(21) International Application Number: PCT/US88/00711
(22) International Filing Date: 9 March 1988 (09.03.88)
(31) Priority Application Number: 024,205
(32) Priority Date: 10 March 1987 (10.03.87)
(33) Priority Country: US

(71) Applicant: EASTERN VIRGINIA MEDICAL AUTHORITY (US/US); 700 Olney Road, Norfolk, VA 23507 (US).


(54) Title: METHOD AND KIT FOR CONTRACEPTION WITH GnRH-ANTAGONIST AND PROGESTIN

(57) Abstract

A method of providing contraception for and regulation of the menstrual cycle of a gonadal female mammal by a) administering thereto about once a week throughout her menstrual cycle an amount of a GnRH-antagonist effective to block folliculogenesis but less than the amount thereof required to block hormonogenesis; also administering prior to the end of that cycle an amount of a progestin effective to produce a secretory endometrium; and b) then terminating the progestin administration, thereby inducing menses.
FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

<table>
<thead>
<tr>
<th>AT</th>
<th>Austria</th>
<th>FR</th>
<th>France</th>
</tr>
</thead>
<tbody>
<tr>
<td>AU</td>
<td>Australia</td>
<td>GA</td>
<td>Gabon</td>
</tr>
<tr>
<td>BB</td>
<td>Barbados</td>
<td>GB</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>BE</td>
<td>Belgium</td>
<td>HU</td>
<td>Hungary</td>
</tr>
<tr>
<td>BG</td>
<td>Bulgaria</td>
<td>IT</td>
<td>Italy</td>
</tr>
<tr>
<td>BJ</td>
<td>Benin</td>
<td>JP</td>
<td>Japan</td>
</tr>
<tr>
<td>BR</td>
<td>Brazil</td>
<td>KP</td>
<td>Democratic People's Republic of Korea</td>
</tr>
<tr>
<td>CF</td>
<td>Central African Republic</td>
<td>KR</td>
<td>Republic of Korea</td>
</tr>
<tr>
<td>CG</td>
<td>Congo</td>
<td>LI</td>
<td>Liechtenstein</td>
</tr>
<tr>
<td>CH</td>
<td>Switzerland</td>
<td>LK</td>
<td>Sri Lanka</td>
</tr>
<tr>
<td>CM</td>
<td>Cameroon</td>
<td>LU</td>
<td>Luxembourg</td>
</tr>
<tr>
<td>DE</td>
<td>Germany, Federal Republic of</td>
<td>MC</td>
<td>Monaco</td>
</tr>
<tr>
<td>DK</td>
<td>Denmark</td>
<td>MG</td>
<td>Madagascar</td>
</tr>
<tr>
<td>FI</td>
<td>Finland</td>
<td>ML</td>
<td>Mali</td>
</tr>
<tr>
<td>MR</td>
<td>Mauritania</td>
<td>MW</td>
<td>Malawi</td>
</tr>
<tr>
<td>NL</td>
<td>Netherlands</td>
<td>NO</td>
<td>Norway</td>
</tr>
<tr>
<td>RO</td>
<td>Romania</td>
<td>SD</td>
<td>Sudan</td>
</tr>
<tr>
<td>SE</td>
<td>Sweden</td>
<td>SN</td>
<td>Senegal</td>
</tr>
<tr>
<td>SU</td>
<td>Soviet Union</td>
<td>TD</td>
<td>Chad</td>
</tr>
<tr>
<td>TG</td>
<td>Togo</td>
<td>US</td>
<td>United States of America</td>
</tr>
</tbody>
</table>
METHOD AND KIT FOR CONTRACEPTION
WITH GnRH-ANTAGONIST AND PROGESTIN

Background of the Invention

This invention relates to a method and kit for achieving contraception in gonadal female mammals with GnRH-antagonists without inducing an agonadal state.


As reported by us in J. Clin. Endocrinol. Metab. 62: 734 (April, 1986), the contents of which article are incorporated herein by reference, we have now found that reliably effective contraception (by inhibition of ovulation) can be achieved with GnRH antagonists in gonadal female mammals without blocking hormonogenesis, thereby
avoiding the side effects associated with a long term agonadal state.

For more than a decade, reproductive endocrinologists have sought an adequate strategy to employ GnRH analogs (agonists and antagonists) for contraception in women. Almost uniformly, enthusiasm to move ahead has been thwarted by the knowledge that a persistent functional hypopituitary status, induced by GnRH analogs, is associated with relative ovarian quiescence and a hypoestrogenic milieu. In turn, the expected consequences include hot flushes, urogenital tissue atrophy and bone mineral loss (osteoporosis). Among women of reproductive age this would surely require steroidal replacement therapy (estrogen and progestin), mimicking familiar treatment regimens in postmenopausal women. Indeed, there seems little gain in a method based on such complex pharmacology, where the potential consequences of side effects may exceed those of the common oral contraceptives containing similar or the same synthetic steroids. Also, there have been questions about the long-term tolerance to and safety of the GnRH analogs themselves at dosages which achieve an agonadal state.

In accordance with this invention, it is now possible to employ GnRH antagonists for reliable ovulation inhibition, while avoiding frank estrogen deficiency and relying on natural ovarian steroids to maintain normal metabolic homeostasis.

**Objects of the Invention**

It is an object of this invention to provide a method for achieving contraception in a gonadal female mammal with a GnRH-antagonist without blocking hormonogenesis.

Another object is to provide such a method which does not interfere with menses, and which regulates the onset thereof.

Still another object is to provide such a method in which the medication is self-administered.
A further object is to provide such a method which does not have the side effects associated with estrogen-progestin oral contraception.

Another object is to provide kits for practicing the method of this invention.

Other objects will be apparent to those skilled in the art to which this invention pertains.

Summary of the Invention

In a method aspect, this invention relates to a method of contraception in and regulation of the menstrual cycle of a gonadal female mammal, which comprises:

a) administering thereto periodically at predetermined intervals longer than daily throughout her menstrual cycle an amount of a GnRH antagonist effective to block folliculogenesis but less than the amount thereof required to block hormonogenesis;

b) also administering thereto prior to the end of that cycle an amount of a progestin effective to produce a secretory endometrium; and

c) then terminating the progestin administration, thereby inducing menses.

In a kit aspect, this invention comprises a kit in the form of a dispenser-type package containing (a) sufficient dosage units of a GnRH-antagonist, in a dosage form suitable for self-administration, to block gametogenesis but not hormonogenesis in a gonadal female mammal for about one calendar month; (b) sufficient dosage units of a progestin, also in a dosage form suitable for self-administration, to produce a secretory endometrium in the female mammal and to induce menses after administration of the progestin is terminated; and, optionally, (c) sufficient dosage units of a placebo of the same dosage form as that of the GnRH-antagonist and/or of the progestin to permit the administration daily of a single dosage unit of the GnRH-antagonist and/or the progestin and/or of a placebo for
about 20-25 days of the menstrual cycle prior to onset of menses.

**Detailed Disclosure**

The intermittent administration of the GnRH-antagonist achieves fertility control in gonadal female mammals, e.g., husbandry animals and humans, by acutely suppressing the release of endogenous luteinizing hormone and follicle stimulating hormone from the pituitary gland, thereby preventing maturation of the dominant follicle and release of oocytes. However, the intermittent administration of the GnRH-antagonist does not impair adequate ovarian estadiol secretion. To distinguish the regulation of the two ovarian activities, viz., as an organ of reproduction and a gland of internal secretion, the term "folliculogenesis" is used herein to refer to the gametogenic activity and "hormonogenesis" refers to the secretory activity. It is known that hypoestrogenic states can cause hot flashes, increase the risk of osteoporosis, and precipitate urogenital atrophy in women. The intermittent use of the GnRH antagonist for fertility control according to this invention obviates these symptoms by maintaining a near normal follicular phase level of serum estradiol by distinguishing blockage of hormonogenesis (which is sustained) from gametogenesis (which is inhibited).

In the normal menstrual cycle, progesterone is produced from the corpus luteum created after ovulation. Since ovulation is prevented, progesterone secretion remains low. An oral progestin supplement converts proliferative endometrium to the secretory phase, thereby reducing the risks of endometrial carcinomas associated with unopposed estrogen action.

Examples of progestins which can be employed in this invention are micronized progesterone (50-150 mg/day), norethindrone (0.5-2.5 mg/day), norethynodrel (1 mg/day);
ethynodiol diacetate (1-2 mg/day), norgestrel (0.2-0.5 mg) and levo-norgestrel (0.1-0.3 mg/day).

The progestin can be administered in the conventional manner by any route that the selected progestin is active. Most synthetic progestins are orally active and therefore are preferably administered by that route, e.g., in the form of a tablet, dragee, capsule or pill. If the progestational agent of this invention is to be administered in tablet or dragee form, it may contain a pharmaceutically-acceptable diluent which includes a binder, such as tragacanth, corn starch or gelatin; a disintegrating agent, such as alginic acid; and a lubricant, such as magnesium stearate. If administration in liquid form is desired, sweetening and/or flavoring may be used as part of the pharmaceutically-acceptable diluent, and intravenous administration in isotonic saline, phosphate buffer solutions or the like may be effected. The progestin can also be administered intranasally, as a suspension or dispersion in an appropriate vehicle, by inhalation as an aerosol discharged from a conventional inhalator, sublingually, in the form of a lozenge, rectally or intravaginally, e.g., in the form of a suppository, alone or in admixture with the GnRH antagonist, when appropriate according to the day of the menstrual cycle. Other means are by an implant which discharges the progestin, for about 7-15 days, about 14-16 days after implant, (e.g., at the onset of menses); intradermally, as a skin patch containing a mixture of the progestin and a skin penetrant, e.g., DMSO; and imbedded in a vaginal ring which discharges the progestin for about 14-16 days after insertion.

An example of a GnRH antagonist which can be employed in this invention is \((\text{Ac-}\text{p-Cl-Ph}^1,\text{p-Cl-Phe}^2,\text{D-Trp}^3,\text{D-Arg}^6,\text{D-Ala}^{10})\text{NET-GnRH}\). Others are known in the prior art. See, e.g., 4,409,208; 4,547,370; 4,565,804; 4,569,927; and the 619,914, whose disclosures are incorporated herein by reference. Such other antagonists include:
D2Nal-DPhe-D3Pal-Ser-Arg-d2nAL-Leu-Arg-Pro-DAla-NH₂
Ac 4Cl

D2Nal-DPhe-D3Pal-Ser-Arg-DGlu-Leu-Arg-Pro-DAla-NH₂
Ac 4Cl A.A.

DNal₂-Phe-DTr-Ser-Tyr-DArg-Lue-Arg-Pro-Gly-NH₂
Ac 4FD

[N-Ac-d-Na(2)₁, d-pCl-Phe², d-Trp³, d-hArg(Et₂)⁶,
d-Ala₁⁰]LHRH

Ac[D-B-Nal¹, D-p-Cl-Phe², D-Trp³, D-Arg⁶, D-Ala₁⁰] LHRH

Ac-D⁻ Me-₄⁻ D-Pal Ser Tyr D-Arg Leu ᵃPr⁻ Pro D-Ala NH₂
Na Cl-Phe Lys

After a GnRH antagonist is administered, a
gametogenesis blocked state is usually achieved within about
1 day thereafter, provided the first dose of the GnRH-
antagonist is administered on day 1, 2 or 3 of menses. A
longer delay may not abate selection of the oncoming
dominant-follicle during that menstrual cycle and therefore
another form of contraception should be practiced during
that cycle, or at least for the first 7 days thereof.

The GnRH-antagonists employed in this invention can be
administered in the form of pharmaceutically acceptable,
nontoxic salts, such as acid addition salts, or of metal
complexes, e.g., with zinc, barium, calcium, magnesium,
aluminum or the like, or of combinations thereof.

Illustrative of such acid addition salts are hydrochloride,
hydrobromide, sulphate, phosphate, nitrate, oxalate,
fumarate, gluconate, tannate, maleate, acetate, citrate,
benzoate, succinate, alginate, malate, ascorbate, tartrate
and the like.

The pharmaceutical compositions employed in the process
of this invention will usually contain the GnRH-antagonist
in conjunction with a conventional, pharmaceutically
acceptable carrier. Usually, the effective dosage of the
GnRH-antagonist is from about 1 to about 100 micrograms of
the GnRH-antagonist per kilogram of the body weight of the
host when given intravenously; therefore, oral dosages and
dosages by other routes will be higher. Overall, treatment

SUBSTITUTE SHEET
of subjects with these GnRH-antagonists is generally carried out in the same manner as other clinical treatments using GnRH antagonists, except for the total amount thereof which is administered.

The GnRH-antagonists can be administered to the mammal intravenously, subcutaneously, intramuscularly, sublingually, orally by inhalation, percutaneously, rectally, intranasally or intravaginally, to achieve contraception. Effective dosages will vary with the selected mode of administration and the particular species of mammal being treated. An example of one typical dosage form is a bacteriostatic water solution containing the GnRH antagonist which solution is administered as a nasal mist to provide a dose in the range of about 0.1 to 2.5 mg/kg of body weight. Oral administration of the GnRH-antagonist may be in the form of a lozenge, to be dissolved under the tongue, or as an aerosol, to be inhaled.

Optionally, one or two of the doses of GnRH-antagonist can be administered in admixture with doses of the progestin on appropriate days of the cycle, e.g., day 16 and day 23.

In its article of manufacture embodiment, this invention relates to materials, reagents and kits for practicing the method of this invention.

In one form of its kit aspect, this invention is directed to a two-stage multiple dosage unit article of manufacture comprising a) four dosage units, adapted for self-administration, each dosage unit containing an amount of a GnRH-antagonist effective when one unit thereof is administered every seven days, the first on day 1, 2 or 3 of mensus, to block follicultogenesis for one cycle but which is less than the an thereof effective to block hormonogenesis; and b) 5-7 units, adapted for self-administration, collectively containing an amount of a progestin effective, when a dosage unit is administered daily on successive days, beginning on day 14, 15 or 16 of the cycle until all of the units have been administered, to
produce a secretory endometrium and thereafter induce menses. Optionally, the kit also comprises a plurality of placebo dosage units corresponding to those of a) which collectively therewith total about 23 to about 28 units, the GnRH-antagonist-containing, the progestin-containing and the placebo units being arranged in appropriate sequential order. Optionally also, the third and fourth GnRH-antagonist-containing dosage units are contained in same dosage units which contain the progestin. Preferably, the GnRH-antagonist units are adapted for sub-lingual, rectal, vaginal or topical (on the skin) application. Preferably also, the progestin units are adapted for oral ingestion e.g., tablets, capsules or dragees, or for administration by the same route as the GnRH antagonist units. Preferably further, the GnRH-antagonist units, the progestin dosage units and any placebo dosage units all are adapted for administration by the same route.

In another form of its kit aspect, this invention is directed to a combination of (a) a nasal spray plastic squeeze bottle containing a GnRH-antagonist in a vehicle adapted for administration thereof as a mist, at a concentration effective upon squeezing the bottle either once, twice or three times while the dispensing tip is inserted into a nostril of the female mammal, once every seven days, an amount of the antagonist effective to block folliculogenesis but less than the amount required to block hormonogenesis; and either 5-11 individual dosage units, preferably adapted for oral ingestion, e.g., tablets or capsules, collectively containing an amount of a progestin effective upon administration thereof during the third week of the menstrual cycle to produce a secretory endometrium and to thereafter induce menses, or 23 or 28 such individual dosage units arranged in appropriate sequential order, 5 to 11 of which units contain the progestin and the remainder are placebos, to be taken daily for the first 23 days of a menstrual cycle or every day of a 28 day menstrual cycle.
In a further kit form, instead of the nasal spray squeeze bottle a plurality of skin patches or an aerosol inhaler is employed to dispense the required amount of GnRH-antagonist.

In still another kit form, both the GnRH and progestin are in a unit dosage form adapted for vaginal or rectal administration, e.g., suppositories. For example, such a kit comprises 4 suppositories containing the GnRH-antagonist and either 5-8 separately identified suppositories containing the progestin for administration on successive days of the third week of one menstrual cycle, or the kit comprises 23 to 28 such suppositories, arranged for sequential administration, every 7th one of which contains the GnRH-antagonist; the 16th through the 22nd of which contain the progestin; and the remainder are placebos.

In alternative embodiments, the test kit contains the GnRH-antagonist incorporated in another delivery system, e.g., adhesive skin patches containing the medication in an intradermal and delivery system for application to the skin; suppositories for administration of the medication rectally or vaginally; an inhaler for delivery of the medication as an aerosol, or lozenges for administration sublingually; in each case once or twice daily on the same or on two successive days of each week.

The materials, reagents and kits optionally and preferably also contains instructions for administering the GnRH-antagonist and the progestin.

A specific example of materials, reagents and kits of this invention intended for use by a gonadal female who is to be rendered infertile but not agonadal is a box with lid containing (a) a conventional nasal mist squeeze bottle containing a GnRH-antagonist, in an inert, physiologically acceptable carrier in which the antagonist is stable, at a concentration effective to deliver into the nostril, when the bottle is squeezed twice while the dispensing tip thereof is inserted into the nostril, an amount of the GnRH-
antagonist effective to place the female in a folliculogenesis blocked state for 7 days, and containing an amount thereof sufficient for four such administrations on a weekly basis throughout one menstrual cycle, and (b) seven to 11 tablets of an orally active progestin effective when taken orally on successive days, e.g., beginning on day 15 of the menstrual cycle, to produce a secretory endometrium and thereafter to induce menses. If eleven tablets are employed, about a 30 day cycle will usually be achieved whereas a lesser number will produce a correspondingly shorter cycle.

Intermittent GnRH-antagonist therapy alone according to this invention blocks folliculogenesis while sustaining hormonogenesis. The maintenance of hormonogenesis provides circulating estrogen levels sufficient to preclude hypoestrogenic effects. The blockage of folliculogenesis prevents the selection and maturation of a functional dominant follicle. Consequently, anovulation ensues concurrently with subnormal levels of luteal phase serum progesterone. Although metabolic homeostasis is not maintained by intermittent GnRH-antagonist therapy alone, it is maintained by the combination of intermittent GnRH-antagonist and a progestin, which combination inhibits ovulation while assuring that proliferative endometrium will be shed during menses. This action negates the risk of endometrial carcinoma due to unopposed estrogen. The combination therapy 1) synergistically enhances the contraceptive action of each, 2) reliably inhibits ovulation, and 3) provides for cyclic menstruation.

Although this invention employs progestin as well as GnRH-antagonist administration, it will be apparent that because contraception may be achieved primarily although not exclusively by the GnRH-antagonist, if a normal menstrual cycle is not mandated, for example, where the GnRH-antagonist is to be administered only on a short term basis, e.g., when only temporary contraception is desired for
medical reasons, the progestin can be omitted, in which case a normal menstrual cycle will not occur until GnRH-antagonist administration is terminated. However, because the GnRH-antagonist and the progestin not only collectively mimic a normal menstrual cycle, they act synergistically in achieving contraception, they are preferably employed together in accordance with this invention to ensure an unplanned pregnancy does not occur and to avoid the potential adverse effects of a retained endometrium.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. In the following examples, all temperatures are set forth uncorrected in degrees celsius; unless otherwise indicated, all parts and percentages are by weight.

EXAMPLES
Materials and Methods

Fifteen adult (3.8-6.1 kg) cynomolgus monkeys (Macaca fascicularis) with regular ovulatory menstrual cycles were subjected to protocols of GnRH-antagonist treatment. The methods of feeding, housing, blood collection, and drug administration were described previously. Goodman, A.L. et al. Composite pattern of circulating LH, FSH, estradiol, and progesterone during the menstrual cycle in cynomolgus monkeys, Proc. Soc. Exp. Biol. Med 155:479

Study I. The GnRH-antagonist employed was (Ac-pClPhe, pClpPhe, DTrp, DArg, DA1a)GnRH. Based on previous dose studies demonstrating profound suppression of serum LH and FSH levels by this agent at a daily dose of 1 mg/kg, a higher single dose was chosen for weekly administration. Since the dominant follicle was likely to be selected by cycle day 7, the intermittent dose of GnRH-
antagonist might achieve its ablation, thereby causing initiation of a new follicular phase. The GnRH-antagonist was suspended in sesame oil and injected im.

Eight monkeys were used to test ovulation inhibition. Drug administration began on day 3 after spontaneous onset of menses, when a single dose of 10 mg/kg was injected. This initial injection was followed at weekly intervals with single doses of 5 mg/kg on days 10, 17, and 24. During the first 42 days after initiation of the pretreatment menstrual flow, femoral blood (3.5 ml) was obtained on alternate days. on day 43, the frequency of blood drawing was decreased to twice weekly through day 64, when the study was terminated. Throughout, sera were harvested and frozen for subsequent RIA. During the 4 weeks of GnRH-antagonist treatment, laparoscopies were performed at weekly intervals to assess ovarian status.

Study II. To clarify the mechanisms(s) by which the GnRH-antagonist blocked estrogen-induced LH surges in Study I, normal ovulatory monkeys received the GnRH-antagonist daily (2 mg/kg in sesame oil) from either days 2 through 9 or days 2 through 6 of the menstrual cycle, respectively, and estradiol (E$_2$) benzoate 50 µg, im; n=3) or GnRH 50 µg, iv; n=4) was given on day 6. Because the weekly treatment regimen used in study I was so inherently volatile, with GnRH-antagonist suppression of pituitary function followed by transient recovery in a series of four episodes, we selected a daily regimen in study II to strive for steadier, more consistent conditions for estrogen challenge and GnRH challenge tests provided an evaluation of pituitary gonadotropin secretory status during the GnRH-antagonist regimen; serum LH concentrations served as the principal end point. The same test was given to three control monkeys (not given GnRH-antagonist) on day 6 of the normal menstrual cycle.

RIAs for E$_2$, progesterone (P$_4$), LH, and FSH were performed as described previously. Goodman A.L. et al.,
Endocrinology 1977, 100:155; Niswender, G.D. et al.,
Endocrinol 1971, 88:1327. The monkey LH RIA had an
intraassay variation of 1.9-5.0% and an interassay variation
of 9.3-18.7%.

Tests for significant differences (P<0.01) of the
intervals from the onset of menstruation to the next LH
surge and/or ovulation and the menstrual interval were
performed using the F statistic. Freund, J.E., "Manual of
Experimental Statistics", (Prentice-Hall, Pub., Englewood
Cliffs (1960).

During the 4-week course of intermittent GnRH-
antagonist treatment in study I, there were no ovulation
sites seen at laparoscopy. Moreover, the absence of
ovulation was confirmed by the lack of serum P₄ elevations;
also, no LH surges were found.

It was evident from mean peripheral serum E₂ levels
immediately before and on the day after the four GnRH-
antagonist treatments are shown there was no sustained
suppression of E₂ in serum; that is, E₂ concentrations
remained near the level found in the early follicular phase
of normal ovarian/menstrual cycles (-90-125 pg/ml).

None of the animals with ovulation inhibition and
absence of LH surges during treatment as well as resumption
of an ovulatory ovarian cycle after treatments, after
discontinuation of treatment with the GnRH-antagonist, serum
E₂ levels rose within 3 weeks and were followed by timely LH
surges in six of eight monkeys. Presumed ovulation was
indicated by typical P₄ elevations in serum and an ensuing
luteal phase of normal length. The levels of E₂ and P₄ in
the circulation were consistent with single ovulations. The
mean interval from the last GnRH-antagonist treatment to the
subsequent preovulatory E₂ peaks and LH surges was
approximately 14 days among the six monkeys who resumed
ovulatory menstrual cycles during the study interval. The
remaining two monkeys did not resume regular menstrual
cycles until nearly a month after completion of this study.
Pituitary refractoriness was exhibited (three of three monkeys) to the $E_2$ benzoate challenge test given on cycle day 6 during the daily GnRH-antagonist regimen spanning days 2 through 9 (study II). There was an initial negative feedback effects of exogenous estrogen on LH and FSH secretion, the absence of familiar preovulatory-like LH surges, and finally, the resumption of apparent ovulation about 3 weeks later.

In contrast, administered GnRH (as a 50-ug bolus dose given on cycle day 6) during the daily GnRH-antagonist regimen elicited unambiguous LH responses (four of four monkeys) of seemingly normal strength and duration. All responses exceeded 4-fold elevations and did not differ significantly from the responses of control monkeys given the same iv bolus dose of GnRH.

In six of the eight monkeys, ovulation resumed promptly after the last GnRH-antagonist administration; the first posttreatment estradiol peak occurred $14.3 \pm 3.8$ (±SEM) days after the final dose. The interval for resumption of ovulatory menstrual cycles after the final GnRH injection is summarized in Table I.

These tests established that GnRH-antagonists achieve female contraception when given using an intermittent (weekly) treatment regimen.

Monkeys previously having regular ovarian/menstrual cycles had no ovulation (32 of 32 treatment intervals) when the GnRH-antagonist was administered once weekly. After cessation of GnRH-antagonist therapy, ovulation resumed promptly in 6 of 8 monkeys. That some monkeys regained ovulatory inhibition as soon as 8-10 days after the final dose of GnRH-antagonist suggests that a weekly treatment interval may be near the upper limit for maintenance of ovulation inhibition at the dose tested here. These observations correlate well with earlier experience in monkeys (Goodman AL & Hodgen GD, Endocrinology 19779 104:1304; diZerega GS et al., J. Clin Endocrinol Metab 1980,
50:1046) and women (Nilsson L. et al., Fertil Steril 1982, 37:30) undergoing surgical ablation of the putative dominant follicle, in whom destruction of the largest visible follicle delayed ovulation by causing reinitiation of a new follicular phase. It is possible that each weekly dose of GnRH-antagonist caused atresia of the newly selected dominant follicle. The intermittent elevations of E₂ in serum may reflect its secretion by newly recruited follicles in the next growing cohort. Goodman & Hodgen, supra.

Although two monkeys given the GnRH-antagonist had longer delays before resuming regular menstrual cycles, their suppressed status ended less than 60 days after the cessation of treatment.

In regard to the mechanism(s) by which the intermittent (weekly) GnRH-antagonist regimen prevented ovulation, the data from study II indicate that the estrogen-positive feedback for the LH surge was negated. During the normal follicular phase, E₂ benzoate will induce midcycle-like LH surges within 48 h; the GnRH-antagonist clearly negated this estrogen-positive feedback. Williams RF et al. J. Clin. Endocrinol Metab 49:422; Hodgen GD Fertil Steril 1982 38:281. In contrast, the response to GnRH was normal. It is possible that the iv bolus dose of GnRH displaced the GnRH-antagonist from gonadotroph receptors, so that the exogenous GnRH elicited normal LH secretory responses. An alternative interpretation is that the dose of GnRH-antagonist was submaximal, since previously we achieved a "medical hypophysectomy"-like status with only 50% of the dose used in these experiments. Kenigsberg et al., supra (1984).

**Example 1**

A kit is prepared which contains four unit doses adapted for self-administration of the GnRH-antagonist (Ac-p-ClPhe¹, pClDphe², DTrp³, DArg⁶, DAla¹₀)-NET.GnRH; 11 conventional tablets adapted for oral ingestion, each containing 0.1-0.5 mg. Of the progestin norgestrel; a 4-
week chart on which the first day of menses can be oriented with the day of the week on which it occurs as day 1 of the 4 week chart and administration instructions. Days 2, 9, 16, and 23 on the chart (which are aligned with the same day of the week) bear indica informing the female to self-administer, in the manner and by the route indicated in the instructions, a unit dosage of the GnRH-antagonist on those days. Days 15 through 25, inclusive, bear indica informing the female to ingest one of the progestin tablets on each of those days. The instructions inform the female to obtain a second kit for the next menstrual cycle.

A gonadal female who self-administers the GnRH-antagonist and progestin according to the above-described schedule achieves contraception while maintaining an otherwise normal menstrual cycle of about 30 days.

a) In one form, the GnRH-antagonist is in admixture with a dry diluent, e.g., dextrose, in the form of a dry micronized powder which is dispensed from a conventional pressurized (under CO₂) container which is springload mounted in a conventional inhalator which dispenses about 10 mg of the GnRH-antagonist when the container is depressed into the inhalator. The instructions instruct the female to activate the inhalator twice while inhaling, on the same day of each week as indicated by the chart.

b) In another form otherwise corresponding to a), the inhalator dispenses about 75 mg of the GnRH-antagonist each time the container is squeezed.

c) In another form otherwise corresponding to a), the GnRH-antagonist is present in four conventional foil wrapped anhydrous suppositories, each containing 150 mg. thereof, and the instructions instruct the female to insert one of the suppositories into the rectum on each day of the week indicated on the chart.

d) In another form otherwise corresponding to a), the GnRH-antagonist is present in four conventional dry, foil wrapped saliva dissolvable lozenges, each containing 150 mg
thereof, and the instructions instruct the female to
dissolve a lozenge under the tongue on the same day of each
week as indicated by the chart.

e) In variations of each of the kits described
hereinabove, the norgestrel in the tablets is replaced by 1
mg of norethindrone, 5 mg of norethynodrel, 1 mg of
ethynodiol diacetate or 0.2 mg of levonorgestrel.

f) In other variations of each of the kits described
hereinabove, the GnRH-antagonist therein is replaced by a
GnRH-antagonist of U.S. 4,409,208; 4,444,759; 4,547,370;
4,565,804; 4,569,927; or 4,619,914.

The preceding examples can be repeated with similar
success by substituting the generically or specifically
described reactants and/or operating conditions of this
invention for those used in the preceding examples.

From the foregoing description, one skilled in the art
can easily ascertain the essential characteristics of this
invention, and without departing from the spirit and scope
thereof, can make various changes and modifications of the
invention to adapt it to various usages and conditions.
WHAT IS CLAIMED IS:

1. A method of providing contraception for and regulation of the menstrual cycle of a gonadal female mammal, which comprises a) administering thereto periodically at predetermined intervals longer than daily throughout her menstrual cycle an amount of a GnRH-antagonist effective to block folliculogenesis but less than the amount thereof required to block hormonogenesis; b) also administering thereto prior to the end of that cycle an amount of a progestin effective to produce a secretory endometrium; and c) then terminating the progestin administration, thereby inducing menses.

2. The method of claim 1, wherein the GnRH-antagonist is administered once every 5–9 days.

3. The method of claim 1, wherein the GnRH-antagonist is administered about once every 7 days.

4. The method of claim 1, wherein the GnRH-antagonist is administered initially on day 1 or 2 of menses.

5. The method of claim 1, wherein the GnRH-antagonist is administered sublingually.

6. The method of claim 1, wherein the GnRH-antagonist is administered intranasally.

7. The method of claim 1, wherein the GnRH-antagonist is administered by inhalation as an aerosol.

8. The method of claim 1, wherein the GnRH-antagonist is administered vaginally or rectally.

9. The method of claim 1, wherein the GnRH-antagonist is (Ac-pClPhe₁,p-ClPhe²,DTrp³,DArg⁶,DAla¹⁰)-NET GnRH.
10. The method of claim 1, wherein the progestin is orally active and is administered orally.

11. The method of claim 1, wherein the progestin is administered daily during at least the third week of the menstrual cycle.

12. The method of claim 1, wherein the progestin is administered from about day 15 to about day 25 of the menstrual cycle.

13. The method of claim 1, wherein the progestin is nortindrone, norethynodrel, ethynodiol diacetate, ethynodiol diacetate, norgestrel or levo-norgestrel.

14. The method of claim 1, wherein the GnRH-antagonist is administered about once every 7 days of the menstrual cycle, initially on day 1 or 2 of menses.

15. The method of claim 14 wherein the GnPH antagonist is \( \text{Ac-pClPhe}^1, \text{p-ClPhe}^2, \text{DTrp}^3, \text{DArg}^6, \text{DAla}^{10} \)-NET GnRH.

16. The method of claim 14 wherein the GnPH antagonist progestin is nortindrone, norethynodrel, ethynodiol diacetate, ethynodiol diacetate, norgestrel or levo-norgestrel and is administered daily during at least the third week of the menstrual cycle.

17. The method of claim 16, wherein the GnPH antagonist is \( \text{Ac-pClPhe}^1, \text{p-ClPhe}^2, \text{DTrp}^3, \text{DArg}^6, \text{DAla}^{10} \)-NET GnRH.
# INTERNATIONAL SEARCH REPORT

**International Application No.** PCT/US88/00711

## I. CLASSIFICATION OF SUBJECT MATTER

According to International Patent Classification (IPC) or to both National Classification and IPC:
- **INT. Cl. (4):** C07K 7/06; A61K 37/38
- **U.S. Cl.:** 530/328; 514/12

## II. FIELDS SEARCHED

<table>
<thead>
<tr>
<th>Classification System</th>
<th>Classification Symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S.</td>
<td>530/328, 324, 398; 514/843, 12</td>
</tr>
</tbody>
</table>

Documentation Search other than Minimum Documentation to the extent that such Documents are Included in the Fields Searched:

## III. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of Document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to Claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>US, A, 4,143,136 (DE JAGE) 06 March 1979 See the entire document.</td>
<td>1-8, 10-14, 16</td>
</tr>
<tr>
<td>A</td>
<td>US, A, 4,481,190 (NESTOR) 06 November 1984 See the entire document.</td>
<td>5, 6, 8, 9, 15, 17</td>
</tr>
<tr>
<td>A</td>
<td>US, A, 4,292,315 (VORYS) 29 September 1981 See the entire document.</td>
<td>1-17</td>
</tr>
<tr>
<td>A</td>
<td>US, A, 4,409,208 (RIVIER) 11 October 1983 See the entire document.</td>
<td>5, 6, 8, 9, 15, 17</td>
</tr>
</tbody>
</table>

* Special categories of cited documents:
  - “X” document defining the general state of the art which is not considered to be of particular relevance
  - “E” earlier document but published on or after the international filing date
  - “L” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  - “O” document referring to an oral disclosure, use, exhibition or other means
  - “P” document published prior to the international filing date but later than the priority date claimed

“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

“X” document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

“Y” document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

“Z” document member of the same patent family

## IV. CERTIFICATION

**Date of the Actual Completion of the International Search:** 03 JUNE 1988

**Date of Mailing of this International Search Report:** 18 JUL 1988

**International Searching Authority:** ISA/US

**Signature of Authorized Officer:**

T. D. Wessendorf

Form PCT/ISA/210 (second sheet) (Rev.11-87)
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of Document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to Claim No</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>DE, A, 2,253,916 (BIOLOGICAL CONCEPTS INC.) O6 September 1973 See the abstract.</td>
<td>13 and 16</td>
</tr>
<tr>
<td>Category</td>
<td>Citation of Document</td>
<td>Relevant to Claim No</td>
</tr>
<tr>
<td>----------</td>
<td>----------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>A</td>
<td>US, A, 4,569,927 (RIVIER)</td>
<td>5,6,8, 9,15,17</td>
</tr>
<tr>
<td></td>
<td>11 February 1986 See the entire document.</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>US, A, 4,547,370 (ROESKE)</td>
<td>5,6,8, 9,15,17</td>
</tr>
<tr>
<td></td>
<td>15 October 1985 See the entire document.</td>
<td></td>
</tr>
</tbody>
</table>

V. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. Claim numbers , because they relate to subject matter not required to be searched by this Authority, namely:

2. Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out , specifically:

3. Claim numbers , because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This International Searching Authority found multiple inventions in this international application as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest:
- The additional search fees were accompanied by applicant's protest.
- No protest accompanied the payment of additional search fees.