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Preovulatory luteinization during induction of follicular maturation with menotrophin and menotrophin–clomiphene combination

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Late follicular phase levels of 17β-oestradiol (E_2) and progesterone (P) in serum were studied during the induction of follicular maturation with human menopausal gonadotrophin (HMG) (n = 23) and HMG–clomiphene citrate (CC) (n = 18). For each patient one hormonal profile was studied between 6 days to 2 h before administration of human chorionic gonadotrophin (HCG). Ultrasonographic follicular measurement at the time of evaluation demonstrated preovulatory ovarian follicles of various number and size in all patients studied. Sixteen endometrial biopsies were performed at the time of evaluation (eight for each subgroup), before HCG administration. Mean late follicular phase levels of E_2 did not differ significantly between the two groups (436 ± 348 and 475 ± 267 pg/ml for the HMG and HMG–CC groups respectively). The mean progesterone levels in the HMG–CC group (1.233 ± 0.67 ng/ml) was significantly higher than that for the HMG group of (0.86 ± 0.55 ng/ml, $P \leq 0.04$). The mean time interval between ovulation and HCG administration for the two groups was 2.5 and 2.4 days for the HMG and HMG–CC groups, respectively. In all eight biopsies taken from the HMG group, various stages of endometrial proliferation were demonstrated. Premature glandular secretory transformation and oedematous stroma were observed in three out of eight biopsy specimens obtained from the HMG–CC group. Taken together, these results demonstrate that subtle or full premature luteinization in the late follicular phase occurs more frequently with the use of HMG–CC for induction of follicular maturation.

Key words: follicular maturation/HMG/HMG–clomiphene citrate/preovulatory luteinization

Introduction

The use of human menopausal gonadotrophin (HMG) alone or in combination with clomiphene citrate (CC) is effective in inducing ovulation in as many as 98% of treatment cycles (Schwartz and Jewelewicz, 1981; Kemmann and Jones, 1983; Kurachi *et al.*, 1985). The addition of CC to HMG has been reported to improve ovulation rates and reduce the amount and duration of HMG needed (March *et al.*, 1976; Edwards and Ellis, 1983; Kemmann and Jones, 1983). The conception rates per ovulatory cycle of HMG alone and HMG–CC regimens are rather similar and range between 13.3 and 17% (March *et al.*, 1976; Schwartz and Jewelewicz, 1981; Kemmann and Jones, 1983; Kurachi *et al.*, 1985). These rates are lower than the expected conception rates per ovulated ovum (Witschi, 1971; Warburton, 1987). The rates of egg loss, preimplantation and second week, and early and late embryonal wastage are also increased (Witschi, 1971; Schwartz and Jewelwicz, 1981; Kemmann and Jones, 1983; Warburton, 1987). The possible factors which may affect pregnancy rates and the reproductive toxicity following the use of these drugs have been reviewed and discussed (Hammond *et al.*, 1983; Birkenfeld *et al.*, 1986. Scialli, 1986). Among these factors, endometrial physiology has been suggested as a possible cause contributing to the discrepancy between ovulation and conception rates. Morphological luteal phase defects, probably reflecting functional disturbances, have been attributed to the use of HMG and CC (Birkenfeld *et al.*, 1986; Scialli, 1986).

Possible deviations from normal reproductive processes may also be evident during or shortly after the induction of follicle maturation. We have previously demonstrated premature secretory endometrial transformation following CC treatment, occurring before ovulation (Birkenfeld *et al.*, 1986). We have therefore evaluated the sex steroidal pattern and performed complementary endometrial biopsies during induced cycles with HMG and HMG–CC, before the administration of human chorionic gonadotrophin (HCG).

Patients and methods

Forty-one anovulatory patients (group II WHO classification) (Lunenefeld and Insler, 1978) attending the infertility unit at the Hadassah University Hospital, Jerusalem, Israel between July and October, 1988, have participated in the study. All patients, aged 26–38 years with hypothalamic anovulation had either a normal cycle length or oligomenorrhoea. Patients with primary or secondary amenorrhoea (group I) were excluded from the study. The patients were randomized systematically (according to referral date) into two groups; 23 patients were treated with HMG only (Pergonal, Ikapharm, Teva Pharmaceutical Industries Ltd, Tel Aviv, Israel) and 18 patients with HMG–CC (Pergonal and Ikaclomin, Ikapharm, Teva Pharmaceutical Industries Ltd,

Tel Aviv, Israel) for the induction of follicular maturation. Induction of follicular maturation with HMG was initiated between the 4th and 7th days of the cycle at a dose of 2–5 ampoules per day. Clomiphene (50–150 mg) was administered daily between the 4th and 9th days of the cycle. Low dose Parlodel treatment (1.25–2.5 mg/day) was added when mild hyperprolactinaemia (20–40 ng/ml) was detected. Dexamethasone, (1 mg/day) was added in two cases according to previously described guidelines (Evron *et al.*, 1983). The ovarian response was monitored by serial serum E_2 and ultrasonographic follicular measurements. The target values for HCG administration were a serum level E_2 of 500–1200 pg/ml and ultrasonographic demonstration of one to three follicles ≥ 18 mm. Serum E_2 and progesterone (P) were determined by standard RIA (Isodan, Jerusalem, Israel) and Coat-a-Count (Diagnostic Products Corporation, Los Angeles, CA), respectively. Biopsies were obtained by a sharp Novak curette placed high in the uterine cavity. Progesterone determinations (one per patient) were performed 12 h to 6 days before HCG administration. All biopsies were performed between days 11 and 17 of the cycle, 12 h to 4 days before HCG administration. Tissue was immediately fixed in formaldehyde and processed for haematoxylin and eosin staining. Histological evaluation was performed according to the criteria of Noyes *et al.* (1950) by a gynaecological pathologist who was blind to the clinical data. In Figure 1, the correlations of induction of ovulation, reproductive events and the timing for a safe endometrial sampling are illustrated. Although post-ovulatory endometrial biopsies are performed for the evaluation of the endometrium during treatment cycles, we have evaluated the endometrium prior to HCG administration, so that premature follicular phase transformation would be detected. Statistical analysis was performed using the unpaired one-tailed Student's *t*-test. The level of significance was $P < 0.05$. Values are expressed as mean ± standard deviation (SD).

Results

Mean serum levels of E_2 were similar for the HMG and HMG–CC groups (436 ± 348 pg/ml and 475 ± 267 pg/ml, respectively). The mean interval between the time of evaluation and HCG administration was also similar for both groups, 2.5 days for the HMG group and 2.4 days for the HMG–CC group.

In the HMG–CC group, serum P levels ranged between 0.4 and 2.2. ng/ml. Mean preovulatory serum P was significantly higher in the HMG–CC than in the HMG only group. 1.23 ± 0.67 pg/ml and 0.86 ± 0.55 ng/ml, respectively ($P < 0.05$) (Figure 2). The E_2/P ratio did not differ between the two groups.

In the HMG–CC group, premature ovulation or complete luteinization was observed by ultrasound in three patients 24–48 h prior to HCG administration. Their P values (5.4, 12, 16.3 ng/ml) were excluded when the mean P value was calculated for the HMG–CC group.

All endometrial biopsy specimens taken during HMG treatment between the 11th and 17th cycle days and before the administration of HCG for the induction of ovulation were classified as early or midproliferative with no signs of secretory transformation (Table I). Progesterone levels in this group ranged between 0.2 and 1.7 ng/ml. These low levels were observed despite three of the biopsies being performed only 12 h before HCG administration. E_2 levels ranged between 134 and 1006 pg/ml.

In three out of eight specimens taken during combined HMG–CC treatment between the 11th and 14th cycle days and before the administration of HCG for the induction of ovulation, different patterns of secretory transformation were seen (Table II). In one case, the endometrial glands corresponded to cycle days 18–19 with an even more advanced oedematous stromal appearance (day 20–21). In the other two biopsy specimens, subnuclear vacuoles were abundantly observed (day 17–18). However, some of the glands still possessed the tubular shape corresponding to early–mid-proliferative endometrium. Also in these biopsies, a more advanced oedematous stromal appearance was observed (Figure 3). At least two of these biopsies probably reflect premature ovulation or complete luteinization of an unruptured follicle.

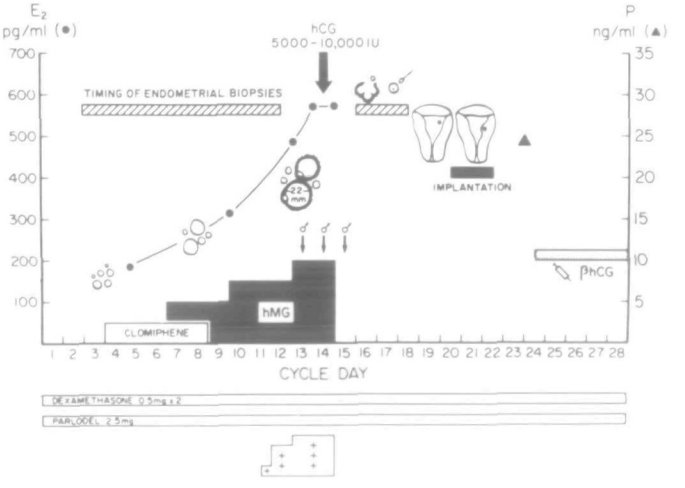

Fig. 1. Induction of follicular maturation and ovulation. Suggested timing for a safe endometrial sampling.

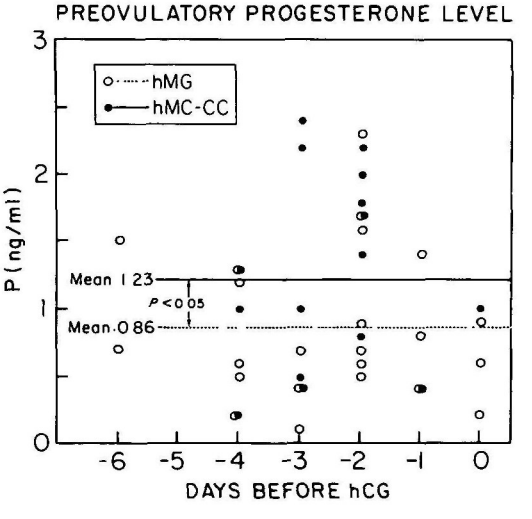

Fig. 2. Progesterone levels of the HMG and HMG–CC groups before HCG administration.

Table I. Induction of ovulation with HMG: endometrial dating and endocrine correlations

Patient	Cycle length	HMG (amp. prior to biopsy)	Cycle day	E_2 (pg/ml)	P (ng/ml)	Endometrial glands	Stroma
1. SV	Normal	30	11(−3)[b]	270	0.4	Mid-proliferative	Mid-proliferative
2. SM	Oligomenorrhoea	48	16(0)	1006	0.2	Mid-proliferative	Mid-proliferative
3. NM	Normal	4	11(0)	792	0.9	Early−mid-proliferative	Mid-proliferative
4. SS	Oligomenorrhoea	28[a]	17(−1)	832	1.4	Early proliferative	Early proliferative
5. YR	Oligomenorrhoea	45	17(−2)	199	1.7	Early proliferative	Early proliferative
6. CS	Normal	23[a]	12(−2)	450	1.6	Late proliferative	Late proliferative
7. NH	Normal	12[a]	13(−2)	478	0.5	Early−mid-proliferative	Mid-proliferative
8 NZ	Normal	4	11(0)	134	0.6	Mid-proliferative	Mid-proliferative

[a]Treatment combined with Parlodel, 1.25−2.5 mg/day.
[b]Days before HCG administration (day −0).

Table II. Induction of ovulation with HMG−CC; endometrial dating and hormonal correlations

Patient	Cycle length	HMG (amp. prior to biopsy)	CC (mg/day)	Cycle day	E_2 (pg/ml)	P (ng/ml)	Endometrial glands	Stroma
1. ZM[a]	Normal	7[b]	50	12(−4)[d]		1 3	Early-proliferative	Mid-proliferative
2. RA	Normal	10[c]	100	12(−2)	750	2.2	Mid-proliferative	Mid-proliferative
3. AB	Normal	28	100	14(−1)	224	16.3	Day 18−19	Oedematous
4. AN	Normal	5	100	12(−3)	849	0.5	Mid-proliferative	Mid-proliferative
5 EM	Oligomenorrhoea	13	100	13(−1)	490	0.4	Mid-proliferative	Mid-proliferative
6. IH	Normal	10[b,c]	100	11(−1)	344	12	Tubular glands, subnuclear vacuoles	Oedematous
7. KH	Normal	28	150	12(−2)	250	2	Early proliferative	Early proliferative
8. YS	Oligomenorrhoea	7	150	11(−3)	432	2.4	Tubular glands, subnuclear vacuoles	Oedematous

[a]Conceived during same cycle.
[b]Treatment combined with Parlodel, 1.25−2 5 mg/day.
[c]Treatment combined with dexamethasone, 1 mg/day.
[d]Days before HCG administration (day −0).

Discussion

Luteal phase deficiency is one of the most frequently encountered problems associated with the use of gonadotrophins and clomiphene citrate for the induction of follicular maturation and ovulation (Hammond *et al.*, 1983; Birkenfeld *et al.*, 1986). These defects may be explained either by a failure of these drugs to establish adequate follicular maturation through a direct ovarian effect (Adashi, 1984; Collins *et al.*, 1984), or by their interference at the target tissue receptor level (Birkenfeld *et al.*, 1986; Scialli, 1986). However, aberrant oestradiol secretion patterns and premature ovulation or luteinization of unruptured preovulatory follicles may also be detected before ovulation and may affect pregnancy rates (Marik and Hulka, 1978; Coulman *et al.*, 1982; Bergquist *et al.*, 1983; Jones *et al.*, 1983; Zimmermann *et al.*, 1984; Janssen-Caspers *et al.*, 1986; Wolf *et al.*, 1986; Navot *et al.*, 1987). The differentiation between ovulation and full lutenization of an unruptured follicle can only be determined by ultrasound imaging or determination of peritoneal fluid hormones (Coulman *et al.*, 1982; Janssen-Caspers *et al.*, 1986). We have proposed that the whole range between subtle preovulatory luteinization to overt ovulation may occur during ovulation induction and alter the physiological steroidal

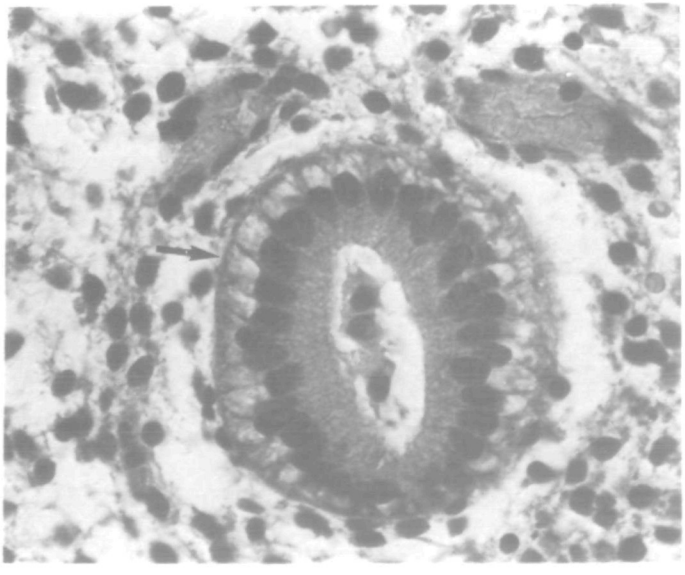

Fig. 3. Endometrial response to premature ovarian luteinization occurring on day 11 during HMG−CC treatment before HCG administration. Tubular gland with subnuclear vacuolation (black arrow) (patient IH, ×160).

milieu. Combined treatment with HMG–CC results not only in supraphysiological production of E_2 but also in elevated and sustained LH levels (Wu, 1977), a combination which may in turn be responsible for the observed premature luteinization. Taylor *et al.* (1986) reported pregnancies after superovulation with HMG–CC, all of which had significantly higher P levels before and during oocyte recovery. However, P levels were determined at induction of anaesthesia (34 h after HCG injection). The mean P values before HCG injection were not reported.

The observed alterations in the steroidal hormone patterns may affect endometrial maturation and transformation. Premature secretory changes in the follicular phase endometrium have also been demonstrated subsequent to CC treatment. These secretory changes were observed in tubular glands and were associated with low serum P values, indicating the absence of luteinization. It was therefore speculated that these changes were the result of a direct antioestrogenic effect on the endometrial cells, rather than premature luteinization (Birkenfeld *et al.*, 1986).

In the present study, it is demonstrated that follicular phase secretory changes may be detected in the human endometrium during combined HMG–CC treatment prior to the induction of ovulation with HCG. These changes probably reflect substantial premature luteinization or ovulation in this group, but not subtle luteinization. Of particular interest is the fact that asynchronous glandular/stromal maturation was observed in all secretory endometria, irrespective of P levels. It is not known whether the subtle luteinization and relatively elevated prevoulatory P levels also affect endometrial physiology and in turn, its receptivity of the developing embryo.

In conclusion, subtle or full luteinization may occur during induction of follicular maturation with HMG–CC rather than with HMG alone. These changes may affect endometrial cyclicity and could be linked with reproductive failure associated with asynchronous endometrial maturation and transformation.

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