

Ultrasonic Evaluation of Uterine Endometrial Morphology in the Normal Menstrual Cycle

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Abstract—Recent advancement of ultrasonographic technology enables vivid visualization of the female reproductive tract and the indirect detection of ovulation. This technique is now applied in induction of ovulation, timing artificial insemination, oocyte retrieval for in vitro fertilization and embryo transfer (IVF & ET).

Twenty normal ovulatory cycles were investigated in order to elucidate the cyclic ultrasonographic changes of uterine endometrial morphology (dates: March, 1984 to February, 1985).

Demonstration of ultrasonic endometrial changes revealed that the early proliferative phase was characterized by a long thin endometrium which became much thicker, giving evidence of growth in endometrial glands and stroma. The stromal cells of the superficial layer may be separated by transudate which was ultrasonically demonstrated by a hyporeflexive area. Following ovulation, we observed the 'ring' sign as an evidence of ovulation. The thickness of the central midline uterine echogenic area was 6.6 ± 1.2 mm in the early proliferative phase. It became 11.8 ± 1.4 mm on the day of ovulation (detected by ultrasound examination) and 12.3 ± 1.2 mm in the late secretory phases.

We concluded that ultrasonic evaluation of the endometrial morphology accompanying ovarian follicle monitoring is helpful in determining the dating as well as the confirmation of the progestational effect on the endometrial tissue and in detecting other organic pelvic disease related to infertility and menstrual disorders.

Key Words: *Endometrial morphology, Menstrual cycle, Ultrasonography, Ovulation*

INTRODUCTION

The increasing use of the ultrasound with high resolution properties may possibly expedite the monitoring of the entire process of reproduction. One of the most recent advances in the field of infertility involves the use of ultrasound for ovarian follicle monitoring (Hackeloer et al. 1979; de Crespigny et al. 1981; Queenan et al. 1982; Hammond 1984; Sanders et al. 1985). In the light of such monitoring, the condition of the endometrium and related aspects of fertility can be assessed with regard to processes involved in implantation.

A review of the ultrasound literature revealed that little had been published on the cyclic changes of the endometrium in the normal menstrual cycle (Sample et al. 1977; Hall et al. 1979; Duffield et

al. 1981; Nakano et al. 1982).

Ultrasonic evaluation of uterine endometrial morphology in the normal menstrual cycle has not been studied in Korea. Therefore this study was carried out to investigate the cyclic morphologic changes of the endometrium and the follicular growth pattern in the normal ovulatory menstrual cycle.

MATERIALS AND METHODS

1. Patients: Twenty healthy women with infertility were studied. They were between 24 and 36 years of age and had 30 ± 4 day normal menstrual cycles. Their infertility work-up disclosed azoospermia, and poor post-coital test. Artificial insemination was performed through follicle monitoring with serial ultrasound and plasma estradiol de-

termination.

2. Ultrasound examination:The patients were examined on a contact scanner using a 3.5 MHz (Aloka S.S.D. 710, Tokyo) employing the full-bladder technique. The patients were scanned at either 0.5 or 1.0 cm intervals in transverse and longitudinal sections. A base-line ultrasonographic examination was performed on MCD #3~#5.

Thereafter ultrasonographic examination was done every 3 to 4 day until the selection of a dominant or co-dominant follicle was apparent. During the periovulatory period the ultrasonographic examination was performed daily at 8:00 a.m. until the evidence of ultrasonographic ovulation was confirmed.

In the luteal phase ultrasonographic examination was done to evaluate the change of endometrial morphology and the recruitment of the new follicles. Longitudinal scanning allowed the long axis of the uterus to be defined and the central uterine cavity echo was searched for. Then ultrasonographic determination of endometrial maturation was made by measuring the distance between both sides of translucent areas around the central uterine cavity echo (Fig. 1)

3. Plasma estradiol(E2) determination:Plasma estradiol levels were measured in peripheral blood samples obtained daily between 8:00 to 9:00 a.m.. Unconjugated plasma E2 was measured by radioimmunoassay, using rabbit antiserum to 17beta-E2-6-(carboxymethyl)oxim-bovine serum albumin obtained with Estradiol-ter kit (Serono Diagnostics, Switzerland & Internat., SA). The limit of sensitivity of the assay was from 20 pg/ml to 2000pg/ml. Cross-reaction with estrone was 1.3% and with estriol 0.4%. The inter-assay and intra-assay coefficients of variation for estradiol were 4.2% and 5.5%, respectively.

4. Plasma progesterone(P) determination:Plasma progesterone levels were measured in the active luteal phase i.e. 6 or 7 days after the occurrence of ultrasonographic evidence of ovulation. By a radioimmunoassay using antisera against 11 alpha-hydroxyprogesterone with Progesterone-ter kit (Serono Diagnostics, Switzerland & Internat., SA). The limit of sensitivity of the assay was from 0.5 ng/ml to 80 ng/ml.

The inter-assay and intra-assay coefficients of variation for progesterone were 6.5% and 9.4%, respectively.

RESULTS

1. Ultrasonographic pattern of uterine endometrium:Initially the central uterine cavity echo was less conspicuous, but could be traced in all cases. During the early proliferative phase, there was a narrow linear, less echogenic band which became more echogenic during the late proliferative phase. Translucent areas appeared on both sides of the central echogenic layer. Especially a rapid thickening was observed on day 0 and it coincided with the day of ultrasonographic ovulation. During the early secretory phase the central uterine cavity echo became more echogenic and was surrounded by a more prominent sonolucent area presumed to be endometrial fluid or vascular congestion. These ultrasonographic changes made a 'ring' structure just after ovulation in 8 cases. During the late secretory phase the thickness of central echogenic area plus sonolucent area was revealed in the maximum dimension.

During the menstrual phase, the central echogenic area became separated by a layer of decreased echogenicity with an even translucent pattern.

2. Thickness of uterine endometrium:The thickness of central midline uterine echogenic area was 6.6 ± 1.2 mm (Mean \pm S.E.M.) in the early proliferative phase. Day 0 was defined as the day that the leading follicle was seen to be ruptured on the ultrasonographic examination. The sequential change of the thickness of uterine endometrium on the longitudinal scanning were as follows: 8.8 ± 0.8 mm on Day -5, 10.0 ± 1.7 mm on Day -4, 10.4 ± 1.1 mm on Day -3, 10.8 ± 1.1 mm on Day -2, 11.4 ± 1.4 mm -1, 11.8 ± 1.4 mm on Day 0 and 1.20 ± 0.9 mm on Day +1. It was 12.3 ± 1.2 mm in the late secretory phase.

3. Ultrasonographic evidence of ovulation:A line of decreased reflectivity around the follicle was appeared in 3 cases (15%) and the crenation pattern within the follicle was observed in 9 cases (45%). These patterns were presumed to be as the sign of imminent ovulation.

Follicular collapse or disappearance was observed in 10 cases (50%) and decreased follicular diameter was observed in 4 cases (20%). Appearance of corpus luteum cyst was observed in 6 cases (30%). The fluid track or level in the posterior cul-de-sac or endometrial 'ring' sign were observed in 9 cases (45%) and 8 cases (40%), re-

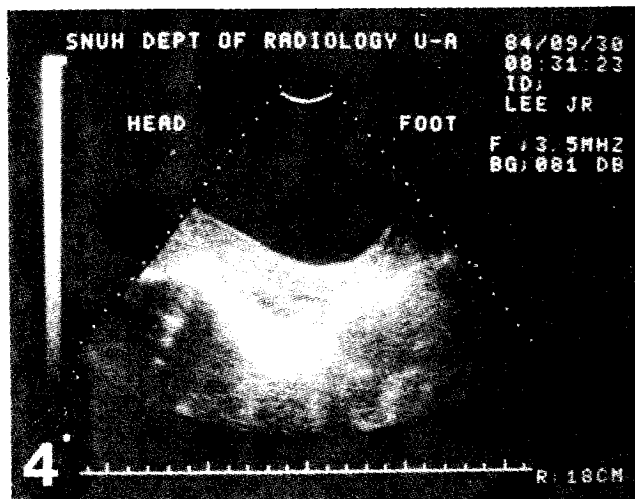
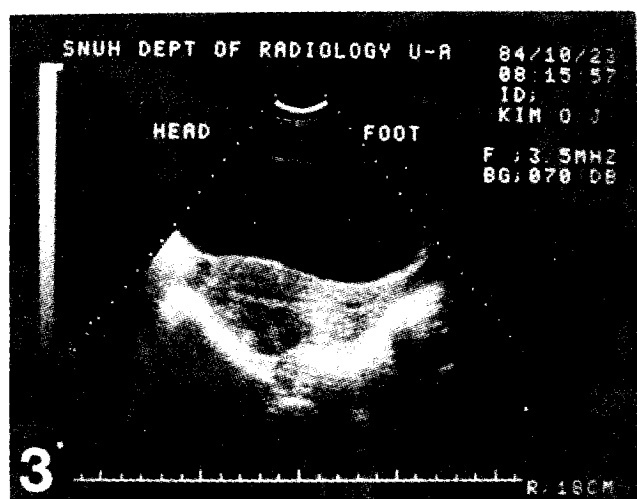
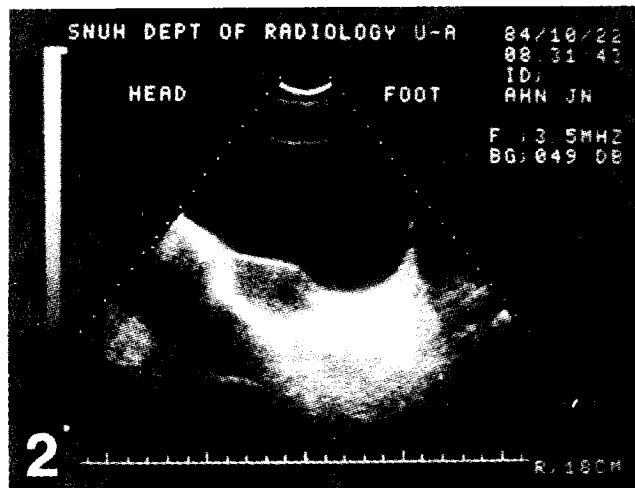
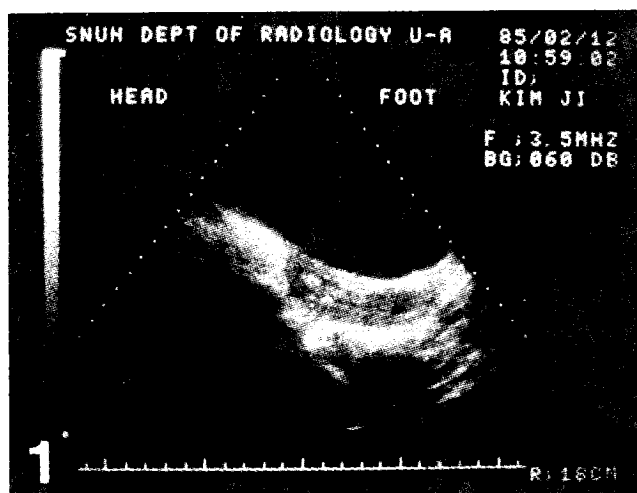


Fig. 1. Early proliferative phase-Thin endometrium.
 A and C: Margins of hyporeflective area
 B: Midline central uterine cavity echogenic area
 Fig. 2. Mid-proliferative phase.
 Fig. 3. Late proliferative phase-Thick endometrium.
 Fig. 4. Late proliferative phase-Thick endometrium with endometrial fluid.

Table 1. Growth pattern of thickness of uterine endometrium and follicular diameter(mm)

	Day relative to follicular rupture									
	Basal	-6	-5	-4	-3	-2	-1	0	+1	LS*
Mean	6.6	8.8	9.6	10.0	10.4	10.8	11.4	11.8	12.0	12.3
UT** S.E.M.	1.2	0.5	0.8	1.7	1.1	1.1	1.4	1.4	0.9	1.2
Mean	—	10.0	11.2	11.6	12.9	15.0	17.3	19.2	—	—
FD*** S.E.M.	—	0.7	0.9	1.2	1.9	1.1	0.4	1.7	—	—

* LS: Late secretory phase

** UT: Uterine endometrial thickness

*** FD: Follicular diameter

spectively.

4. Mean follicular diameter: Mean follicular dia-

eters of the leading follicle in the periovulatory period (from Day -6 to Day 0) were $10.3 \pm$

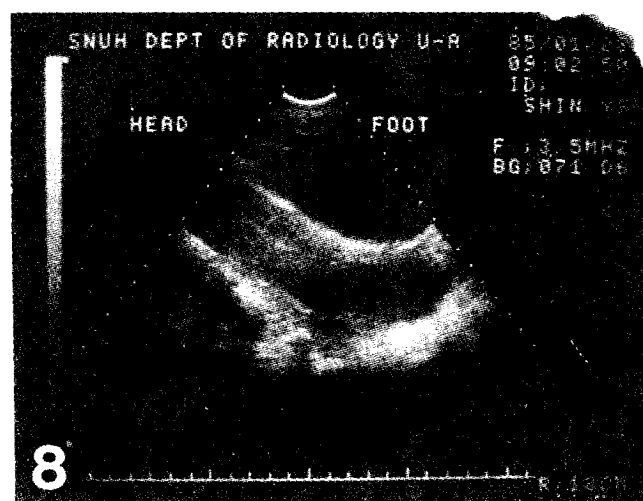
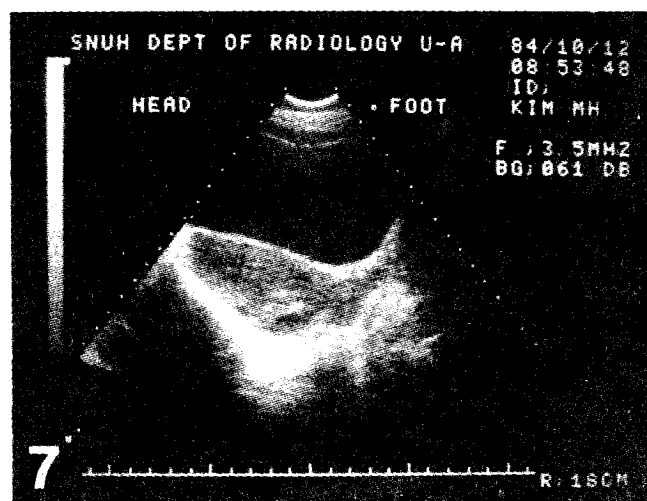
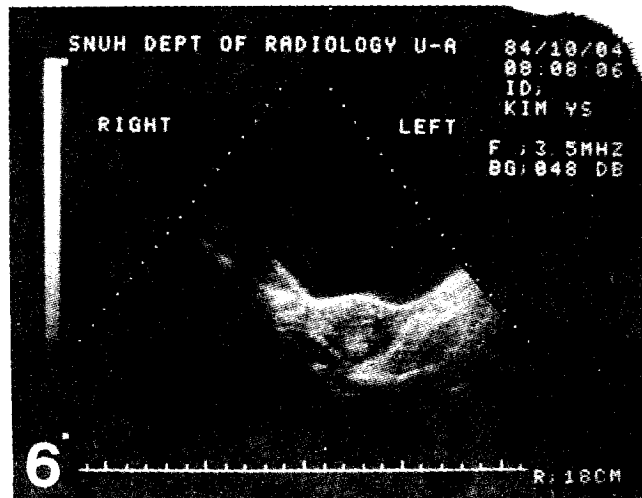
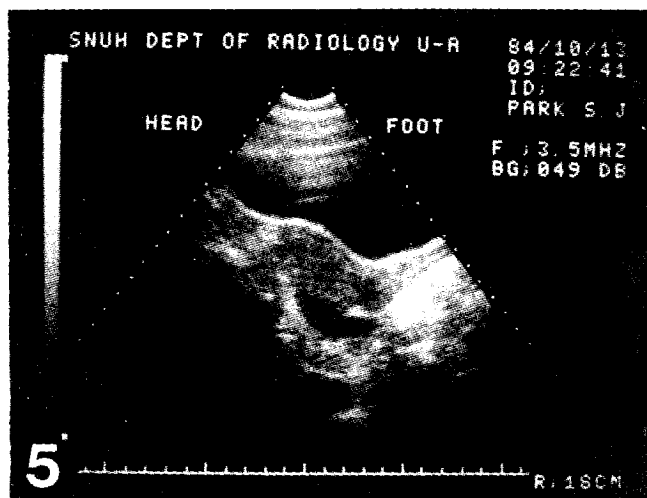


Fig. 5. Early secretory phase with PCDS fluid level.
 Fig. 6. 'Ring' sign in early secretory phase.
 Fig. 7. Late secretory phase.
 Fig. 8. Ultrasonographic appearance of biopsy-proven cystic hyperplasia.

Table 2. Plasma estradiol levels(pg/ml)

	Day relative to follicular rupture							
	Basal	-6	-5	-4	-3	-2	-1	0
Mean	46.	100.1	134.5	159.1	227.4	246.2	296.6	176.5
S.E.M.	26.2	35.9	40.2	84.5	64.4	84.7	96.7	187.8

0.7mm(Mean±S.E.M.), 11.2±0.9mm, 11.6±1.2mm, 12.9±1.9mm, 15.0±1.1mm, 17.3±0.4mm, and 19.2±1.7mm, respectively.

5. Plasma estradiol level:The plasma estradiol(E2) level was 46.2±26.2 pg/ml(Mean±S.E.M.) in the early proliferative phase. During the periovulatory phase(from Day -6 to Day 0) plasma E2 levels were 100.1±35.9 pg/ml, 134.5±40.2

pg/ml, 159.1±84.5 pg/ml, 227.4±64.4 pg/ml, 246.2±84.7 pg/ml, 296.6±96.7 pg/ml, and 176.5±187.8 pg/ml, respectively.

6. Plasma progesterone level:Plasma progesterone level was 16.2±2.7(Mean±S.E.M.) ng/ml during the active luteal phase. The lowest value was 11.2 ng/ml and the highest value was 22.7 ng/ml.

DISCUSSION

The normal uterus and ovaries undergo cyclic changes in response to hormonal stimulation through the hypothalamic pituitary-ovarian axis.

The ability to confirm ovulation and assess luteal function is of paramount importance especially in the management of infertile women with ovulatory failure.

The sequential maturation and rupture of the dominant ovarian follicle constitutes the central event of the human female reproductive cycle. Although the elaborate hypothalamic and pituitary biochemical mechanisms influencing follicular ripening have been well documented over the past two decades, insight into the exact timing of ovulation and the physical changes in the ovary preceding and immediately following ovulation has developed less rapidly, due to a paucity of appropriate noninvasive techniques.

Ultrasound was first introduced into gynecologic practice over twenty years ago, but it is only in recent years that technologic developments in imaging capability and resolution have facilitated follicle monitoring. The first ovarian studies employed compound B-mode "static" scanners. While these offer superior tissue penetration, particularly in obese subjects, the rapidly developing real-time mode is probably now more appropriate, at least for routine clinical practice. This is for a number of reasons: 1) its moving image facilitates the differentiation of the preovulatory follicle from other pelvic structure; 2) the apparatus is portable, so that bedside examination is possible in a clinical setting; 3) it has a wider gray-scale field and variable plane of section through the pelvis; and 4) it is relatively easier to master in comparison with compound B-mode scanning, which required manipulation of the gantry-mounted side arm (de Crespigny et al. 1981).

The texture of the normal myometrium is consistent throughout all age groups and is of homogenous, low to medium echogenicity. The innermost layers of the endometrium appear as a central linear echogenicity, most prominent during menses (Callen et al. 1979). This echo has been ascribed to the uterine cavity or interface between adjacent layers of endometrium. This echo was traced in all the cases of follicle monitoring and this result was comparable to that of Callen (1979). They demonstrated central uterine cavity echo in 100% of patients prospectively. They suggested

that this echo could be diagnostically useful in excluding an intrauterine pregnancy or identifying the uterus when confusing pelvic pathology was present. Although the cavity may be localized more easily and its thickness altered when blood clot, necrotic debris, decidual casts, or intrauterine contraceptive devices are present, none of these was required to demonstrate this linear echo (Marks et al. 1979; Sailer et al. 1979; Nyberg et al. 1983).

This echo lies relatively perpendicular to the beam direction and the endometrium (Duffield et al. 1981). Surrounding this echogenic interface is a band of sonolucency that, most likely, corresponds to the spongiosa and basal layers of the endometrium. The endometrium thickens from 2 to 3 mm in the proliferative phases to 3 to 6 mm in the secretory phases (Callen et al. 1979; Duffield et al. 1981; Sanders et al. 1985). Our observation was comparable to these findings. The thickness of central midline uterine echogenic area was 6.6 mm in the early proliferative phase and 12.3 mm in the late secretory phase which was measured bidirectionally.

During the early proliferative phase of the cycle, glandular reconstruction occurs by proliferation and differentiation of cells derived from the basal layer of the endometrium. Two basic layers of differential reflectivity can be recognized within the uterine cavity with ultrasound: a basal layer of decreased reflectivity and a central layer with reflectivity similar to that of myometrium. As endometrial proliferation advances, the endometrial echo within the uterus becomes thicker, corresponding to the growth of the endometrial glands. The highest rates of endometrial proliferation were recorded on cycle days of 8 to 10 in the upper one third of the functional layer. This was done by a radioautographic analysis of the number and distribution of labeled nuclei after *in vitro* incorporation of radiothymidine. The endometrium of the isthmus and cornual regions and of the basal zone demonstrated relatively constant and comparatively lower rates of proliferation throughout the cycle (Ferenczy et al. 1979).

In the normal cycle, a progressive increase in estrogen production before ovulation causes parallel development of all elements of the endometrium—stroma, glands, and superficial arteries. Birnholz (1984) reported that stripping movement of mucin within the endometrium were observed in 19 of 26 women with a recently developed high-resolution ultrasonic imaging system.

In the secretory phase, the endometrium, in-

duced by progesterone, was seen as a central echo complex that became more reflective than the surrounding myometrium and gradually thickened. This increased reflectivity was due to the development of multiple reflective interfaces as the endometrial glands became convoluted and filled with secretion under the influence of progesterone from the corpus luteum. The compound structure of the endometrial glands during the secretory phase produced many more reflective interfaces than the shallow endometrial glands seen in the proliferative phases.

Prior to menstruation, regressive changes are found to coincide with the decrease and final cessation of function of the corpus luteum.

In the menstrual phase, the thickness of the central uterine cavity echo was not conspicuous and blood clots imitated the 'ring' sign. This was due to the separation of the dense echo complex by a layer of decreased reflectivity due to blood and debris. Small intrauterine fluid collections were seen most frequently in non-pregnant patients who had vaginal bleeding or inflammation of the endometrium or adnexa (Laing et al. 1980). The endometrial fluid is made of 1) components from the transudation of the blood serum and 2) protein, carbohydrate, and other metabolites synthesized within the endometrial cells and discharged through the apical cell membrane. The endometrial secretions play a major role in the capacitation of spermatozoa and the nutrition of the blastocyst (Hafez et al. 1975).

We observed follicular collapse or disappearance as a ultrasonographic evidence of ovulation most frequently. But the ultrasound criteria that were adopted were a change in the appearance of the ovary that was consistent with follicular rupture. The formation of an early corpus luteum was variable in the patients group, so the distribution of the ultrasonographic ovulation did not coincide with that of other reports (Hackeloer et al. 1979; Queenan et al. 1982).

Following ovulation, the endometrium shrunk in thickness and the edema of the superficial layer was lost. This produced an ultrasound 'ring' sign. Our experience indicated that the 'ring' sign had never been visualized in the absence of ovulation, and is, therefore, believed to be an additional sign that ovulation had taken place, probably within the previous 12 hours. Picker et al. (1983) reported ultrasonic signs of imminent ovulation. The appearance

of a line of decreased reflectivity around the follicle was apparent within 24 hours of ovulation. It progressed to advance separation of the granulosa cell layer of the follicle producing a crenation pattern within the follicle when ovulation was imminent, within 6 to 10 hours.

Mean follicular diameter of the leading follicle was 19.2 ± 1.7 mm and this was comparable to those of Hackeloer and Queenan.

Midluteal phase (around day 20 to 22 of the cycle = the phase estimated 5 to 10 days prior to the next menstrual period) plasma progesterone concentrations were between 11.2 ng/ml and 22.7 ng/ml and mean value was 16.7 ± 2.7 ng/ml. These values are considered as the indirect evidence of ovulatory cycle (Radwanska et al. 1981).

We concluded that ultrasonic evaluation of endometrial morphology accompanying follicle monitoring is helpful in determining dating as well as the confirmation of the progestational effect of the endometrial tissue and in detecting other organic pelvic disease related to infertility and menstrual disorders.

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= 국문초록 =

正常 月經週期에서의 超音波 撮影術에 依한 子宮內膜의 形態的 變化에 關한 研究

서울대학교 醫科大學 產婦人科學教室

文信容 · 林龍澤 · 李珍鏞 · 張潤錫

最近에 들면서 超音波 撮影術의 發展으로 女性 生殖器官을 觀察할 수 있게 되었으며 排卵을 間接적으로 探知할 수 있게 되었다.

著者들은 1984년 3월부터 1985년 2월 사이에 20名の 正常 月經週期를 가진 女性에서 超音波 撮影術을 利用한 子宮內膜의 形態的인 變化에 關한 研究를 實施하였다.

研究 結果, 子宮內膜은 增殖期 初期에 細長形을 나타내다가 子宮 內膜腺 및 間質組織의 成長으로 肥厚하게 된다. 子宮內膜 表在層의 間質組織細胞가 滲出液에 依해 分離되어 超音波 撮影像으로는 hyporeflective area로 나타난다. 排卵 後에는 排卵에 따른 子宮內膜의 變火로 'ring' sign이 觀察된다. 또한 子宮內膜 中心部位의 肥厚度를 測定한 結果 初期 增殖期에는 $6.6 \pm 1.2\text{mm}$ 이었으며 超音波像에 依한 排卵日에는 $11.8 \pm 1.4\text{mm}$ 에 이르고 分泌期 後半에는 $12.3 \pm 1.2\text{mm}$ 에 到達하였다.

卵巢卵胞에 對한 모니터링에 隨伴하여 子宮內膜의 月經週期에 따른 形態的 變化를 觀察하는 것은 子宮內膜의 成熟度에 따른 月經週期 및 子宮內膜의 黃體機能을 確認 및 決定하는데 도움을 주며 不妊 및 月經異常에 連關된 骨盤疾患을 알아내는 데에 도움을 준다.