy before radical hysterectomy
X	Presence of one of the defined structures

FII, MF, CL, PF, CF, FC : see TABLE I.

be due to the older age of most of them (48 to 52 years). Nonetheless, since 2 patients were younger (41 and 42 years), the hazard of the section plane might probably also have played a role.

III. Patients within 24 days after discontinuation of lynestrenol (GROUP C and TABLE IV)

One of the aims of present study was to investigate the behavior of the ovaries during the first cycle after discontinuation of lynestrenol therapy. The reappearance of cyclic structures was observed during the first menstrual cycle in 9 women, an incidence of 39.1% (versus 61.1% in the controls). When therapy had been stopped for more than 14 days, ovulation did occur in 2 out of 4 cases. The appearance of antral follicles (F II) was in no way delayed since they were seen as early as D7 to D15, which compares favorably with D9 – D14 of controls. Mature follicles (MF) appeared sooner in the treated group (D9 – D12) than in the controls (D13 – D14). Non-cyclic structures were observed in 17 of the 23 cases, i.e. 73.4% (versus 61.1% in controls).
Since endometrial thickening in the proliferative phase of the cycle is an indirect indicator of follicular maturation, comparison of endometrial proliferation between GROUP C ($r = 0.468$) and GROUP A ($r = 0.283$) patients before day 14 yielded comparable regression lines.

DISCUSSION

The absence of antral or mature follicles and corpora lutea under administration of lynestrenol (5 mg/day) reveals the inhibitory effect of the drug on follicular maturation. Although LAUWERIJNS and FERIN (12) concluded that lynestrenol (5 mg/day) interrupted the follicular growth at an early stage, they also found some secondary and tertiary follicles. Accordingly, a continuous oral 5 mg/day lynestrenol regimen has a similar impact on the finding of follicular structures to that reported with intramuscular depot-progestative (13). Although in women under combined oral contraceptive treatment corpora lutea are absent, secondary and mature follicles are present though rare (13, 14). Contrary to high dose (5 mg/day) lynestrenol, low dose gestagen contraception does not impair follicular maturation morphologically (15, 16). However, even as with other low dose gestagen (17, 18), a 0.5 mg/day regimen of lynestrenol is able to modify follicular steroidogenesis (19).

TABLE IV. Radical hysterectomy performed within 24 days after discontinuation of lynestrenol (Group C)

Age	Dur	Day	FII	MF	CL	PF	CF	FC	Endometrium Proliferative (Thickness in mm)
41	6 M	4	.	.	.	x	x	.	1
33	1 Y	4	.	.	.	.	x	.	1
49	1 M	4	.	.	.	x	.	.	2
46	4 M	4	.	.	.	.	.	x	1
48	1 M	4	.	.	.	.	.	.	1
44	6 M	5	.	.	.	xx	.	.	3.5
46	1 Y	7	.	.	.	xx	.	.	4.5
46	6 M	7	x	.	.	x	x	.	4.5
47	2 M	8	.	.	.	.	.	.	5.5
51	2 M	9	.	.	.	.	.	x	2
39	2 Y	9	.	xx	.	xx	xx	.	7.5
44	4 M	9	.	.	.	xx	x	.	3.5
50	5 M	9	.	x	.	.	.	.	3.5
43	1 M	10	x	.	.	x	.	xxx	2
47	11 Y	11	.	.	.	.	.	x	1
48	9 M	11	.	x	.	.	x	.	4.5
50	1 M	11	.	.	.	.	.	.	2
49	2 M	12	x	.	.	x	x	.	3.5
46	1 Y	12	.	x	.	.	.	.	4
37	1 Y	15	x	.	x	.	x	.	secretory
47	3 M	18	.	.	x	.	.	.	secretory
44	1 M	21	.	.	.	x	.	x	7.5
51	3 M	24	.	.	.	.	x	.	4.5

Classification of patients following number of days since withdrawal of lynestrenol therapy

Age	Age of patients
Dur	Duration of lynestrenol therapy before radical hysterectomy
Day	Number of day since withdrawal of lynestrenol
X	Presence of one of the defined structures

FII, MF, CL, PF, CF, FC : see TABLE I.

Plasma oestrogen values (unpublished data) in patients of the present series, i.e. under treatment (5 mg/day), suggest the functional absence of antral or mature follicles and corpora lutea. Indeed oestrone and oestradiol concentrations fluctuate around the lower limits of normal menstrual levels, as under depot-progestatives (20).

As reported with depot-progestatives (13), we did not find any influence of 5 mg/day lynestrenol regimen on the frequency of the non-cyclic follicles (PF, CF, FC).

Combined contraceptive regimens however increase these non-cyclic structures (13, 14), but low-dose progestatives do not. The apparently weaker impact on follicular growth of combined contraceptives as compared to a single progestative treatment may possibly be explained by the discontinued schedule of bihormonal therapy, where the seven-day withdrawal period might be sufficient to permit a rapid resumption of follicular maturation. Another explanation could be the presence of mature follicles under bihormonal contraceptives, in contrast to the stronger inhibition caused by a continuous progestative treatment.

It is important to note the absence of any delay of follicular growth after cessation of a high-dose oral lynestrenol therapy, despite the marked inhibition during treatment. After withdrawal of lynestrenol, the endometrial proliferation, which reflects the steroidogenesis of the follicles, as well as the time-course of the appearance of antral and mature follicles and also the corpus luteum ratio, are in good correlation with the results observed in the control group.

The absence of delay in the resumption of normal follicular maturation is all the more surprising since lynestrenol in the dose utilized strongly depresses the hypothalamo-pituitary-ovarian axis. In animal experiments, hypothalamic and pitiutary implants of northisterone, a progestative closely related to lynestrenol and an intermediate in lynestrenol metabolism (17), decreased the LH-release (21, 22). The low L –FSH levels in the human under high-dose lynestrenol (1) result, at least partly, from this direct inhibiting effect of lynestrenol on ovarian steroidogenesis (2).

REFERENCES

1. Kutten, F., Moufarege, A. and Mauvais-Jarvis, P. Bases hormonales de la contraception progestative discontinue. Nouv Press Méd 7 : 3109 – 3113 (1978)
2. Lacroix, E., Eechaute, W. and Leusen, I. Influence of lynestrenol on oestrogen production by rat ovaries. Arch Int Pharmacodyn 172 : 239 – 243 (1968)
3. Fotherby, K. Metabolism of synthetic steroids by animals and man. Acta Endocrinol 185 : 119 – 142 (1974)

4. Ferin, J. Orally active progestational compounds. Human studies : effects on the utero-vaginal tract. I E P T, Tausk, M., Editor. Pergamon Press, Oxford & New-York, 1972, vol. 2, p. 245 – 272
5. Ferin, J. Hypo-estrogenic amenorrhea and/or sterility induced by lynestrenol. Int J Fertil 9 : 29 –34 (1964)
6. Saey, A., Vanderick, G., and De Muylder, E. Orgametril ; Computerstudie over 1161 patienten onder continue behandeling. Ars Medici 5 : 10 – 45 (1976)
7. Novak, E. R., and Woodruff, B.S. Novak's Gynecologic and Obstetric Pathology. 7th edition, W B Saunders Co, Philadelphia, 1974, p. 313 – 318
8. Brux, J. Histopathologie Gynécologique, Masson & Co, Paris, 1971, p. 311 – 312
9. Hertig, A.T. and Gore, H. Atlas of Tumor Pathology, Armed Forces Institute of Pathology, Washington DC, 1961, p. 11 – 18
10. Gompel, C. Anatomie Pathologique Gynécologique et Obstétricale, Arscia & Maloine, Brussels, 1963, p. 414 – 416
11. Gompel, C. and Silverberg, S. Pathology in Gynecology and Obstetrics, European Academic Press, Brussels, 1977, p. 145 – 146
12. Lauwerijns, J. and Ferin, J. Effects on the ovary of prolonged administration of lynestrenol : a histological study. Int J Fertil 9 : 35 – 39 (1964)
13. Maqueo, M., Rice-Wray, E. J., Calderon, J. J. and Golzieher, J. W. Ovarian morphology after prolonged use of steroid contraceptive agents. Contraception 5 : 177 – 185 (1972)
14. Starup, J. and Visfeldt, J. Development of ovarian follicles correlated to levels of serum FSH and LH during long-term oral contraception. Europ J Obstet Gynec Reprod Biol 4 : 147 – 151 (1974)
15. Moghissi, K. S. Morphological changes in the ovaries of women treated with continuous microdose progestogens. Contraception 7 : 503 – 513 (1973)
16. Aref, I., Hefnawi, F. and Kandil, O. Changes in the human ovaries after long-term administration of microdose progestogens. Contraception 7 : 503 – 513 (1973)
17. Moghissi, K. S. and Marks, C. Effects of microdose norgestrel on endogenous gonadotropic and steroid hormones, cervical mucus, vaginal cytology and endometrium. Fertil Steril 22 : 424 – 434 (1971)
18. Saunders, D. M., Marcus, D. L., Saxena, B. B., Beling, C. G. and Connell, E. B. Effects of daily administration of 0.5 mg of chlormadinone acetate on plasma levels of FSH, LH and progesterone during the menstrual cycle. Fertil Steril 22 : 332 – 344 (1971)

19. Friedrich, E., Keller, E. R., Jaeger-Whitegiver, E. R., Joel, E. W. and Schindler, A. E. Effects of 0.5 mg of lynestrenol daily on hypothalamic-pituitary-ovarian function. Am J Obstet Gynecol 122 : 642 – 649 (1975)

20. Mishell, D. R., Kharma, K. M., Thorneycroff, I. H. and Nakamura, R. M. Estrogen activity in women receiving an injectable progestagen for contraception. Am J Obstet Gynecol 113 : 372 – 376 (1972)

21. Hilliard, J., Croxatto, H. B., Hayward, J. M. and Sawyer, C. H. Norethindrone blockade of LH-release to intrapituitary infusion of hypothalamic extract. Endocrinol 75 : 411 – 419 (1966)

22. Kanematsu, S. and Sawyer, C. H. Blockade of ovulation in rabbits by hypothalamic implants of norethindrone. Endocrinol 76 : 691 – 699 (1965)