GYNECOLOGY

The Optimum Time for Exogenous Human Chorionic Gonadotropin to Rescue the Corpus Luteum

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Purpose: Our purpose was to study the optimum time to administer exogenous human chorionic gonadotropin (hCG) to rescue the human corpus luteum during the luteal phase of normal menstrual cycles.

Methods: Groups of normally cycling women were given 4-day regimes of exogenous hCG by daily injection beginning 4 (Group A), 8 (Group B), and 12 (Group C) days after the midcycle luteinizing hormone surge. The hCG regime used was designed to mimic hCG levels following a spontaneous implantation. All subjects acted as their own controls in a preceding normal menstrual cycle.

Results: Group A subjects exhibited patterns and levels of salivary progesterone concentration similar to those seen in the control cycles throughout the normal luteal phase. In contrast, subjects in both Group B and Group C demonstrated a rapid and sustained increase in progesterone production following the hCG injections. Furthermore, subjects in Group B achieved the highest mean peak progesterone concentrations and the total amount of salivary progesterone secreted was significantly higher than in the control cycles ($P < 0.05$). Although the mean luteal-phase length was greatest in Group C, the response of the corpus luteum was suboptimal, with a delayed rise in salivary progesterone.

Conclusions: These data show that the qualitative and quantitative response of corpus luteum to an early pregnancy-type hCG signal is maximal around the midluteal phase, coincident with the time of implantation.

KEY WORDS: human chorionic gonadotropin; human corpus luteum; progesterone.

INTRODUCTION

Exogenous human chorionic gonadotropin (hCG) is commonly administered in an attempt to increase the pregnancy rate following in vitro fertilization (IVF) where it is known or suspected that the luteal phase will be inadequate (1). hCG is also given to women with a history of recurrent pregnancy loss potentially to stimulate or prolong corpus luteum steroidogenesis (2). In spite of its widespread clinical application, the optimal dosage timing and frequency of hCG administration remain unexamined. Therefore, hCG treatment schedules are empirical and can vary widely. In the human the pattern of progesterone secretion that accompanies a single injection of exogenous hCG is known to vary with the postovulatory age of the corpus luteum (3). Similarly, a 5-day incremental course of exogenous hCG in animal models yielded different patterns of progesterone secretion in the early and midluteal phases (4). Evidence that even subtle changes in the steroid milieu may influence the rate and extent of endometrial development (5) and that implantation can succeed only within a narrow "window of endometrial receptivity" (6) suggests that such hCG-induced alterations in the pattern of steroidogenesis may have major clinical implications.

Traditionally, progesterone levels have been determined in serum or plasma. The measurement of progesterone in saliva offers an alternative method for monitoring the reproductive status of the human female. The obvious advantage of salivary progesterone over venous sampling is that the procedure is noninvasive, which allows easier repeated daily progesterone measurement. Furthermore, sample collection and storage can be performed by the patient at

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home, which is administratively and financially easier for a busy clinic (7).

In this study, hCG injections mimicking an early pregnancy signal were administered and the response of the corpus luteum was monitored. A group of healthy ovulatory women was assigned to receive exogenous hCG at precisely defined times during their luteal phase and the resulting salivary progesterone secretion was compared with the profiles observed in matched but untreated cycles.

MATERIALS AND METHODS

Study Subjects. Fourteen subjects aged 24–36 years were recruited and each gave informed consent for the study. All subjects demonstrated regular spontaneous menstrual cycles and none were taking any form of steroidal contraception.

Experimental Design. Beginning on cycle day 9, all subjects tested a daily early-morning urine sample for luteinizing hormone (LH) to identify the midcycle urinary LH surge using commercially available home-based LH detection kits (Clearplan One Step; Unipath Ltd., Bedford, England). The day of the LH surge was designated cycle day 0. Subjects were also provided with a box containing numbered tubes and were asked to collect 2-ml samples of saliva into each tube between 1800 and 2000 hr daily, starting from day 0 and continued for the next 18 days. Subjects were instructed to record the day of menstruation. Each patient acted as her own control by collecting similar samples during a pretreatment cycle.

In the treatment cycle, all subjects received exogenous hCG, 250, 500, 1000, and 2000 IU, administered intramuscularly on consecutive days. The injections were given at 0900 hr every day for 4 days. This particular dose regime was chosen because it simulates the normal rise in hCG seen in spontaneous conception cycles, where the hCG concentrations double every 30 hr (8). The subjects were divided into three groups, starting hCG administration on luteal day 4 (Group A; $n = 5$), luteal day 8 (Group B; $n = 5$), and luteal day 12 (Group C; $n = 4$).

Assays. Saliva samples were stored at -20°C until assayed. All samples from each individual were assayed together. The saliva progesterone radioimmunoassay was a simple, direct steroid assay, employing reagents available from Steranti Laboratories (Steranti Research Ltd., St. Albans., UK). These comprised progesterone- ^{125}I -tyramine glucuronide (code No. 205)

and an antibody against progesterone glucuronide 11 β -hemisuccinate bovine serum albumin (code No. ER 125), which was covalently bound to a solid phase support. Solutions of progesterone, initially dissolved in ethanol and then progressively diluted in assay buffer (phosphate/gelatin, pH 7.0), were used as standards (giving a range of 10 to 2600 pM). Quality controls (QC) were prepared from male saliva by the addition of progesterone-q (high QC = 732 pM, low QC = 380 pM).

Prior to analysis, all samples of saliva were thawed and centrifuged at 900g to precipitate mucins. Reagent volumes employed in the assay were as follows: antibody, 50 μl ; ^{125}I -progesterone, 50 μl ; and saliva or standard solution, 150 μl ; giving a total of 250 μl . Assay tubes were incubated overnight at room temperature, then centrifuged at 1400g for 20 min. The supernatants were decanted and the precipitate (containing the bound fraction) was counted in a gamma-counter for 1 min. The sensitivity was 20 pM and interassay coefficients of variation of high and low QC were 8.0 and 7.5%, respectively.

Data Analysis. Hormonal data are presented in the form of geometric means. The total serum progesterone concentration ($\Sigma\text{P}_{0-\text{M}}$ per cycle) was determined by summing the daily individual progesterone values from cycle day 0 till the day of menstruation (M). Parametric (paired Student's t test) statistical tests were applied as appropriate. A probability level <0.05 was used to indicate statistical significance.

RESULTS

The duration of the luteal phases in the three study groups and their corresponding control cycles are shown in Table I. Compared with their control cycles, the luteal phases were significantly prolonged in Groups B (17.0 ± 1.0 days) and C (18.5 ± 0.6 days) ($P < 0.005$) after hCG injections. No difference in luteal-phase length was seen in the group that received the earliest hCG treatment (Group A).

Treatment with exogenous hCG starting on luteal day 4 (Group A) had no consistent effect on the salivary progesterone concentration. Although salivary progesterone concentrations on luteal days 6, 7, and 8 were moderately higher in Group A than in the corresponding control cycles (Fig. 1a), the $\Sigma\text{P}_{0-\text{M}}$ per cycle was not significantly different (Table I).

In Group B, an immediate response by the corpus luteum with an increase in salivary progesterone from the first day of hCG injection was seen. Concentrations

Table I. Luteal-Phase Duration and Total Serum Progesterone Concentrations in the Study and Corresponding Control Groups^a

Group	hCG administration on luteal day	Luteal-phase duration (days)	ΣP_{0-M} (pM)
A ($n = 5$)	+4	$13.8 \pm 1.1^{(NS)}$	$3970 \pm 556.9^{(NS)}$
Control ($n = 5$)	Nil	13.8 ± 1.1	3226 ± 409.3
B ($n = 5$)	+8	$17.0 \pm 1.0^{**}$	$6495 \pm 1967^*$
Control ($n = 5$)	Nil	13.8 ± 0.5	4211 ± 675
C ($n = 4$)	+12	$18.5 \pm 0.6^{**}$	$6109 \pm 1565^{(NS)}$
Control ($n = 4$)	Nil	14.8 ± 0.5	4726 ± 1215

^a Values are means $\pm$ SD. NS, not significant compared to control data using the paired *t* test.

* $P < 0.05$.

** $P < 0.005$.

reached a mean peak value of 790 pM by day 11, the day of the last hCG injection. Progesterone levels then declined steadily and fell below the assay sensitivity of 20 pM by luteal day 17 (Fig. 1b).

Prior to hCG administration on luteal day 12 in Group C, progesterone profiles were similar in the control subjects and the treated cycles. However, from luteal day 12, the level of progesterone increased sharply following exogenous hCG administration. Progesterone concentrations reached a mean peak value of 580 pM on luteal day 15 and then declined (Fig. 1c). Again, saliva progesterone concentrations fell immediately after discontinuation of the hCG injections even though there would still have been hCG in the circulation. Overall, the pattern of decline of progesterone was similar in the two groups. Significant differences were seen in the ΣP_{0-M} per cycle values in Group B compared with their control cycles ($P < 0.05$), but in Group C the increase in progesterone production did not reach statistical significance (Table I).

DISCUSSION

Lenton *et al.* have shown that the concentration of progesterone in saliva can be used as an alternative to the measurement of the hormone in the plasma and they have also defined the reference values for the hormone during the luteal phase (7). Salivary progesterone levels during the midluteal phase of all 14 subjects in this study reached values of 400–500 pM, indicative of ovulation. The hCG regime used was designed to mimic the hormonal changes in early pregnancy. It has been shown to produce peripheral serum

Fig. 1. Geometric mean of serum progesterone concentration pooled to the day of the midcycle urinary LH surge (luteal day 0). (a) Group A ($n = 5$) subjects, who received exogenous hCG from luteal day 4; (b) Group B ($n = 5$) subjects, who received exogenous hCG from luteal day 8; (c) Group C ($n = 4$) subjects, who received exogenous hCG from luteal day 12. (■) Control subjects; (□) subjects who received hCG injections.

hCG concentrations that are similar in terms of both the absolute values obtained and the rate of rise of hCG seen in normal conception cycles (9).

The response of the human corpus luteum to exogenous chorionic gonadotropin on luteal day 4 is of par-

ticular interest. Despite the presence of specific binding sites for LH in human corpora lutea 4 days after the midcycle surge (10), hCG failed to elicit a significant steroidogenic response. Fisch and co-workers (11) have demonstrated that human luteal cells isolated in vitro several days after ovulation show a marked increase in progesterone production when exposed to hCG in vitro, yet the in vivo hCG challenge used in this study did not clearly increase serum progesterone concentrations. Richardson *et al.* (12) have demonstrated that hCG and low-density lipoprotein (LDL) act in a synergistic manner to bring about a sustained increase in steroidogenesis. Therefore the failure of the developing corpora lutea to respond to hCG in vivo suggests a physical barrier, such as poorly developed vasculature in the early luteal phase, limiting the precursor cholesterol in the form of LDL and hence restricting the rate of steroid biosynthesis. Alternatively, Fisch (11) has postulated that human corpora lutea may function autonomously for a period of days after ovulation. Failure of the corpora lutea to respond steroidogenically to hCG on luteal day 4 lends support to either of these hypotheses.

The human corpus luteum of the midluteal phase was the most responsive to the hCG challenge (as seen in Group B). This result correlates well with animal models, where monkey as well as human corpora lutea of the midluteal phase synthesize the greatest quantities of progesterone and are the most sensitive to hCG (3,4). The highest binding capacity for LH/hCG has been found in the midluteal phase of the monkey corpus luteum (13). Luteal weight, luteal progesterone content, and numbers of binding sites for LDL all achieve maximal values in monkeys at this time (10,13). It is therefore not surprising that the response of the human corpus luteum to exogenous gonadotropin should be greatest at a time when all other parameters of luteal function indicate maximal steroidogenic capacity. Physiologically, this part of the luteal phase also coincides with the time of human implantation where the embryo is attaching to the maternal endometrium and hCG produced by the trophoblastic cells is gaining access to the maternal circulation (8).

Although the corpus luteum responds to hCG with an increase in steroidogenesis in late luteal phase (Group C), the total amount of progesterone produced was not statistically significantly different compared with that in matched control cycles. The extended duration of the luteal phase seen in these subjects was merely a reflection of the low continuing progesterone activity remaining in the circulation (Fig. 1c). From this observation, it is tempting to speculate that an

embryo implanting during this "less-responsive" period is more likely to fail to initiate an adequate "rescue" response. As demonstrated by Lenton *et al.* (14), early pregnancy wastage is seen more commonly in women undergoing IVF where embryo implantation may be slightly delayed. Therefore, the responsiveness of the maternal corpus luteum is an important factor in determining the outcome of assisted reproduction and spontaneous pregnancies.

The number of available binding sites for LH/hCG in the human corpus luteum remains the same during the mid and late luteal phases (9), suggesting that the relative insensitivity of the older corpus luteum may be associated with an increasing block to steroidogenesis downstream of LH/hCG receptor binding. Desensitization of the LH/hCG receptor from its ligand, with uncoupling from second-messenger systems, has been reported by Segaloff and Ascoli (15). Such desensitization may explain why hCG concentrations normally need to increase exponentially to maintain progesterone production during early pregnancy. The immediate fall in progesterone concentrations after the discontinuation of hCG seen in the current study further substantiates the fact that luteal progesterone production is highly dependent on hCG. It is not yet known whether such changes in hCG/LH sensitivity are dependent solely upon the age of corpus luteum or whether they are related to specific agents such as estradiol, inhibin, or prostaglandins.

In IVF cycles using gonadotropin releasing hormone analogue and hMG, luteal supplementation using exogenous hCG is often given on the day of oocyte recovery, 2 and 4 days later (16). These three doses of hCG supplement are given in the early luteal phase, when, from the data shown here, the corpora lutea may be refractory to such an external stimulus. Based on our observations, we question whether luteal support at this time is either necessary or effective. Potentially administration of hCG in the midluteal phase would be a more logical regime.

In summary, our study has shown that the optimal time for exogenous gonadotropin to "rescue" the corpus luteum is around the midluteal phase in normally cycling women, coincident with the physiological time of normal embryo implantation.

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REFERENCES

1. Buvat J, Marcolin G, Guittard C, Herbaut JC, Dehaene JL, Verbecq P, Fourlinnie JC: A randomised trial of human chorionic gonadotrophin support following in vitro fertilisation and embryo transfer. *Fertil Steril* 1988;49:458–461
2. Quenby S, Farquharson RG: Human chorionic gonadotrophin supplementation in recurring pregnancy loss: A controlled trial. *Fertil Steril* 1994;62:708–710
3. Fritz LCMA, Hess DL, Patton PE: Influence of corpus luteum age on the steroidogenic response to exogenous human chorionic gonadotrophin in normal cycling women. *Am J Obstet Gynecol* 1992;167:709–716
4. Wilks JW, Noble AS: Steroidogenic responsiveness of the monkey corpus luteum to exogenous chorionic gonadotrophin. *Endocrinology* 1982;112:1256–1266
5. Seif M W, Pearson J M, Ibrahim Z H, Buckley C H, Aplin JD, Buck P, Matson P L, Lieberman BA: Endometrium in in vitro fertilisation cycles: morphological and functional differentiation in the implantation phase. *Hum Reprod* 1992;7:6–11
6. Navot D, Scott T, Driesch K, Veeck LL, Liu HC, Rosenwaks Z: The window of embryo transfer and the efficiency of human conception in vitro. *Fertil Steril* 1991;55:114–118
7. Lenton EA, Gelsthorpe CH, Harper R: Measurement of progesterone in saliva: Assessment of the normal fertile range using spontaneous conception cycles. *Clin Endocrinol* 1988;28:637–646
8. Lenton EA, Woodward AJ: The endocrinology of conception cycles and implantation in women. *J. Reprod Fert Suppl* 1988;36:1–15
9. Illingworth PJ, Reddi K, Smith K, Baird DT: Pharmacological “rescue” of the corpus luteum results in increased inhibin production. *Clin Endocrinol* 1990;33:323–332
10. Duncan WC, McNeilly AS, Fraser HM, Illingworth PJ: Luteinising hormone receptor in the human corpus luteum: Lack of down-regulation during maternal recognition of pregnancy. *Hum Reprod* 1996;11:2291–2297
11. Fisch B, Margara RA, Winston RML, Hillier SG: Cellular basis of luteal steroidogenesis in the human ovary. *J Endocrinol* 1988;122:303–311
12. Richardson MC, Davies DW, Watson RH, Dunsford ML, Inman CB, Masson GM: Cultured human granulosa cells as a model for corpus luteum function: Relative roles of gonadotrophin and low density lipoprotein studied under defined culture conditions. *Hum Reprod* 1992;7:12–18
13. Cameron JL, Stouffer RL: Gonadotrophin receptors of the primate corpus luteum. II. Changes in available luteinising hormone- and chorionic gonadotrophin-binding sites in macaque luteal membranes during the non-fertile menstrual cycle. *Endocrinology* 1982;110:2068–2073
14. Lenton EA, Hooper M, King H, Kumar A, Monks N, Verma S, Osborn J: Normal and abnormal implantation in spontaneous in-vivo and in-vitro human pregnancies. *J Reprod Fert* 1991;92:555–565
15. Segaloff DL, Ascoli M: The lutropin/chorio-gonadotrophin receptor. 4 years later. *Endocrinol Rev* 1993;14:324–347
16. Buvat J, Marcolin G, Guittard C, Herbaut JC, Louvet AL, Dehaene JL: Luteal support after luteinising hormone-releasing hormone agonist for in vitro fertilisation: Superiority of human chorionic gonadotrophin over oral progesterone. *Fertil Steril* 1990;53:490–494