# Relationship between Progesterone Receptor Level in Endometrium and Bleeding Pattern in Depot Medroxyprogesterone Acetate Users†

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**Abstract**

Bleeding disturbance is the major reason for discontinuation among depot medroxyprogesterone acetate (DMPA) users. However, the causes of progestin-induced bleeding are not well understood. The aim of the study was to examine the correlation between the occurrence of uterine bleeding and progesterone receptor (PR) levels in the endometrium. Forty-five matched pairs of age and body mass index in DMPA users with bleeding and amenorrhea were studied. The endometrial PR levels were evaluated. The PR score was assessed semi-quantitatively. Forty-two subject pairs met the criteria. There was no difference in serum estradiol and progesterone levels between the groups. No correlation between the number of bleeding days and PR score nor between the number of bleeding days and serum estradiol and progesterone level was detected. The stromal PR score in DMPA subjects with amenorrhea was significantly higher than those with bleeding ($p < 0.05$). By contrast, the PR score in glandular endometrium was not significantly different between the groups ($p > 0.05$). In conclusion, after a second dose of DMPA, subjects with amenorrhea had a higher stromal PR score than those with uterine bleeding.

**Key word :** DMPA, Endometrium, Uterine Bleeding, Progesterone Receptor, Amenorrhea, Stroma

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Over 20 million women worldwide currently use progestin-only contraceptives. The injectable depot medroxyprogesterone acetate (DMPA) is the most widely used (approximately 13 million women worldwide)[1]. While it is highly effective and has long-acting properties, DMPA also has a significant problem, namely, bleeding disturbance.

Bleeding disturbance is the major reason for treatment discontinuation[2,3]. Several treatment regimens have been used but, unfortunately, over the long-term, no effective treatment has been noted[4]. This is due to the fact that the exact mechanisms of progestin-induced bleeding problem have not yet been extensively elucidated.

Many studies have been conducted to define the factors that result in endometrial bleeding. The progesterone receptor (PR), which may play an initial role in the signaling cascade leading to endometrial bleeding, has been studied with conflicting results [5,6]. However, PR levels in the endometrium of DMPA users have never been adequately evaluated. In this study, the authors aimed to investigate whether the presence or absence of bleeding was related to the levels of PR in the endometrium.

## MATERIAL AND METHOD

Ninety healthy, fertile, non-lactating women, aged between 25 and 35 years, were recruited from the Family Planning Clinic at King Chulalongkorn Memorial Hospital between April 2000 and January 2001.

All subjects had no known medical or gynecological disorders and no previous history of chronic anovulation. They also had no previous history of abortion, or endometrial curettage. Subjects reporting any other hormonal treatment were excluded. It was mandatory that all subjects had one child and had received 150 mg DMPA as a contraceptive at 6 weeks postpartum, and had an injection every 84 days. Menstrual data were recorded on a menstrual diary chart. Ethical approval for the study was obtained from the Ethics Committees of Chulalongkorn University.

On the day of the third DMPA injection, subjects were divided into two groups according to the occurrence of bleeding in the reference period; an amenorrheic group and a bleeding group. Both groups had a duration of DMPA use of 168 days. The reference period was defined as 84 days after the last DMPA injection. Amenorrhea was defined as no uterine bleeding in the reference period. The presence of bleeding was defined as a significant amount of uterine bleeding such that the use of sanitary pads was anticipated in the reference period. If a subject had irregular bleeding, counseling was given. In the bleeding group, women who had no uterine bleeding on the day of biopsy were excluded. It was mandatory that each participant had missed less than 10 days of menstrual record data in the reference period. In case of missing bleeding data, subjects were asked to recall the presence or absence of bleeding per vagina on those days.

Forty-five subjects in each group were matched for age and body mass index (BMI). All subjects were asked to give a written informed consent. Before DMPA injection, 5/10 ml of venous blood was collected. Serum was separated, stored at -20°C and subsequently analyzed for estradiol (Delfia estradiol kit, Wallace OY, Turku, Findland) and progesterone (Delfia progesterone kit, Wallace OY, Turku, Findland) by time-resolved fluoroimmunoassay. The interassay and intra-assay variations for estradiol were 3.89 per cent and 3.73 per cent, respectively. The interassay and intra-assay variations for progesterone were 6.00 per cent and 5.34 per cent, respectively.

Endometrial specimens were obtained by Endocell biopsy (Wallach Surgical Devices, Inc., Orange, CT). All endometrial tissue samples were fixed overnight in 10 per cent buffered formalin (at 4°C) and wax embedded using the routine method. Five micron sections were placed on 3-aminopropyltriethoxysilane-coated slides (Sigma Chemical Co., St.Louis, MO), then dewaxed and dehydrated with xylene and ethanol. Endogenous peroxidase was blocked with 30 per cent hydrogen peroxide in 100 per cent methanol. Slides were microwave-heated and then incubated at room temperature with a 1 : 200 dilution of anti-PR monoclonal antibody (BioGenex, San Ramon, CA) for 90 minutes. Washed sections were treated with antimouse antibody and color was developed with the vectastain ABC kit (Vector Laboratories, Inc., Burlingame, CA). Hematoxylin was used for counter staining (Fig. 1). PR-positive breast cancer specimens were employed as positive controls.

All tissue sections were assessed semi-quantitatively by a blinded gynecologic pathologist. Modified H-scores were assigned[7]. Firstly, the fraction (F) of stained cells in each compartment were estimated: 0 = 0-9 per cent, 1= 10-39 per cent, 2 = 40-69 per cent, 3 = 70-89 per cent, and 4 = 90-100 per cent. Secondly, the stained intensity (I) was scored: 0 = no

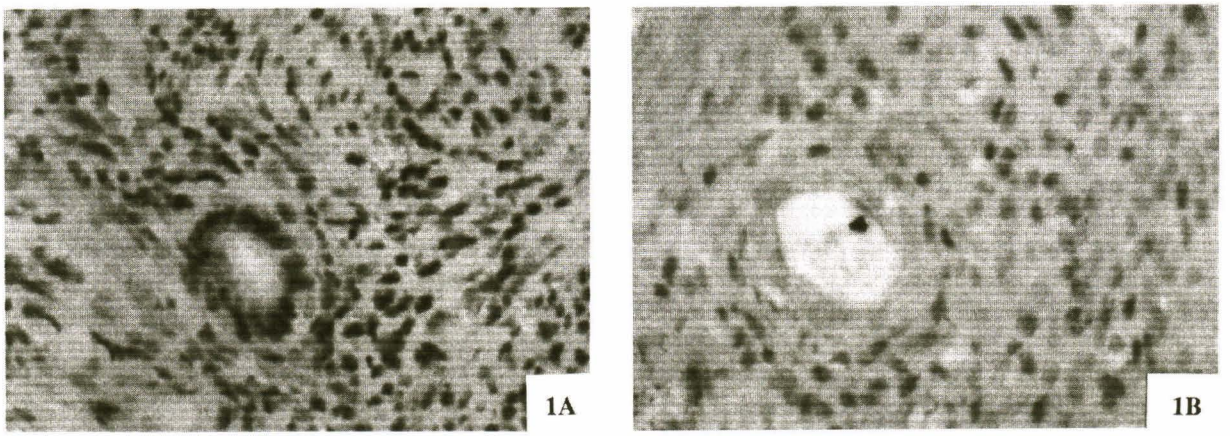


**Fig 1. Immunohistochemical staining for the progesterone receptor in endometrial specimens from depot-medroxyprogesterone acetate users (a) progesterone receptor in endometrial specimens from depot-medroxyprogesterone acetate users with amenorrhea (b) progesterone receptor in endometrial specimens from depot-medroxyprogesterone acetate users with bleeding.**

staining, 1 = weak but definite staining, 2 = moderate staining, 3 = pronounced staining, and 4 = intense staining. Finally, the H-score was calculated by the formula (FxI)/4.

Data were analyzed using the Statistic Package for Social Science (SPSS) software for window 10.0 (SPSS Inc., Chicago, IL). The baseline characteristics and hormone level of the two groups were reported as mean ± standard deviation (SD) and compared with the use of student's *t*-test. The correlation between the number of bleeding days and either endometrial PR score or serum hormone levels was analyzed by Pearson correlation. Differences between groups with respect to the H-scores were analyzed with the use of the Mann-Whitney U-test. Two-sided values of less than 0.05 were considered to indicate statistically significant differences.

## RESULTS

Forty-five participants in each group were recruited. Three cases (two in the amenorrheic group, one in the bleeding group) were excluded due to inadequate endometrial tissue being obtained for immunohistochemistry. As this was a matched case-control study, the matched subjects were excluded simultaneously. Forty-two age- and body mass index-matched pairs were thus evaluated. Table 1 summarizes the baseline characteristics of the patients in both groups. There were no statistically significant differences in weight or height between the groups. There were also no significant differences in the serum levels of estradiol and progesterone on the day of biopsy between the amenorrheic and bleeding groups. The number of bleeding days in the bleeding group was 31.8 ± 23.9 days (mean ± SD), ranging from 4 to 84 days. No signification difference of the correlation between the number of bleeding days and either serum estradiol or progesterone levels were detected ($p > 0.05$). The PR immunoreactivity score between the amenorrheic group and the bleeding group was compared. The PR immunoreactivity score was significantly higher in the endometrial stroma of subjects with amenorrhea (Table 2). In contrast, the PR score in endometrial glands was not different between the groups (Table 2). There was no correlation between the number of bleeding days and PR levels in both gland and stroma ($p > 0.05$).

## DISCUSSION

It has been demonstrated that the PR is down regulated by its own ligand in breast cancer cell lines and human epithelial glands[8,9]. Tseng et al [10], however, demonstrated that progestin up-regulated PR levels in human endometrial stromal cells. Lau et al[5] showed that subjects with amenorrhea who used Norplant had higher concentrations of stromal PR mRNA. In the present study, it was found that DMPA users with amenorrhea had significantly higher stromal PR immunoreactivity scores than those with genital bleeding. This may be due to the rate of

**Table 1. Baseline characteristics of the subjects.**

| Characteristic | Amenorrheic group* (N = 42) | Bleeding group* (N = 42) | Difference between groups (95% CI†) |
|---|---|---|---|
| Age (yr) | 28.3 ± 2.5 | 28.3 ± 2.5 | -1.1 to 1.1 |
| Weight (kg) | 53.2 ± 7.0 | 52.5 ± 6.3 | -2.2 to 3.6 |
| Height (cm) | 154.9 ± 4.6 | 154.1 ± 5.7 | -1.5 to 3.1 |
| BMI‡ | 22.1± 2.5 | 22.1 ± 2.5 | -1.1 to 1.1 |
| Serum estradiol§ (pg/ml) | 27.82 ± 15.08 | 21.90 ± 19.23 | -1.58 to 13.40 |
| Serum progesteroneπ (ng/ml) | 0.22 ± 0.14 | 0.23 ± 0.22 | -0.09 to 0.07 |

* Plus-minus values are mean ± SD. There were no significant differences between the groups with the use of student's *t*-test.
† CI denotes confidence interval.
‡ The body mass index (BMI) is the weight in kilograms divided by the square of the height in meters.
§ To convert the values for serum estradiol to picomoles per liter, multiply by 3.671.
π To convert the values for serum progrsterone to nanomoles per liter, multiply by 3.188.

**Table 2. Progesterone receptor scores in the endometrial stroma and gland in depot medroxyprogesterone acetate users with amenorrhea and bleeding.**

| | Amenorrheic group (N = 42) | | | Bleeding group (N = 42) | | | P-value* |
|---|---|---|---|---|---|---|---|
| | Median PR score† | 25th percentiles | 75th percentiles | Median PR score† | 25th percentiles | 75th percentiles | |
| Endometrial stroma | 1.50 | 0.25 | 2.00 | 1.0 | 0.25 | 1.5 | 0.04 |
| Endometrial gland | 1.00 | 0.25 | 2.25 | 0.50 | 0.25 | 1.5 | 0.08 |

* The Mann-Whitney U-test was used for group comparisons; $p < 0.05$ considered statistically significant.
† PR denotes progesterone receptor.

progestin enhancement of PR levels in endometrial stromal cells being higher in DMPA users with amenorrhea. The high PR levels in stroma may support the surrounding vascular matrix. This effect may be mediated through the expression of certain matrix metalloproteinase and tissue inhibitors[11,12]. In other words, a higher level of progestin suppression of matrix metalloproteinase or progestin enhancement of tissue inhibitors of matrix metalloproteinase may be seen in DMPA users with amenorrhea. These effects promote endometrial vascular support, with resultant enhancement of vascular integrity.

The limitation of the present study was its cross-sectional design. Although standardized criteria of eligibility and matched cases were used, the endometrial PR levels may have been different before the use of DMPA.

From another point of view, if the rate of PR up-regulation is similar in all patients, those starting DMPA with high PR levels would tend towards rapid amenorrhea. This reasoning may change our practice to injecting DMPA in the luteal phase of the menstrual cycle, which has a high level of PR[13], to ensure that patients become amenorrheic rapidly.

Endometrial PR levels may change over time when progestin administration is prolonged. Runic et al[6] demonstrated that after one year of Norplant administration, subjects with hysteroscopic-defined bleeding sites had higher levels of PR at these than at non-bleeding sites.

PR expression may vary in different tissue compartments of the endometrium after progestin therapy. The present results show that there was no difference in glandular PR levels between DMPA users with amenorrhea and genital bleeding. It is likely that endometrial glandular PR levels play no role in genital bleeding.

There was no correlation between PR scores and number of bleeding days. The implication of this finding is that other factors may act in concert to produce bleeding such as the tissue factor[14] or vascular endothelial growth factor[15].

To the authors' knowledge, this study is the first to demonstrate a correlation between PR levels

and the occurrence of genital bleeding in DMPA users. However, the authors did not differentiate the expression of PR isoforms A and B in the endometrial specimens. Further studies should address the alteration of levels of PR and other associated mediators such as vascular endothelial growth factor before and after treatment between amenorrheic and bleeding groups.

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# ความสัมพันธ์ระหว่างระดับโปรเจสเตอโรน รีเซ็พเตอร์ ในเยื่อบุโพรงมดลูกกับภาวะเลือดออกทางช่องคลอดจากยาฉีดคุมกำเนิดดีเอ็มพีเอ†

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**วัตถุประสงค์** : เพื่อศึกษาความสัมพันธ์ระหว่างภาวะเลือดออกทางช่องคลอดระหว่างคุมกำเนิดด้วยยาฉีดคุมกำเนิด Depotmedroxyprogesterone acetate (DMPA) กับระดับ progesterone receptor (PR) ในเยื่อบุโพรงมดลูก

**วัสดุและวิธีการ** : จับคู่ระหว่างสตรีที่มารับยาฉีดคุมกำเนิด DMPA เข็มที่ 3 ที่มีและไม่มีเลือดออกทางช่องคลอดที่อายุและดัชนีมวลกายเท่ากันจำนวน 45 คู่ เพื่อตรวจระดับฮอร์โมนโปรเจสเตอโรนและเอสโตรเจน และดูดชิ้นเนื้อเยื่อบุโพรงมดลูกเพื่อตรวจระดับ PR ในเยื่อบุโพรงมดลูก นำผลที่ได้มาวิเคราะห์โดยใช้ student *t*-test, Pearson correlation และ Mann-Whitney U test

**ผลการศึกษา** : สตรีที่มารับยาฉีดคุมกำเนิด DMPAจำนวน 42 คู่ ที่มีปริมาณชิ้นเนื้อเพียงพอสำหรับตรวจ ทั้งสองกลุ่มนี้มีระดับฮอร์โมนโปรเจสเตอโรนและเอสโตรเจนไม่แตกต่างกัน แต่สตรีที่ไม่มีเลือดออกทางช่องคลอดมีระดับ PR ใน stroma มากกว่า สตรีที่มีเลือดออกทางช่องคลอดอย่างมีนัยสำคัญทางสถิติ ขณะที่ระดับ PR ใน gland ไม่แตกต่างกันระหว่างสองกลุ่ม และไม่พบความสัมพันธ์ระหว่างจำนวนวันที่เลือดออกทางช่องคลอดกับที่ระดับ PR ในเยื่อบุโพรงมดลูก

**สรุป** : สตรีที่มารับบริการคุมกำเนิดด้วยยาฉีดคุมกำเนิด DMPA เข็มที่ 3 ที่ไม่มีเลือดออกทางช่องคลอดจะมีปริมาณ PR ใน stroma มากกว่าในกลุ่มที่มีเลือดออกทางช่องคลอด

**คำสำคัญ** : ตัวรับโปรเจสเตอโรน, เยื่อบุโพรงมดลูก, ยาฉีดคุมกำเนิด, เลือดออกทางช่องคลอด, ภาวะขาดระดู, สโตรมา

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