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Antiprogestin-releasing intrauterine devices:

a novel approach to endometrial contraception

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Abstract

Intrauterine devices (IUDs) that release progestins are highly effective contraceptives, but they induce breakthrough bleeding that some women find unacceptable. Because progesterone (P) antagonists (AP) are known to suppress the endometrium, induce amenorrhea, and inhibit fertility, AP IUDs may provide an effective contraceptive that also controls endometrial bleeding. Here we assessed the effects of empty (blank) vs AP-releasing (ZK 230 211) IUDs on bleeding patterns and endometrial growth in ovariectomized, artificially cycled macaques. The AP IUDs (but not the blank controls) induced extended, frank menstruation when inserted during the late luteal phase, an indication of local AP action. Over time, endometrial glandular and arterial proliferation were inhibited, steroid receptors were elevated, spiral arteries showed degenerative changes, progesterone withdrawal bleeding was prevented and estradiol-dependent proliferation was suppressed by the AP IUDs. In sum, AP IUDs suppressed the effects of P on endometrial progestational development and blocked the effects of estradiol on endometrial proliferation as previously shown for systemic treatment with APs. Therefore, AP IUDs may provide novel contraceptive devices with minimal breakthrough bleeding.

Keywords

Antiprogestins; progesterone withdrawal; uterine bleeding; macaque monkeys; endometrial changes

1. Introduction

Intrauterine devices (IUDs) are highly effective contraceptives that act primarily to block fertilization [1]. However, patient compliance is sometimes less than optimal because of heavy menstrual bleeding, breakthrough bleeding (BTB) and cramping that can occur, especially with various nonhormonal IUDs. The recently developed levonorgestrel-releasing intrauterine system (LNG-IUS, [Mirena]) is an extremely effective contraceptive that reduces overall menstrual blood loss but many women find the amount of BTB that typically occurs during the first three months of use to be unacceptable [2]. Consequently, there is a need for a contraceptive IUD that could completely suppress BTB.

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Several studies indicate that progesterone (P) antagonists (APs) can act as contraceptive agents [3-5] and are also associated with amenorrhea [6-8]. Therefore, AP-releasing IUDs may provide a novel form of intrauterine contraception with minimal BTB. Chronic, systemic administration of low-dose APs including RU486 (mifepristone) and the potent Schering AG progesterone antagonists, ZK 137 316 and ZK 230 211 [9], induce endometrial atrophy in non-human primates [10]. Such treatment is contraceptive in macaques and its effects on menstruation are dose-dependent [4]. In women, chronic oral administration of mifepristone suppressed endometrial mitotic activity, induced amenorrhea, and was contraceptive [11]. Periodic treatment with mifepristone in women treated with Norplant reduced BTB [12].

APs suppress endometrial growth in primates by two mechanisms: blockade of progesterone action and suppression of the proliferative effects of estrogen; the latter effect is known as the endometrial antiproliferative effect [13,14]. Although the exact mechanism of the endometrial antiproliferative effect remains unclear, reduction in uterine blood flow [14], along with an elevation in the androgen receptor [15], are important factors. Overall, the evidence indicates that chronic, systemic administration of low-dose AP can block endometrial proliferation and suppress endometrial bleeding, effects that are desirable in a contraceptive IUD. In a preliminary report, we noted that an AP-releasing IUD could inhibit the macaque endometrium [16]. Here we describe the effects of AP-releasing intrauterine devices (AP IUD) on endometrial growth and bleeding patterns in ovariectomized macaques during hormonally induced cycles.

2. Materials and methods

2.1. The Macaque IUD

Prior to the experiments, we measured the distance from internal os to fundus, cervical length, and uterine luminal diameter in a number of macaques. Based on these measurements, Leiras OY, Finland manufactured AP-releasing (ZK 230 211) IUDs that consisted of straight Silastic tubes 1.2 cm in length that were either empty (controls) or filled with different amounts of steroid to allow release at either low dose (LD; 3.3-4.5 µg/day) or high dose (HD; 24.6-30.2 µg/day) rates. A thread, attached to each IUD, was sewn through the myometrium to hold the IUD within the uterine lumen.

2.2. Animal species and insertion of IUDs

All animal care was provided by the Division of Animal Resources at the Oregon National Primate Research Center under protocols approved by the Institutional Animal Care and Use Committee. We initiated the study in stump-tailed macaques (*Macaca arctoides*) because of reports that their cervix is straight compared to the S-shaped cervix of other common laboratory macaques [17]. However, we found that the canal of the internal os in this species is not straight but sigmoidal, which made it impossible to reliably insert IUDs by the vaginal route. Consequently, we placed IUDs in the uterine lumen of all animals by hysterotomy and anchored the IUD in place by sewing the attached thread through the inner myometrial wall. Pig-tailed macaques (*Macaca nemestrina*) were used when stump-tailed macaques became unavailable. Fig. 1 illustrates the position of an AP-IUD in the uterine lumen.

2.3. Experimental procedures

Nine animals including six stump-tailed and three pig-tailed macaques were ovariectomized and allowed to rest several weeks. All animals were treated sequentially first with an estradiol (E₂) Silastic implant (6 cm) for 14 days and then a progesterone (P) Silastic implant (9 cm) for 14 days to simulate a full cycle and induce an endometrium that would menstruate if P action was blocked. The E₂ implant remained in place continuously throughout all treatments in all

animals. Serum samples were obtained from each animal biweekly to assess their E₂ and P levels.

On the 28th day of the cycle, the animals were randomly assigned to three groups, each containing three animals (two stump-tailed and one pig-tailed macaque). Each group of three received either blank IUDs (controls), LD or HD IUDs. All animals were checked daily for evidence of vaginal bleeding. If no blood was evident on the cage floor or in the perineum region, the vagina was swabbed with cotton-tipped swabs to detect spotting.

In all animals, the systemic P implant remained in place for the next 7 days during which time we recorded menstrual bleeding patterns to determine whether the AP-releasing IUDs (compared to control IUDs) could induce menstrual bleeding in the P-primed endometrium. After 7 days, the P implants were removed, bleeding patterns were assessed, and all animals were again hormonally cycled with sequential E₂ and P to induce a second 28-day cycle. Endometrial biopsies were taken from all animals on day 28 to determine the histological effects of continuous treatment with the IUDs on endometrial growth and development. Subsequently, the P implant was removed and all animals were hormonally cycled to induce a 3rd cycle. At the end of the 3rd cycle, P was removed and menstrual bleeding patterns were again assessed. After this point, all nine animals were treated solely with E₂ for 28 days to determine whether the proliferative effects of E₂ on the endometrium could be blocked by the AP-releasing IUDs. On day 28 of continuous E₂ treatment, all animals were necropsied. The entire reproductive tract was removed, the uterus was quartered along the longitudinal axis, and cross-sections (2 mm thick) were cut from each quarter and prepared for immunocytochemistry (ICC) and morphological study. Fig. 2 depicts the above experimental design.

2.4. Histology and immunocytochemistry

Samples were processed for morphological and ICC study as described previously [18]. Briefly, samples for morphology were fixed in 2% glutaraldehyde and 3% paraformaldehyde, embedded in glycol methacrylate (GMA), sectioned, and stained with hematoxylin. Fresh tissue samples for ICC were microwave stabilized for 7 sec and embedded in Tissue Tek II OCT (Miles Inc., Elkhart, IN, USA) and frozen in liquid propane. Cryostat sections (7 μ m) were thaw-mounted on Superfrost Plus (Fisher Scientific, Pittsburgh, PA, USA) slides, placed on ice and microwave irradiated for 2 s. The microwave-treated sections were fixed in 0.2% picric acid and 2% paraformaldehyde in phosphate buffered saline (PBS) for 10 min at room temperature, immersed twice for 2 min each in 85% ethanol + 1.5% polyvinylpyrrolidone (PVP) at 4°C, rinsed in PBS, immersed twice 7 min each in 0.37% glycine in PBS + PVP, and then immersed in 0.1% gelatin in PBS + PVP at 4°C. To inhibit endogenous peroxidase activity the sections were incubated with a solution containing glucose oxidase (1 unit/mL), NaAzide (1 mM) and glucose (10 mM) in PBS for 45 min. Sections were then incubated with blocking serum for 20 min and then with the primary monoclonal anti-ER (1D-5; Biogenex, San Ramon, CA, USA), anti-PR (JZB-39; courtesy of Geoffrey Greene, University of Chicago [this antibody recognizes both isoforms of A and B of PR]), anti-Ki-67 antigen (Dako Corp., Carpinteria, CA, USA) and anti-AR (F39.4; Biogenex, San Ramon CA) overnight at 4°C. Omission of the first antibody and incubation of tissue sections with irrelevant and isotype-matched mouse IgG were used as method controls. In each case, primary antibody was detected with either biotinylated anti-mouse IgG (for 1D-5, anti Ki-67, F39.4) or anti-rat IgG (for JZB-39) secondary antibody. Final visualization was achieved using the ABC kit (Vector Laboratories, Burlingame, CA), 0.025% 3,3'-diaminobenzidine/4HCl (Dojindos DAB; Wako Chemicals, Richmond, VA) in Tris buffer and 0.03% H₂O₂ (Fisher Scientific, Pittsburgh, PA), and 0.026% osmium tetroxide (Electron Microscopy Sciences, Hatfield, PA). Sections were

then postfixed with 2% paraformaldehyde and lightly counterstained with hematoxylin to facilitate identification of cellular elements.

2.4.1. Archived slides—To provide a baseline for the effects of E₂ on endometrial proliferation, we used a set of archived slides from a set of ovariectomized rhesus macaques (n=10) that had previously been treated with systemic E₂ for 28 days in the absence of an IUD. Sections on these slides were prepared and stained for the Ki-67 antigen exactly as above. The number of Ki-67 positive glandular epithelial cells was assessed to derive a Ki-67 index as previously described [19], and this measure of glandular proliferation was used to compare the archived with the experimental endometria in the current study.

3. Results

3.1. Serum steroid levels

In preliminary work, serum levels of E₂ and P were measured bi-weekly for one complete cycle in adult cycling stump-tailed and pig-tailed macaques before the animals were ovariectomized. In the follicular phase, serum E₂ and P levels averaged 98.2±8.5 pg/mL and 0.21±0.04 ng/mL, respectively. In the luteal phase, E₂ and P levels averaged 86.0±16.8 pg/mL and 6.1±1.41 ng/mL respectively. In ovariectomized monkeys implanted with 6 cm E₂ and 9 cm P Silastic implants, E₂ levels were 73.2±8.5 pg/mL and P levels were 6.4±0.9 ng/mL throughout treatment, similar to physiological levels. There were no significant species differences.

3.2. Effects on bleeding patterns

Fig. 3 shows the bleeding patterns detected in this study. Some bleeding occurred immediately after acute insertion of all the IUDs, but this stopped within a few days in the blank IUD controls. Because it was immediate and stopped quickly, it was considered due to the surgical trauma associated with hysterotomy. However, bleeding persisted in the LD and HD animals, continuing for 7 days in one of the LD and 2 of the HD animals. The third HD animal showed frank menses on days 3-4 after insertion. Overall, these data indicate that while surgical trauma induced short term bleeding in all animals, the AP-IUDs induced bleeding that continued for an extended period. Also, the HD IUDs were more effective at inducing bleeding than the LD IUDs.

Seven days after the IUDs were placed and after all bleeding had stopped, the P implants were withdrawn to begin the 2nd cycle. All 3 animals with control IUDs menstruated for 5-7 days in a typical P withdrawal response. Of those animals with LD IUDs, one showed no bleeding and 2 spotted for either 3 or 6 days. Of the animals with HD IUDs, one didn't bleed at all and the other two showed minor spotting. These data suggest that the antiprogesterone-releasing IUDs had reduced the amount of tissue available to menstruate after P withdrawal, as there was no frank menstruation in these groups.

At the end of the 3rd cycle, the P implants were removed and bleeding patterns assessed again. The animals with blank IUDs menstruated for 4-10 days indicating a normal P withdrawal response. Two HD and two LD animals did not bleed at all. One LD animal spotted for 6 days and one HD animal spotted for 1 day. These data indicate that over time, the AP IUDs had suppressed the endometrium and greatly reduced its ability to respond to P withdrawal.

An Analysis of Variance was performed to compare the total number of bleeding days (frank menses + spotting) during cycles 2 and 3 (combined) for each group. The mean number of bleeding days (mean ± SEM) was as follows: blank IUD (12.5 ± 2.3), LD (5.0 ± 3.6) and HD (1.3 ± 0.8). The HD and LD IUDs significantly decreased the number of bleeding days compared to the blank IUD group (P<0.05). Fig. 4 depicts the bleeding patterns seen during

cycle 3 in diagrammatic form to emphasize that continued exposure to the AP-IUDs suppressed all frank menstruation after P withdrawal.

3.3. Effects on endometrial histology and steroid receptor immunocytochemistry

3.3.1. Luteal phase samples—The endometrial biopsies taken at the end of the luteal phase (day 28) of the second cycle showed that the blank IUD allowed development of a typical progestational endometrium, except that the amount of endometrial tissue was somewhat less than normally seen at the end of the cycle, and there was an infiltration of leucocytes in the upper functionalis indicating a foreign body reaction. However, the antiprogestin-releasing IUDs induced a dramatic, dose-dependent inhibition of endometrial development (Fig. 5), marked by an overall reduction in endometrial height (thickness), inhibition of glandular saccululation, and moderate compaction of the stroma. There were also degenerative changes including hyaline degeneration in the walls of the spiral arteries. This vascular degeneration was especially evident after treatment with the HD IUD (Fig. 6).

Immunohistochemical staining for estrogen receptor (ER) and progesterone receptor (PR) in the control IUD samples showed that ER and PR staining was weak in the glands and detectable in the stroma, as expected for luteal phase samples; this staining was greatly increased in both glands and stroma by the LD and HD IUDs (Fig. 7). The AP-IUDs also enhanced staining for AR, especially in the glands. A similar increase in AR staining occurred in the 28 day E₂ samples and is illustrated below in Fig. 10.

In the luteal phase, glandular and stromal proliferation was minimal regardless of the type of IUD. Vascular proliferation in the spiral arteries, which is normally elevated in the luteal phase due to high serum P [20] was characteristically high in animals treated with the blank IUD, but was dramatically inhibited in animals treated with AP-IUDs (Fig. 8).

3.3.2. 28-day E₂ samples—After systemic treatment with E₂ for 28 days, the endometrial histology in the blank IUD group was normal, similar to that in the archived samples. But the AP-IUD treated group showed extensive shrinkage and stromal compaction (Fig. 9). There was also a dose-dependent inhibition of glandular proliferation (Fig. 10). For example, the mean Ki-67 index $\pm$ SEM in the various groups were as follows: archived samples (22.23 ± 3.9), blank IUD (25.3 ± 5.0), LD IUD (25.1 ± 3) and HD IUD (13.9 ± 4.1). The Ki-67 index in the HD IUD group was significantly lower than in the LD group ($P < 0.05$, Student's-t). Therefore, in animals treated with E₂ alone, local treatment with AP-IUDs induced typical endometrial antiproliferative effects.

Immunocytochemistry showed that ER and PR immunostaining in the blank IUD group was elevated and was not further enhanced by treatment with the AP-IUDs. However, AR staining, which was nondetectable in the glandular nuclei in the blank IUD group, was dramatically increased in these nuclei by AP-IUD treatment (Fig. 10).

4. Discussion

These experiments had several aims. The first was to determine whether the amount of AP released into the intrauterine environment was adequate to antagonize P action and force an effective P-withdrawal menstruation. If menstruation had not occurred upon insertion, we would have remanufactured the IUDs to release higher doses of AP. Because the AP-IUDs induced much more sustained menstruation than the blank IUDs, we concluded that the devices were releasing effective levels of AP. The second aim was to determine whether, after remaining in place for one full cycle, the AP IUD could suppress endometrial growth and development just as systemic AP treatment does. As with systemic treatment, there was considerable endometrial atrophy and vascular degeneration evident at the end of the luteal

phase in the AP-treated but not the blank IUD group., Progestational effects such as glandular sacculation were inhibited, and the spiral arteries showed hyaline degeneration similar to that observed after long-term systemic AP treatment.

A third aim was to determine whether such a suppressed endometrium would undergo P-withdrawal bleeding at the end of a cycle. In all animals exposed to AP-releasing IUDs, there was minimal bleeding when the P implant was removed at the end of a cycle, while in those exposed to blank IUDs, normal menstruation followed P withdrawal. Also, the suppression of bleeding was dose-dependent, as the HD IUDs suppressed P withdrawal bleeding more effectively than the LD IUDs. We concluded that local administration of an AP can suppress progestational development of the endometrium and render it insensitive to P withdrawal.

A fourth aim was to determine whether an AP-releasing IUD would induce the so-called endometrial antiproliferative effect, namely, the inhibition of endometrial proliferation during E₂ treatment in the absence of P. Results showed a dose dependent, endometrial antiproliferative effect similar to that seen after systemic administration of APs.

A fifth aim was to determine whether the blank IUD had any untoward effects on endometrial responsiveness. Except for a mild influx of inflammatory cells in the uppermost zones, endometria treated with a blank IUD did not differ histologically in any major way from the archived samples and responded normally to hormone administration and P withdrawal. Animals with control IUDs menstruated for a somewhat longer period (6-9 days) after P withdrawal, perhaps due to the mild inflammatory condition.

The final aim was to determine the effects of AP-releasing IUDs on endometrial steroid receptors. In the luteal phase, the well known ability of systemic progesterone to downregulate ER and PR [21] was blocked by the AP-releasing IUDs. As a result, nuclear ER and PR staining was far stronger in the treated groups than in the blank IUD controls during the luteal phase. Also, AR was nondetectable in the glandular nuclei of the controls, but strongly expressed in these cells after AP treatment. These effects on steroid receptors were identical to those seen after systemic treatment with progestin antagonists [22,23].

In the estrogen-only group (28 days E₂) ER and PR were strongly stained in the presence of AP-releasing IUDs, though not greatly different from controls, while AR, which was nondetectable in the glandular nuclei in the blank IUD controls, was dramatically elevated by local AP treatment. Because androgens are known to block the effects of estrogens on the endometrium [24] and because antiandrogens can block the suppressive effects of APs on the rhesus macaque endometrium [25] we have suggested that the AR is involved in mediating the endometrial antiproliferative effect [15]. Although a full explanation of the antiproliferative effect is not at hand, the similarity of the local to the systemic effects of APs suggests that the underlying mechanism is an intrauterine, not a systemic, one.

In sum, AP-releasing IUDs have two important effects: 1) they can suppress the effects of P on endometrial progestational development, render the endometrium insensitive to P withdrawal and consequently induce amenorrhea; and 2) they can suppress the effects of E₂ on endometrial proliferation and avoid unopposed estrogen action in this tissue. Although the contraceptive efficacy of such devices needs to be tested, the nonhuman primate data strongly suggest that AP-releasing IUDs can provide a novel approach for bleeding-free contraception.

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References

- [1]. Ortiz ME, Croxatto HB, Bardin CW. Mechanisms of action of intrauterine devices. *Obstet Gynecol Survey* 1996;51(Suppl):S42–S51.
- [2]. Burton KA, Henderson TA, Hillier SG, et al. Local levonorgestrel regulation of androgen receptor and 17beta-hydroxysteroid dehydrogenase type 2 expression in human endometrium. *Hum Reprod* 2003;18:2610–7. [PubMed: 14645180]
- [3]. Baird DT, Hildt I, RU 486 and other antiprogesterins as postovulatory contraceptives 15–18 June Program of the 76th Annual Meeting of Endocrine Society Anaheim, CA 1994
- [4]. Zelinski-Wooten MB, Chwalisz K, Iliff SA, Niemeyer CL, et al. A chronic, low-dose regimen of the antiprogesterin ZK 137 316 prevents pregnancy in rhesus monkeys. *Hum Reprod* 1998;13:2132–8. [PubMed: 9756284]
- [5]. Borman SM, Schwinof KM, Niemeyer C, Chwalisz K, Stouffer RL, Zelinski-Wooten MB. Low-dose antiprogesterin treatment prevents pregnancy in rhesus monkeys and is reversible after 1 year of treatment. *Hum Reprod* 2003;18:69–76. [PubMed: 12525443]
- [6]. Grow DR, Reece MT, Hsiu JG, et al. Chronic antiprogesterin therapy produces a stable atrophic endometrium with decreased fibroblast growth factor: a 1-year primate study on contraception and amenorrhea. *Fertil Steril* 1998;69:936–43. [PubMed: 9591506]
- [7]. Slayden OD, Zelinski MB, Chwalisz K, Hess-Stump H, Brenner RM. Chronic progesterone antagonist-estradiol therapy suppresses breakthrough bleeding and endometrial proliferation in a menopausal macaque model. *Hum Reprod* 2006;21:3081–90. [PubMed: 16936297]
- [8]. Slayden OD, Chwalisz K, Brenner RM. Reversible suppression of menstruation with progesterone antagonists in rhesus macaques. *Hum Reprod* 2001;16:1562–74. [PubMed: 11473944]
- [9]. Fuhrmann U, Hess-Stump H, Cleve A, Neef G, Schwede W, Hoffmann J, et al. Synthesis and biological activity of a novel, highly potent progesterone receptor antagonist. *J Med Chem* 2000;43:5010–6. [PubMed: 11150172]
- [10]. Slayden OD, Zelinski-Wooten MB, Chwalisz K, Stouffer RL, Brenner RM. Chronic treatment of cycling rhesus monkeys with low doses of the antiprogesterin ZK 137 316: morphometric assessment of the uterus and oviduct. *Human Reproduction* 1998;13:269–77. [PubMed: 9557821]
- [11]. Brown A, Cheng L, Lin S, Baird DT. Daily low-dose mifepristone has contraceptive potential by suppressing ovulation and menstruation: a double-blind randomized control trial of 2 and 5 mg per day for 120 days. *J Clin Endocrinol Metab* 2002;87:63–70. [PubMed: 11788624]
- [12]. Cheng L, Zhu H, Wang A, Ren F, Chen J, Glasier A. Once a month administration of mifepristone improves bleeding patterns in women using subdermal contraceptive implants releasing levonorgestrel. *Hum Reprod* 2000;15:1969–72. [PubMed: 10966997]
- [13]. Brenner RM, Slayden OD. Progesterone receptor antagonists and the endometrial antiproliferative effect. *Semin Reprod Med* 2005;23:74–81. [PubMed: 15714391]
- [14]. Chwalisz K, Brenner RM, Fuhrmann U, Hess-Stump H, Elger W. Antiproliferative effects of progesterone antagonists and progesterone receptor modulators (PRMs) on the endometrium. *Steroids* 2000;65:741–51. [PubMed: 11108885]
- [15]. Brenner RM, Slayden OD, Critchley HO. Anti-proliferative effects of progesterone antagonists in the primate endometrium: a potential role for the androgen receptor. *Reproduction* 2002;124:167–72. [PubMed: 12141929]
- [16]. Nayak NR, Slayden OD, Chwalisz K, Lehtinen M, Brenner RM. Antiprogesterin-releasing intrauterine devices: A novel approach to endometrial contraception. *Fertility and Sterility*, 56th Annual Meeting of the American Society for Reproductive Medicine 2000;74(3S):S70–S71. Ref Type: Abstract
- [17]. Demers LM, Macdonald GJ, Hertig AT, King NW, MacKey JJ. The cervix uteri in *Macaca mulatta*, *Macaca arctoides*, and *Macaca fascicularis* - A comparative anatomic study with special reference to *Macaca arctoides* as a unique model for endometrial study. *Fertil Steril* 1972;23:529–34. [PubMed: 4625654]
- [18]. Slayden OD, Koji T, Brenner RM. Microwave stabilization enhances immunocytochemical detection of estrogen receptor in frozen sections of macaque oviduct. *Endocrinology* 1995;136:4012–21. [PubMed: 7649110]

- [19]. Slayden OD, Hirst JJ, Brenner RM. Estrogen action in the reproductive tract of rhesus monkeys during antiprogesterin treatment. *Endocrinology* 1993;132:1845–56. [PubMed: 8462480]
- [20]. Brenner RM, Slayden OD. Steroid receptors in blood vessels of the rhesus macaque endometrium: a review. *Arch Histol Cytol* 2004;67:411–6. [PubMed: 15781982]
- [21]. Lessey BA, Killam AP, Metzger DA, Haney AF, Greene GL, McCarty KS Jr. Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. *J Clin Endocrinol Metab* 1988;67:334–40. [PubMed: 2455728]
- [22]. Neulen J, Williams RF, Breckwoldt M, Chwalisz K, Baulieu EE, Hodgen GD. Non-competitive anti-oestrogenic actions of progesterone antagonists in primate endometrium: enhancement of oestrogen and progesterone receptors with blockade of post-receptor proliferative mechanisms. *Hum Reprod* 1996;11:1533–7. [PubMed: 8671500]
- [23]. Slayden OD, Brenner RM. Hormonal regulation and localization of estrogen, progestin and androgen receptors in the endometrium of nonhuman primates: effects of progesterone receptor antagonists. *Arch Histol Cytol* 2004;67:393–409. [PubMed: 15781981]
- [24]. Grody MH, Lampe EH, Masters WH. Estrogen-androgen substitution therapy in the aged female. *Obstet Gynecol* 1953;2:36–45. [PubMed: 13073078]
- [25]. Slayden OD, Brenner RM. Flutamide counteracts the antiproliferative effects of antiprogesterins in the primate endometrium. *J Clin Endocrinol Metab* 2003;88:946–9. [PubMed: 12574238]

Fig.1.

An antiprogestin-releasing IUD (AP-IUD) in utero. The photograph shows an IUD in place within the uterine lumen of a pig-tailed macaque after hysterectomy. E= endometrium, M= myometrium, C=Cervix and F= the tines of a Dumont #5 forceps for scale.

Experimental Design

Fig. 2.

The experimental design. Ovariectomized animals were hormonally cycled with Silastic implants of estradiol (E_2) and progesterone (P) for 3 cycles followed by 28 days of E_2 treatment alone. IUDs were inserted at the end of the 1st cycle, endometrial biopsies were taken at the end of the 2nd cycle, and the uteri were removed after treatment with E_2 alone for 28 days.

Fig. 3.

Bleeding patterns throughout the entire experiment. Each row represents the bleeding events occurring over time in each animal. The dark squares represent frank menstruation, the light squares indicate swab-positive bleeding (spotting). Listed from right to left across the top are the 3 cycles (see Fig. 2). From top to bottom on the left are listed the Blank, low dose (LD) and high dose (HD) IUD groups. In cycle 1, the LD and HD IUDs antagonized P and caused an effective P-withdrawal bleeding. By the end of cycle 3, the LD and HD had suppressed the ability of the endometrium to respond to P-withdrawal. The HD IUD was more effective than the LD IUD.

Fig. 4. Bleeding patterns during the 3rd cycle. This diagram emphasizes that continuous exposure of the endometrium to the AP-IUDs suppressed the ability of the endometrium to respond to P-withdrawal with frank menstruation.

Fig. 5. Effects of AP-IUDs on endometrial histology in the luteal phase. Left image = Blank IUD, center image = LD IUD, right image = HD IUD. The blank IUD tissue showed normal progesterational endometrium with sacculated glands. The LD and HD IUDs caused shrinkage, stromal compaction and blockade of progesterational changes. Original magnification = ~100x.

Fig. 6. Effects of IUDs on the spiral arteries during the luteal phase. A) Normal spiral artery with a Blank IUD. B) Beginning hyaline degeneration in spiral artery exposed to the LD IUD. C) Severe hyaline degeneration in an artery exposed to a HD IUD. Original magnifications = ~250x.

Fig. 7. Effects of IUDs on endometrial ER and PR in luteal phase. Top row= ER; bottom row = PR. Blank IUD (a,d), LD IUD (b,e), HD IUD (c,f). GI = glands, S = stroma. ER and PR are weakly expressed in the luteal phase (a,d) and are strongly up-regulated by both the LD and HD IUDs (b,c;e,f). Original magnification = ~400x.

Fig. 8.

Effects of IUDs on vascular proliferation in luteal phase. Top row: low magnification = $\sim 250\times$. Bottom row: higher magnification = $\sim 400\times$. Ki-67 staining is evident in the vascular endothelium (arrow), muscular walls (arrowhead) and perivascular stroma in the blank IUD group (a,d). No, or minimal, staining, is evident in these cells in the LD (b,e) or HD IUD groups (c,f).

Fig. 9.

Effects of IUDs on endometrial histology after 28 days of E_2 . Top row: Blank IUD shows normal proliferative endometrium. LD and HD IUDs suppress endometrial thickness. Bottom row: Blank IUD shows normal gland-stromal spatial relationships. LD and HD show extreme stromal compaction and glandular atrophy. These effects are typical of the endometrial antiproliferative effect. Top row: original magnification = $\sim 50\times$; bottom row: original magnification = $\sim 250\times$. Endo= endometrium. Myo = myometrium.

Fig. 10.

Effects of IUDs on endometrial proliferation (Ki-67) and the androgen receptor (AR) after 28 days of E_2 . Top row = Ki-67. The Blank IUD shows a large number of Ki-67-positive cells, typical of E_2 -dependent proliferation. The LD IUD had a minimal effect while the HD IUD strongly reduced the number of Ki-67-positive cells. Bottom row = AR. The Blank IUD had no effect on AR, which is normally expressed only in the stroma under E_2 influence. Both the HD and LD dramatically increased AR staining in the glandular component. Original magnification = $\sim 250\times$.