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Early luteal serum progesterone concentrations are higher in pregnancy cycles*

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In a consecutive series of 167 patients reaching the stage of embryo transfer after in vitro fertilization and embryo transfer, 19 clinical pregnancies ensued. The serum progesterone (P) levels were significantly greater on the first and second ($P < 0.01$) and third ($P < 0.05$) postaspiration days for those who conceived. Higher circulating levels of P were achieved on days 1, 2, and 3 ($P < 0.05$) by the daily injection of P, 50 mg in oil, given for 5 consecutive days, beginning immediately after follicle aspiration. Both pregnancy and nonpregnancy cycles demonstrated high circulating P levels, but the study implies that relatively higher levels are required for conception, and such levels can be achieved by the use of intramuscular P.
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It has been shown that the hormonal characteristics of successful in vitro fertilization and embryo transfer (IVF-ET) cycles that generate ongoing pregnancies are maintained within a fairly narrow range for clomiphene citrate (CC) and clomiphene citrate/human menopausal gonadotropin (CC/hMG) schedules.[1] The conclusions were drawn from an analysis of the serum levels of various hormones, including progesterone (P), during the follicular phase, the midluteal and late luteal phase, and the early weeks of pregnancy in 24 IVF pregnancies that yielded live infants between 32 and 41 weeks' gestation. The data have been subsequently reanalyzed for 70 IVF pregnancies and the conclusions found to hold true within the original defined limits.[2]

We have recently initiated studies into the early luteal phase and demonstrated differences in the levels of serum P between CC and CC/hMG cycles in the 4 days after ovum aspiration. In this study the periaspiration and early luteal serum P levels are analyzed, and a strictly randomized group allocation of patients was studied for the effects of administered P.

MATERIALS AND METHODS

A consecutive series of 167 patients had oocytes aspirated at laparoscopy and reached the stage of ET. The technical details of the program have been fully described.[3] All patients in this series were given CC (Clomid, Merrell Dow Pharmaceuticals Inc., Cincinnati, OH), 100 mg/day for 5

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days, beginning on days 2 to 4 of the menstrual cycle. Daily injections of hMG (Humegon, Organon [Australia] Pty. Ltd., Lane Cove, New South Wales, Australia) were also given, beginning with two ampules on the third day of the CC course and increasing by one or two ampules every third day until a continuing rise of serum 17β-estradiol was achieved. Most patients responded to two or three ampules daily, with < 20% given four or five ampules daily. The coasting phase between the last day of hMG and the human chorionic gonadotropin (hCG) trigger injection (10,000 IU) varied between 16 and 64 hours, with the majority at about 40 hours. All patients in this series underwent follicle aspiration 36 (± 1) hours after the hCG injection.

Patients having their first or second IVF attempt (137 cases) were allocated randomly (alternate selection at the onset of the treatment cycle) for P support treatment during the early luteal phase; an additional 30 patients undergoing their third to fifth IVF attempt during this series were included for empirical support with P as a defined course of action planned at case review several months earlier, but were excluded from the statistical analysis. P, 50 mg in oil (Proluton, Schering Pty. Ltd., Tempe, New South Wales, Australia), was given daily by deep intramuscular injection, beginning immediately after ovum aspiration (day 0), then daily after a blood sample was taken at 8:00 A.M. for a total of 5 days (0 to 4, inclusive).

The serum P concentrations were analyzed by Coat-A-Count solid phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA). The detection limit was 0.16 nmol/l, and there was minimal cross-reactivity with other steroids (2.4% with 11-deoxycortisol, 2.0% with 20-dihydroprogesterone, and no detection of androstenediol, cortisol, or testosterone). The coefficient of variation for within-run assays was < 8.5%, within the range of 5 to 60 nmol/l. The interassay coefficient of variation was 10% over the same range. In the range of 50 to 500 nmol/l, both the interassay and intraassay coefficients of variation were < 12%. Within the coefficients of variation for the assay, there was total detection of the P from the Proluton ampules when it was diluted and spiked in serum (90% to 110% recovery of observed/expected in samples spiked with from 10 to 250 μl).

The data were recorded with mean and standard error limits. Student's *t*-test was applied for the analysis of differences between P levels in the designated groups for each day.

RESULTS

After ET in this consecutive series of 167 cases, 19 pregnancies were generated that reached the stage of clinical confirmation of a live fetus between the seventh and eighth week by ultrasound scanning of the uterine contents. Only one of the pregnancies aborted in the first trimester; the others are currently well advanced beyond 20 weeks' gestation. Table 1 denotes the serum P concentrations for the 5 observation days in the four subgroups analyzed (pregnant or nonpregnant, ± P therapy). An analysis of the nonpregnant group indicated that the patients treated with P injections had significantly higher circulating levels of P detected on days 1, 2, 3, and 4 after aspiration ($P < 0.05$).

A comparison of the two pregnant groups (± P therapy) indicated no significant difference in the circulating P levels, whether or not P injections

Table 1. *Serum P Levels in the Early Luteal Phase*[a]

Day	No treatment		Proluton injections	
	Nonpregnant (group 1; *n* = 55)	Pregnant (group 2; *n* = 5)	Nonpregnant (group 3; *n* = 66)	Pregnant (group 4; *n* = 11)
	nmol/l		*nmol/l*	
0[b]	21.27 ± 2.21	21.00 ± 2.42	22.43 ± 3.88	25.78 ± 4.77
1	57.06 ± 4.19	112.20 ± 49.99	112.85 ± 7.78	146.33 ± 27.42
2	112.78 ± 9.25	194.00 ± 63.69	173.02 ± 10.99	249.08 ± 54.85
3	165.80 ± 13.83	281.40 ± 93.19	247.27 ± 20.72	298.92 ± 51.68
4	235.43 ± 20.27	305.80 ± 72.91	276.58 ± 17.97	333.33 ± 67.75

[a]Values are means ± standard error of the mean.

[b]Day 0, serum sample before injections. Nonpregnant patients injected with Proluton have higher serum P levels on days 1, 2, 3, and 4 ($P < 0.05$) than nonpregnant patients with no treatment. No difference is seen between pregnant patients who received Proluton injections and patients with no treatment. Of patients with no treatment, serum P levels were higher in pregnant women on days 1, 2, and 3 ($P < 0.05$).

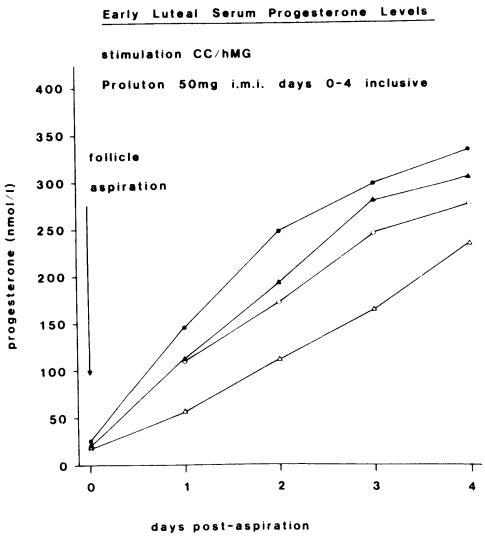

Figure 1
Mean daily serum P levels from day 0 (follicle aspiration) to day 4 of the luteal phase in patients who underwent IVF after stimulation with CC combined with hMG (CC/hMG). Patients given Proluton began treatment after serum sample and follicle aspiration on day 0. The groups are untreated women (Δ, nonpregnant; ▲, pregnant) and women who received Proluton (○, nonpregnant; ●, pregnant).

had been given. However, the small numbers in the pregnant series should be noted, and the findings require confirmation from a larger series of pregnancies.

A comparison of the pregnant patients with the nonpregnant patients who did not have P treatment indicated a clear distinction between the circulating P levels of the two groups (Fig. 1), the pregnant group demonstrating higher levels on the first ($P < 0.01$), second and third ($P < 0.05$) postaspiration days. No difference was noticed on the fourth day, when both groups were equally high. The range and mean values of circulating P for the pregnant group (± P therapy) are shown in Table 2, where it can be seen that there was a fivefold rise in P within the first 24 hours after aspiration and thereafter a doubling of the output on the second day, with a plateau effect thereafter. Three of the 30 women (10%) having their third to fifth IVF attempt conceived, which indicated that the pregnancy data would not be affected by their inclusion. Of the 137 randomized cases, 11 pregnancies ensued in 76 patients treated with injections, whereas only 5 pregnancies occurred in 60 untreated patients. However, this is not a significant difference (χ^2 [1] = 1.63; $0.25 > P > 0.05$). Pregnancy ensued after the transfer of 1 to 5 embryos (mean, 3.4) derived from an average of 4.0 large follicles (≥ 1.6 cm) per patient.

DISCUSSION

Earlier work from this IVF-ET program has reported luteal phase studies in pregnancy and nonpregnancy cycles.[1, 4] In treatment cycles stimulated with CC or CC/hMG, shortened luteal phases were not observed, but 14.3% demonstrated midluteal P levels that were considered to be too low for successful pregnancy.[4] Having now generated over 100 clinical pregnancies, with 50 healthy infants delivered, our data from pregnancy cycles suggest that at least 40% of the embryos transferred in this program are capable of successful implantation[5] if maternal uterine receptivity is optimal. An analysis of the midluteal and late luteal phase of pregnancy cycles indicated that the serum P levels were in the high range for the overall group, and the values were maintained at a high level throughout the late luteal phase. These levels could be generated by the administration of hCG injections to stimulate increased corpus luteal steroid output.

The circulating P levels in both the pregnant and nonpregnant patients of this series are all well within the range one might expect for pregnancy during stimulated cycles.[6] The data do suggest, however, that the optimum level of P should be considered in relation to the stimulation schedule used during the follicular phase. As we have previously shown, this influences the number of large follicles generated; one might speculate that an increased number of follicles creates high circulating steroid levels and perhaps an increased endometrial response that requires relatively higher P levels for adequate secretory change and subsequent decidualization. This hypothesis requires definitive study, by the comparison of P levels with endometrial response by the measurement of endometrial thickness and endometrial histology from biopsy specimens or a circulating marker such as estradiol. Interestingly, Garcia et al.[7] have shown a difference in peripheral P concentrations in pregnant and nonpregnant women, although it did not achieve statistical significance until 4 days after oocyte aspiration (day 4 in the present study). A number of women also showed an advanced pattern of endometrium after ovulation induction with exogenous gonadotropin; this led to the suggestion that

Table 2. *Successful Pregnancy Cycles*

	Serum P levels					Follicles ≥ 1.6 cm	Embryos transferred
	Days after aspiration						
	0	1	2	3	4		
	nmol/l						
1	23	62	97	162	230	4	3
2	22	77	254	225	250	6	4
3	17	122	142	100	210	1	3
4	9.8	100	135	140	165	1	1
5	30	137	200	325	350	6	4
6	55	174	550	500	575	8	4
7	5.6	85	87	130	155	7	4
8	7.2	69	72	115	—	1	4
9	34	210	380	630	810	3	4
10	18	115	150	240	260	3	5
11	25	99	185	330	375	3	3
12	—	305	400	615	550	5	4
13	14	18	34	75	124	4	2
14	—	—	—	170	200	3	1
15	17	132	180	270	330	2	5
16	58	99	140	185	220	2	2
17	17	37	200	265	665	6	3
18	30	415	690	587	—	8	4
19	18	130	135	215	260	4	4
Mean	23.56	132.56	223.94	277.84	337.00	4.05	3.37
SEM[a]	3.55	22.70	40.96	40.82	47.84	0.52	0.27

[a]Standard error of the mean.

the "advanced" endometrium may have some benefit for embryo implantation.

Whereas many IVF-ET groups use P support therapy during the luteal phase, the treatment has been given empirically; its value remains undefined, and a rationale of selecting cases for such treatment is lacking. One of the differences between the IVF-ET units in Australia and the United States is the general lack of luteal support treatment given by the Australian teams. It does now appear that the ongoing pregnancy rate in the Australian series may be lower than that documented by well-established American units,[8] and the role of P support therapy is an implied reason. From our data, it certainly appears that intramuscular P injections contribute to the circulating pool of P during the 3 days after aspiration. A significant difference was not confirmed on the fourth postaspiration day, possibly because the overall circulating levels of P on the final day were higher, and the contribution from intramuscular injections was therefore relatively less. Nonetheless, ETs were undertaken on the second postaspiration day, when the circulating P levels for the pregnancy group were significantly higher ($P < 0.01$). Patients given P injections all had circulating P levels in the pregnancy range, and the implication is that the treatment improves the chance of pregnancy for this reason. However, the conclusion cannot be drawn emphatically from this study because 88% of the patients did not develop a clinical pregnancy, regardless of their circulating P values. We have reported clinical pregnancy rates of up to 23% per ET per session,[1] but the rates can fluctuate markedly for technical reasons within the laboratory, because of variations in timing the hCG trigger injection, and for unexplained reasons. A definitive cause for the lower rate during this series is not obvious; but the conclusions drawn from the data presented should hold true, because no changes were made in any part of the program during this series.

In summary, this study implies that relatively higher circulating P levels are required for pregnancy, and such levels can be achieved by the use of intramuscular P (50 mg daily). However, the data need confirmation from a larger study series of pregnancy cycles, and the circulating P levels should be compared with some measure of the endometrial response to the follicular phase stimulation regimen.

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