Recovery of corpus luteum function after prolonged deprivation from gonadotrophin stimulation

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Three women with hypogonadotrophic hypogonadism, all desiring pregnancy, participated in a prospective open study attempting to assess the ability of the human corpus luteum to recover after 7 days of deprivation from gonadotrophin stimulation. Follicular growth was induced by gonadotrophins. An endogenous luteinizing hormone (LH) surge was induced by the s.c. injection of a gonadotrophin-releasing hormone agonist. For luteal support, 10 mg/day oral medroxyprogesterone acetate were given for 7 days, after which a single i.m. injection of human chorionic gonadotrophin (HCG) was administered. Monitoring during the follicular phase consisted of daily measurements of serum oestradiol, LH and follicle stimulating hormone (FSH) concentrations, and of follicular growth by transvaginal ultrasonography. During the luteal phase, monitoring consisted of measurements of serum concentrations of LH, FSH, oestradiol, progesterone, 17-hydroxyprogesterone and β-HCG. Ovulation and luteinization occurred in two patients, demonstrated by transient marked increases in serum progesterone and 17-hydroxyprogesterone concentrations which decreased to basal pre-ovulatory values and increased again following the administration of HCG 7 days later. In the third patient, ovulation and luteinization did not occur, and the subsequent administration of HCG did not result in an increase in progesterone concentration. Of the two patients who ovulated, one conceived and the second had a luteal phase of 15 days duration. Our preliminary results suggest that the human corpus luteum can be ‘rescued’ and can function normally after 7 days of deprivation from gonadotrophin stimulation in patients with hypogonadotrophic hypogonadism.

Key words: corpus luteum/GnRH agonist/gonadotrophins/luteal support/ovulation induction

Introduction

The formation and function of the corpus luteum is an integral part of the reproductive cycle and an important factor in early pregnancy support. The dependence of corpus luteum function on gonadotrophin stimulation has been repeatedly documented in both animal (Fraser et al., 1985, 1986, 1987; Collins et al., 1986) and human studies (Mais et al., 1986; McLachlan et al., 1989). However, our understanding of this particular aspect of the cycle is as yet fragmentary and incomplete. It has been shown that the withdrawal of luteinizing hormone (LH) support from the primate corpus luteum for at least 3 days induces luteolysis (Hutchison and Zeleznik, 1984). However, corpus luteum function can be rescued if gonadotrophin therapy is reinitiated within 3 days, suggesting that corpus luteum viability can be preserved without LH support for at least 72 h (Hutchison and Zeleznik, 1985). A longer period of gonadotrophin deprivation, induced by the administration of a gonadotrophin-releasing hormone (GnRH) antagonist, has been found to be associated with permanent damage to the corpus luteum, which was unable to recover when stimulated by physiological low doses of human chorionic gonadotrophin (HCG; Dubourdieu et al., 1991). It has been suggested that whereas progesterone secretion is dependent on pituitary LH, the maintenance of the functional capacity of the corpus luteum is independent of gonadotrophin support (Hutchison and Zeleznik, 1985).

In most studies on suppression and stimulation of the corpus luteum, two basic models have been used. Subhuman primates were rendered hypogonadotrophic by hypothalamic lesions and treated by a GnRH pump (Hutchison and Zeleznik, 1984, 1985); alternatively, GnRH antagonists were administered to abolish pituitary gonadotrophin secretion in both animals (Fraser et al., 1985, 1986, 1987; Collins et al., 1986) and women (Mais et al., 1986; McLachlan et al., 1989; Dubourdieu et al., 1991). In neither of the above models was an attempt made to achieve pregnancy, the ultimate proof of normal corpus luteum function.

In the near future, new genetically engineered gonadotrophin preparations are expected to replace urinary gonadotrophins for the induction of ovulation. Recombinant forms of both follicle stimulating hormone (r-hFSH) and LH (r-hLH) are now available and being explored clinically, and pregnancies have already been reported following their use (Germont et al., 1992; Hull et al., 1994). The new understanding of the actions of LH (Shoham et al., 1993) suggests that small amounts of LH are required for steroidogenesis to synergize with FSH in the follicular phase so as to stimulate full functional maturation of the follicles. A single sufficiently large dose of r-hLH could closely mimic the pre-ovulatory LH surge, which has traditionally been induced by HCG. However, the use of r-hLH, with its short half-life, as a substitute for HCG in triggering ovulation raises some questions regarding the luteal phase. Exogenous HCG maintains corpus luteum progesterone and oestradiol secretion for 7–10 days, up to the moment when HCG secreted by the implanting embryo takes

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over the corpus luteum maintenance function. In an environment with significantly low LH and FSH because of pituitary down-regulation by GnRH agonist (GnRH-a), if ovulation is induced by r-hLH and luteal support is provided by exogenous progesterone, would HCG secreted by the ensuing conceptus be sufficient to rescue the corpus luteum after 7 days of gonadotrophin deprivation? In other words, under these conditions would the corpus luteum be able to maintain its function for 10 days following its formation, during which it is deprived of significant LH or FSH stimulation? The answer to this question would provide invaluable information regarding the need for luteal support in cycles where recombinant gonadotrophins will be used in addition to GnRH-a.

We have developed a novel approach to study the ability of the corpus luteum to recover from prolonged gonadotrophin deprivation. Women with isolated hypogonadotropic hypogonadism, who lack endogenous gonadotrophin secretion, were treated with human menopausal gonadotrophin (HMG) for the induction of follicular growth and steroidogenesis. Because r-hLH was not yet available for ovulation induction, we were looking for a model in which ovulation could be induced by endogenous LH (mimicking r-hLH), while the patient would remain deficient of LH and FSH during the luteal phase. We used a single s.c. injection of a GnRH-a for that purpose. The luteal phase was supported by medroxyprogesterone acetate (MPA). Because MPA, in the doses given in our study, does not cross-react with our progesterone assay, it provides luteal phase support and also allows the monitoring of endogenous progesterone secretion. On day 7 of MPA treatment, corpus luteum rescue was attempted by the administration of HCG.

Following treatment, in addition to measuring steroid hormone concentrations, pregnancy was regarded as the best end-point to testify to normal corpus luteum function. Here we report our preliminary experience with three such patients.

Materials and methods

Patient characteristics

Three women (aged 22, 23 and 41 years) with hypogonadotropic hypogonadism volunteered to participate in our study. All suffered from isolated hypogonadotropic hypogonadism, with delayed puberty, amenorrhea, low serum concentrations of gonadotrophins (FSH and LH <1.0 IU/l) and oestradiol <73 pmol/l and a negative progesterone challenge test. Patient characteristics, including baseline hormonal status, are given in Table I. Ovarian function was proved to be normal in all three women during previous gonadotrophin treatment cycles when pregnancies were successfully induced. All three women desired pregnancy, and all refrained from hormone replacement therapy for at least 30 days before entering the study. All women were in good health without chronic illness. They gave their informed consent to participate in the study, which was approved by the Ethical Committee of the hospital (Kaplan Medical Center, Rehovot, Israel) according to the Helsinki Declaration.

Stimulation protocol

For the stimulation of follicular growth (Figure 1), 150 IU LH and FSH (HMG; Pergonal; Teva, Petah-Tikva, Israel) were administered i.m. on a daily basis. The dose was increased every 7 days as necessary. The monitoring of follicular growth consisted of measurements of serum oestradiol concentrations and follicular diameters by transvaginal ultrasonography. Serum concentrations of LH and FSH were also measured during the follicular phase. For triggering final follicular maturation and ovulation, 200 µg buserelin acetate (Suprefact; Hoechst AG, Frankfurt, Germany) were administered by a single s.c. injection when at least one follicle ≥18 mm was present and the endometrial thickness was ≥8 mm.

Luteal phase support and monitoring

Luteal phase support was given in the form of oral MPA (Aragest; Dexon, Haifa, Israel) at a dose of 10 mg/day, commencing on day 3 following buserelin administration, and continuing for 7 days. On the same day that MPA was discontinued, a single i.m. injection of 5000 IU HCG (Chorigon; Teva) was given.

Monitoring of the patients consisted of measurements of serum concentrations of LH, FSH, oestradiol, progesterone, 17-hydroxy-progesterone and the β-subunit of HCG (β-HCG) throughout the luteal phase.

The study blood sampling regimen was not designed to document the GnRH-a-related LH surge. Serum FSH concentrations were elevated as a result of HMG administration. They returned to basal levels within 1 week.

Hormone assays

Serum oestradiol concentrations were measured by a standard radioimmunoassay technique (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA, USA). This was a direct radioimmunoassay (i.e. without extraction) with a solid-phase separation. The sensitivity of the assay was 20 pmol/l (5.5 pg/ml), and the intra- and interassay coefficients of variation (CV) were 4.0–7.0 and 4.0–8.0% respectively.

Serum FSH and LH concentrations were measured by an enzyme-linked immunosorbent assay (ELISA), a one-step sandwich assay

<table>
<thead>
<tr>
<th>Table I. Patient characteristics and baseline hormonal status</th>
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<tr>
<td>Patient</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>BMI (weight/height²)</td>
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<tr>
<td>Serum prolactin (mIU/l)</td>
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<td>Serum testosterone (nmol/l)</td>
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<td>Serum DHEA-S (µmol/l)</td>
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BMI = body mass index; DHEA-S = dehydroepiandrosterone sulphate.
Serum progesterone concentrations were measured by a radioimmunoassay (Coat-A-Count; Diagnostic Products Corporation). The sensitivity was 0.3 nmol/l and the intra- and interassay CV were 2.6% and 5.0% respectively. Serum 17-hydroxyprogesterone concentrations were measured by a radioimmunoassay using a single-antibody direct assay (Coat-A-Count; Diagnostic Products Corporation). The sensitivity was 0.3 nmol/l and the intra- and interassay CV were both <10.0%.

Serum β-HCG concentrations were assessed by an immunoradiometric assay magnetic solid phase (Maia Clione; Serono Diagnostic, Geneva, Switzerland), using a reference standard (1st IRP/3rd IS 75/537). The sensitivity was 5 IU/l. The intra- and interassay CV were 4.1 and 2.7% respectively. Serum testosterone concentrations were measured by a radioimmunoassay (Coat-A-Count; Diagnostic Products Corporation) with a detection rate of 0.27 nmol/l. The intra- and interassay CV were <9.0 and 13.0% respectively.

Serum luteinizing hormone (LH) concentrations were measured by a radioimmunoassay (Coat-A-Count; Diagnostic Products Corporation) using the International Reference Preparation Standards (IRP) 75/505 and 78/549 and 68/40 respectively. The sensitivity was 1 IU/l for FSH and 0.5 IU/l for LH. The intra-assay CV were 2.5–5.0% for both hormones; interassay CV were 1.0 and 2.0% for FSH and LH respectively.

Serum progesterone concentrations were measured by a radioimmunoassay using a single-antibody direct assay (Coat-A-Count; Diagnostic Products Corporation). The sensitivity was 0.3 nmol/l and the intra- and interassay CV were 5.0–8.0 and 6.0–10.0% respectively.

Serum dehydroepiandrosterone sulphate concentrations were measured by a radioimmunoassay using a single-antibody direct assay (Coat-A-Count; Diagnostic Products Corporation). The sensitivity was 0.03 μmol/l and the intra- and interassay CV were <5.3 and <7.0% respectively.

Results

Clinical course

All patients responded favourably to HMG treatment, as demonstrated by follicular growth and an increase in serum oestradiol concentrations. Patient 1 conceived during the treatment cycle; unfortunately, this was a tubal pregnancy. This patient had known severe tubal disease on the affected side from previous surgery. Patient 2 had a luteal phase of 15 days duration with no spotting or irregular bleeding until menstruation. In patient 3, ovulation did not occur, and menstruation occurred 13 days following the administration of buserelin.

Serum progesterone, 17-hydroxyprogesterone, LH and FSH concentrations

Following the s.c. injection of 200 μg buserelin on day 0, luteinization had occurred in two patients, as shown by clear-cut increases in serum progesterone and 17-hydroxyprogesterone concentrations, with peak progesterone concentrations of 11.3 (on day 3) and 28.0 nmol/l (on day 1) in patients 1 and 2 respectively (Figures 2 and 3). Serum LH concentrations fell to baseline levels 24 h following GnRH-a administration. In patient 2, the serum LH concentration was measured 4 h following GnRH-a administration and was found to be 70 IU/l. Serum concentrations of progesterone and 17-hydroxyprogesterone then decreased to basal pre-ovulatory concentrations in both patients, and increased again 9 days after ovulation following the administration of HCG. In patient 1, this increase was sustained because the patient conceived. In the second patient the increase lasted 5 days, and was then followed by a rapid decline until menstruation on day 15. In the third patient, no change in serum LH, progesterone and 17-hydroxyprogesterone occurred following the administration of GnRH-a.

Serum β-HCG concentrations

In patient 1, the administration of HCG resulted in a serum concentration of 80 IU/l on the next morning, with a gradual decline during the next 6 days to 12 IU/l (Figure 2). An increase in β-HCG concentration reappeared 1 week after the administration of HCG, this time representing trophoblastic HCG. Patients 2 and 3 exhibited a similar course, with peak β-HCG concentrations of 86 and 520 IU/l following the administration of HCG, but because pregnancy did not occur the β-HCG concentrations declined gradually until menstruation (Figure 3).

Serum oestradiol concentrations

Serum oestradiol concentrations continued to rise on the day following the administration of buserelin in all patients, and then declined for the next 7 days during the administration of MPA (Figures 2 and 3), reaching nadirs of 80, 110 and 100 pg/ml in patients 1–3 respectively. Following the administration of HCG, serum oestradiol concentrations increased in all three patients. This increase was sustained in patient 1, who conceived, but lasted for only 5 days in patients 2 and 3, and then gradually decreased until menstruation.

Discussion

The results of our study emphasize for the first time that the human corpus luteum is capable of recovering normal function after 7 days of deprivation from LH stimulation. In the absence of any significant LH stimulation, luteal cells were found to be able to retain both their enzymatic steroidogenic properties and their capacity to respond to exogenous HCG, thus allowing the establishment of pregnancy and 'rescue' by trophoblastic HCG. One may reason that the rise in progesterone and 17-hydroxyprogesterone reflects a second ovulation rather than reactivation of the corpus luteum. However, strong arguments prevail against a second ovulation. First, it is well known that the mid-cycle LH peak, whether spontaneous or induced by exogenous HCG, triggers ovulation in the mature Graafian follicles; at the same time the less mature and immature follicles of that cohort become atretic or degenerate and cannot be reactivated later on (Friedrich et al., 1975). Of greater importance is the fact that the 'implantation window' of the endometrium is relatively narrow, and by the time of HCG administration in our study the human uterus has already entered a state of refractoriness where no pregnancy could have been established (Psychopoulos, 1993). The role of pituitary gonadotrophins in corpus luteum function during the luteal phase of the menstrual cycle has
been the subject of controversy in recent years. Central to this issue is whether LH secretion is required for progesterone production throughout the luteal phase. Hutchison and Zeleznik (1984) studied rhesus monkeys in which gonadotrophin secretion was abolished by hypothalamic radiofrequency lesions. Endogenous gonadotrophin secretion was restored by the pulsatile infusion of GnRH. They demonstrated that withdrawal of the circulating gonadotrophins during the early or mid-luteal phase led to a rapid fall in plasma progesterone concentrations followed by premature menstruation. In an extension of their original study (Hutchison and Zeleznik, 1985), they made the remarkable observation that the corpus luteum was capable of recovering from a temporary 3 day withdrawal of gonadotrophin support which had resulted in functional luteolysis; the degree to which luteal function was restored varied with the age of the corpus luteum. They suggested that, whereas progesterone secretion is dependent on pituitary LH, maintenance of the functional capability of steroidogenic enzymes is independent of gonadotrophic support.

Physiological studies on the effects of GnRH antagonists in both women and animal models have indicated that they rapidly and effectively suppress pituitary and ovarian function (Karten and Rivier, 1986). Early studies in which a GnRH antagonist was administered to rhesus monkeys throughout the
luteal phase (Balmaceda et al., 1983), or where rhesus monkeys had been hypophysectomized (Asch et al., 1982), resulted in apparently normal luteal progesterone secretion, suggesting that the corpus luteum does not require further gonadotrophin support after the pre-ovulatory LH surge. However, subsequent studies in stump-tailed macaques showed that a single injection of a more potent GnRH antagonist (Detrelitix) suppressed bioactive LH and progesterone during all stages of the luteal phase (Fraser et al., 1986). When the antagonist was administered as a single dose in the early to mid-luteal phase, significant, although transient, decreases in progesterone concentrations occurred (Fraser et al., 1986).

Of major importance is the study by Dubourdieu et al. (1991), who tried to assess the potential rescue of corpus luteum function by HCG after treatment with the GnRH antagonist Nal-Glu by using different doses and various timings of HCG administration. They confirmed previous observations made in both primates and women (Fraser et al., 1985, 1986, 1987; Collins et al., 1986; Mais et al., 1986; McLachlan et al., 1989) by showing that the suppression of LH support of the corpus luteum for at least 3 days induces luteolysis. After 72 h of suppression, corpus luteum function could not be restored by the administration of small doses of exogenous HCG, but could be restored, at least partially, by pharmacological doses (1500 and 5000 IU) which mimic circulating concentrations of HCG seen in early pregnancy. They concluded that a 3 day period of LH suppression is critical for viability of the corpus luteum. Their data are in agreement with those of Fraser et al. (1987), who found that, following the 3 day administration of a GnRH antagonist in the early luteal phase of the stump-tailed monkey, physiological doses of HCG, given from day 7 (thus mimicking early pregnancy), caused an only partial and small progesterone rise, ~20% of that observed in control monkeys receiving HCG.

The discrepancies in results from studies in both humans and monkeys are probably related to differences and limitations in the methodologies. Antagonists analogues of GnRH are different in potency, leading to variable degrees and durations of pituitary LH suppression. Monkeys with hypothalamic lesions, produced by either radiation or surgical methods, are not by these methods depleted of all sources of LH activity (Baker et al., 1977; Lobo Antunes et al., 1979; Asch et al., 1982). In addition, a direct effect of GnRH and its agonists or antagonists on the ovary cannot be excluded, and this subject has also been under debate (Clayton and Huhtaniemi, 1982; Bramley et al., 1985; Latouche et al., 1989). Moreover, most studies have focused on pituitary LH suppression because it is considered to be the luteinizing gonadotrophin. Interestingly, recombinant FSH has been shown to promote ovulation and luteinization in rats (Galway et al., 1990), suggesting that differences in FSH suppression may also contribute to these discrepancies.

The conflicting results of the above studies have led us to search for a better and more clinically relevant model to study human corpus luteum function in preparation for the replacement of HCG by recombinant human LH to induce the pre-ovulatory surge. Women with hypogonadotrophic amenorrhoea, who on the one hand lack endogenous gonadotrophin secretion and on the other, undergo FSH accumulation in serum when stimulated with HMG, seem well suited for this purpose. Of major importance is the fact that, in addition to the hormonal assays traditionally used, conception is attempted in our model, which can be regarded as the best proof of normal luteal function. Polson et al. (1987) have already tried to assess the recovery of luteal function after a 2 day interruption of gonadotrophin support in a woman with hypogonadotrophic hypogonadism. By using a pulsatile infusion of GnRH, they were able to show that stopping the pump 3 days after ovulation caused gonadotrophin and progesterone concentrations to fall rapidly to very low values. Conversely, restarting the infusion 48 h later caused progesterone concentrations to return to normal mid-luteal values. They concluded that normal progesterone secretion by the corpus luteum can be restored after the temporary withdrawal of gonadotrophin support. Our data are in agreement with their findings.

Since r-hLH is not yet available for clinical use, in our model we used a single injection of a GnRH-a to trigger an endogenous LH surge, lasting for <36 h. This contrasts with the long circulatory half-life of HCG, which allows serum HCG concentrations to remain significant for up to 10 days after administration (Saal et al., 1991). This method has already been reported as safe and successful for triggering ovulation and achieving pregnancy, and it concomitantly reduces the risk for ovarian hyperstimulation syndrome (Itskovitz et al., 1991). In our study, although the blood sampling schedule did not allow the identification of the LH surge peak value, ovulation was achieved in two of our patients after the administration of buserelin, as shown by sharp rises in serum progesterone and 17-hydroxyprogesterone concentrations, followed by a rapid decline to basal values (Figures 2 and 3). To maintain endometrial integrity and prevent its shedding, we administered oral MPA for 7 days, starting 48 h after the buserelin injection. MPA, a pregnane (C21) derivative of progesterone, was chosen because at the dosage used its degradation produces insufficient progesterone to cross-react in the assay. A recent study (Raman-Wilms et al., 1995) has indicated that there is no association between first trimester exposure to sex hormones and fetal genital effects. During the week of MPA treatment, serum concentrations of LH and FSH declined to their low basal concentrations, reflecting the hypogonadotrophic state of our patients. Serum concentrations of oestradiol, progesterone and 17-hydroxyprogesterone gradually declined to very low values, reflecting the inactivation and lack of secretion by the corpus luteum. When 5000 IU HCG were administered after 1 week of MPA therapy, sharp increases were observed in the serum concentrations of all steroid hormones, which reached physiological and supra-physiological values within 3 days. Concentrations of LH and FSH remained low and unchanged. Clearly, these observations imply that luteolysis has not occurred, and there appears to be no effect on the life span of the corpus luteum. This was demonstrated by a resumption of normal secretion of steroid hormones, normal cycle length and the establishment of pregnancy. Therefore it is suggested that maintenance of the steroidogenic properties of the human corpus luteum is largely...
independent of a significant LH secretion. A similar observation in primates was made by Hutchison and Zeleznik (1985).

The development of the corpus luteum involves the differentiation of heterogeneous groups of specialized cells that act as a complex endocrine organ (Jones, 1991). Two major populations of cell are observed in the corpus luteum, comprising large luteal cells (LLC) and small luteal cells (SLC). These two types of cell differ in size, structure and function and probably originate from different sources. LLC presumably arise from granulosa cells and produce higher concentrations of progesterone, whereas SLC are considered to be derived from theca cells, produce less progesterone and are more responsive to stimulation by LH or HCG. In the early and mid-luteal phases, the LLC are the major source of basal progesterone production. Receptors for LH are induced by FSH and oestradiol, and these LLC receptors are distinguished from the LH receptors on SLC in that, once occupied by LH, they continue to transduce signals over a 10 day span, after which the LLC no longer secrete progesterone. These LH receptors are unique in that they do not internalize and, after the LH surge, are not responsive to either an LH pulse or HCG stimulation (Jones, 1991). Therefore, basal progesterone is secreted by the LLC at a constant rate, unrelated to a LH pulse. On the other hand, the SLC secrete both oestradiol and progesterone in response to LH pulses, but their contribution to progesterone production during the early luteal phase is minimal. Only after luteal day 10 do they become the major source of progesterone in response to LH pulses, exogenous HCG (Retamales et al., 1994) or the ‘rescue’ of the corpus luteum by trophoblastic HCG (Alilo and Hansel, 1984). Furthermore, SLC increase in number from the mid-luteal phase, and this subpopulation of cells also displays a preferentially increased binding of HCG starting from that period and leading to increased progesterone production in the presence of HCG (O’Hara et al., 1987; Retamales et al., 1994).

The results from our study can be explained in terms of the above observations (Alilo and Hansel, 1984; Jones, 1991). A pre-ovulatory LH surge of sufficient magnitude, whether endogenous or surrogate, may cause luteinization and occupy enough LH receptors on LLC such that luteolysis does not occur. The corpus luteum can thus preserve its potential secretory function, even though endogenous secretion subsides in the absence of further gonadotrophin stimulation. SLC maintain their capacity to respond to subsequent stimulation by HCG even after 1 week of gonadotrophin deprivation. Therefore, in patients undergoing ovarian stimulation in protocols where GnRH-a are used, if the final stage of ovulation is triggered by r-hLH, then the luteal phase may be supported for 1 week by exogenous progesterone; the corpus luteum maintains its ability to be rescued by HCG, either endogenous or exogenous, even after prolonged deprivation from gonadotrophin stimulation. Further studies need to be conducted to determine the most efficacious regimens for the pre-ovulatory LH surge and for luteal support. It is suggested from our study that a single application of a short-acting GnRH-a (or perhaps r-hLH), followed by progestin supplementation, may be sufficient.

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