Journal of in Vitro Fertilization and Embryo Transfer, Vol. 3, No. 6, 1986

Differences in the Luteal Phases After Failed or Successful in Vitro Fertilization and Embryo Replacement

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Submitted: March 28, 1986
Accepted: July 8, 1986 (European Editorial Office)

Luteal phases after in vitro fertilization (IVF) and embryo replacement have been studied in 241 cycles. A positive correlation was observed between the follicular estradiol (E_2) peak and the progesterone (P) level on day 3 of the luteal phase, but no correlation was found between the E_2-peak value and the luteal-phase duration or midluteal P concentration. When the trials were classified in relation to their outcome (i.e., clinical pregnancies, chemical pregnancies, or failures), the mean P level on day 3 of the luteal phase was significantly higher in clinical pregnancies than in chemical pregnancies and in failures. Mean E_2 levels on day 3 were not significantly different among the three groups. Values of the E_2/P ratio were significantly higher in chemical pregnancies than in the other groups. No significant differences were observed among the three groups on day 8. When comparing trials ending in failure to those leading to clinical pregnancy for the same patients, pregnancies were obtained in cycles in which early luteal P was higher and the early luteal E_2/P ratio was lower than in failures cycles. These data suggest that high P levels and a low E_2/P ratio in the early luteal phase might have a favorable influence on the implantation process in human IVF.

KEY WORDS: in vitro fertilization; luteal phase; implantation.

INTRODUCTION

Although success in in vitro fertilization (IVF) depends on many variables (1), the number and vitality of embryos transferred and the quality of the uterine environment play major roles in the establishment of a pregnancy. Embryos can be evaluated to some extent by their morphology and their speed of development (2). Although the uterine environment may be assessed by endometrial biopsy allowing morphological, ultrastructural, and biochemical studies, this approach is possible only in cycles without embryo replacement (ER) (3–5). However, since the endometrial response is directly related to the endocrinology of the luteal phase (6–8), the present study was undertaken to investigate the hormonal patterns of the early luteal phase of IVF trials, that is, in the few days between oocyte retrieval and the theoretical time of implantation.

MATERIALS AND METHODS

Two hundred forty-one IVF-ER trials, performed between September 1983 and August 1985, were included in our study. Patients were treated for either tubal, idiopathic, or male infertility, which was sometimes associated with endocrinological disorders. When present, hyperandrogenism or hyperprolactinemia was treated by suppressive therapy, that is, 5 mg prednilosone/day or 2.5 mg bromocryptine/day, respectively. All patients received ovarian stimulation by a combination of clomiphene citrate (100 to 150 mg/day from day 3 to day 7) and human menopausal gonadotropin (hMG) (150 to 600 IU/day from day 6 to day 10) (Humegon, Organon, Oss, The Netherlands, or Pergonal, Serono, Milan, Italy) and dosages were eventually adjusted to the individual responses of the patients. Oocyte collection time was defined either by an injection of 5000 IU of human chorionic gonadotropin (hCG) (Pregnyl, Organon, Oss, The Netherlands) or by determination of the "LH [luteinizing hormone] surge initiating rise" (9). Egg retrieval was carried out through laparoscopy or by transvesical puncture under echographic guidance. In vitro fertilization and embryo culture were performed in Earle's medium (Flow Labs, Irvine,

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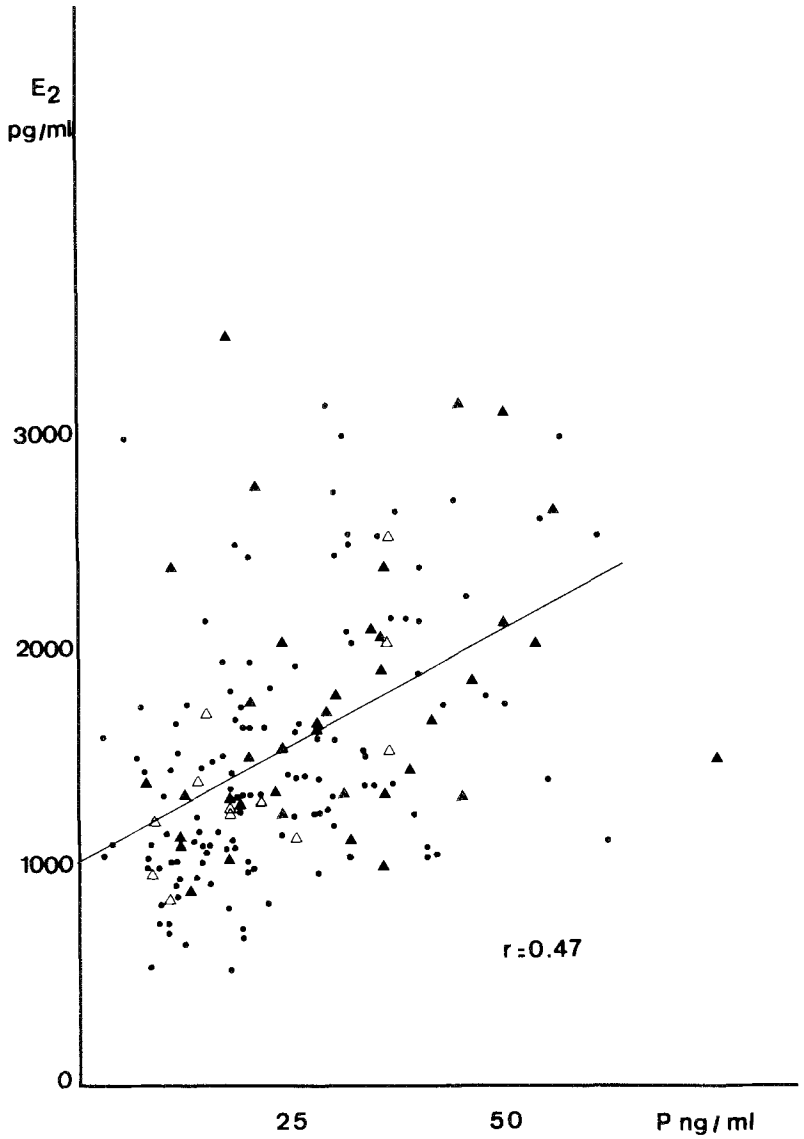

Fig. 1. Correlation between follicular E_2 peak and P on day 3 of the luteal phase.

Scotland) supplemented with pyruvate and 10% human cord serum in a gas-controlled incubator (N_2:CO_2:O_2, 90:5:5). A maximum of three embryos was replaced by the transcervical route in the dorsal or knee-to-chest position (10) and no luteal supplementation was given.

Estradiol (E_2) and progesterone (P) radioimmunoassays (RIA) were performed on sera obtained each morning, from the midfollicular phase to day 4 of the luteal phase (day 0 = day of oocytes aspiration) (for methods, see Ref. 11). hCG, E_2, and P levels were determined on days 8, 12, and 16 after oocyte retrieval and the luteal-phase duration was also recorded. Trials were classified into three categories with respect to their outcome: (i) 185 implantation failures; (ii) 13 chemical pregnancies (hCG levels transiently above 10 mU/ml[3] between day 12 and day 20 after oocyte retrieval; and (iii) 43 clinical pregnancies, i.e., 37 ongoing pregnancies and 6 cases of blighted ova). The correlation between follicular E_2 peaks and progesterone levels on luteal day 3 was evaluated through linear regression on the entire population of assays pertaining to the 241 trials.

[3] hCG levels were measured by a highly specific (cross-reactivity with hLH, 0.24%) monoclonal RIA (Hybritech, Liège, Belgium) with a sensitivity below 2 mU/ml.

Dispersions of values of E_2, P, and the E_2/P ratio were analyzed. Logarithmic transformation was effected before statistical evaluation to obtain Gaussian distributions. Comparisons among differents groups were effected by the use of ANOVA and Tukey test and by Student's t test for paired samples.

RESULTS

Our data are summarized in Figs. 1 to 4 and in Tables I to III. A positive correlation ($r = 0.47$) was observed between the follicular E_2 peak and the P level on day 3 of the luteal phase (Fig. 1), but this relationship did not persist beyond that time. We also observed a positive correlation ($r = 0.44$) between the number of follicles punctured during laparoscopy and the P level on day 3 of the luteal phase. But again, this relationship did not persist beyond that time. The luteal-phase duration was not correlated with maximal values of follicular E_2.

The mean P level at day 3 of the luteal phase was significantly higher in cycles providing a clinical pregnancy than in those ending in failure without detectable hCG ($P < 0.05$) (fig. 2). Although P values were identical in failures and chemical pregnancies, the difference between the latter and clin-

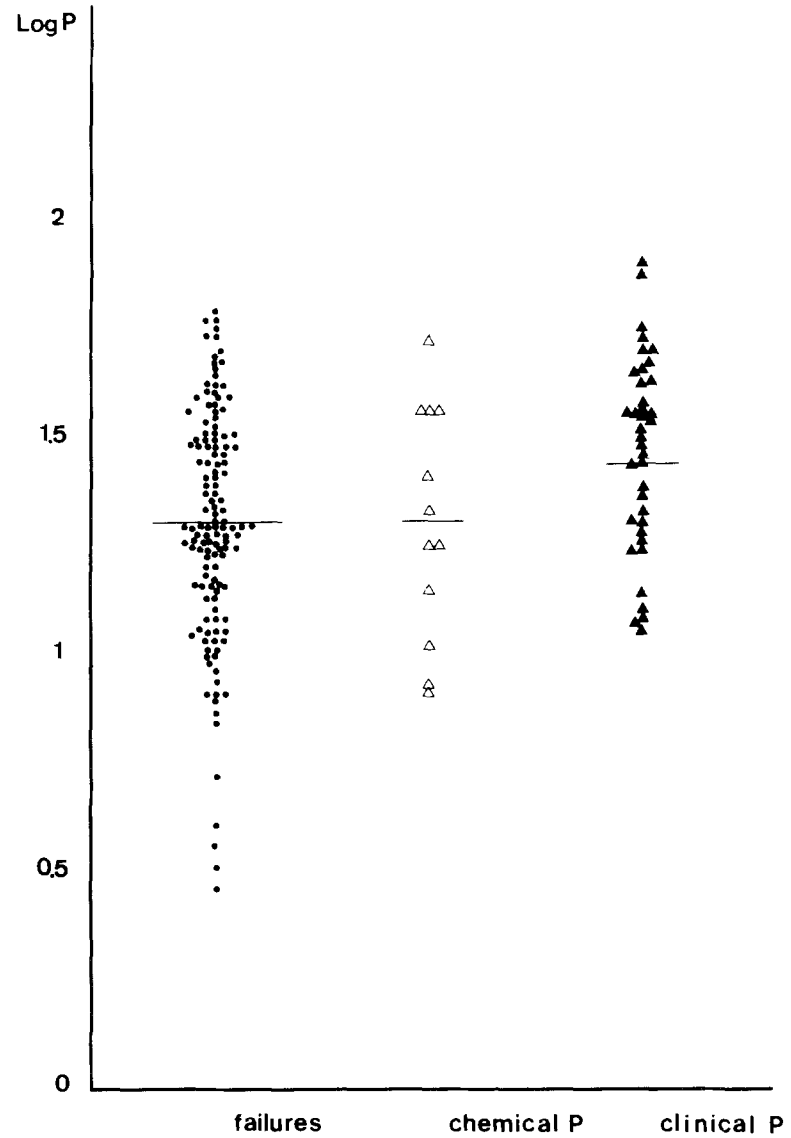

Fig. 2. Comparison of P levels among failures, chemical pregnancies, and clinical pregnancies on day 3 of the luteal phase.

ical pregnancies does not reach significance, as the number of cases is too small. The mean luteal E_2 levels at day 3 were not significantly different in failures, chemical, and clinical pregnancies, although a shift toward higher values was found among chemical pregnancies (Fig. 3). The mean value of the E_2/P ratio on the third day of the luteal phase is significantly elevated in chemical pregnancies ($P < 0.05$) and almost significantly increased in plain failures as compared to clinical pregnancies (Fig. 4).

When the comparison was made between trials ending in failure and those leading to clinical pregnancies for individual patients who underwent several stimulated cycles, pregnancies were obtained in cycles in which the early luteal P was higher and the early luteal E_2/P ratio was lower than in the two other groups (Table II).

On day 8 of the luteal phase no statistical difference were observed among these different endocrine parameters, while on day 12 a clear increase in E_2 and P levels was observed in clinical pregnancies (Table III).

DISCUSSION

Previous studies of the luteal phase of spontaneous cycles have shown that midluteal P levels

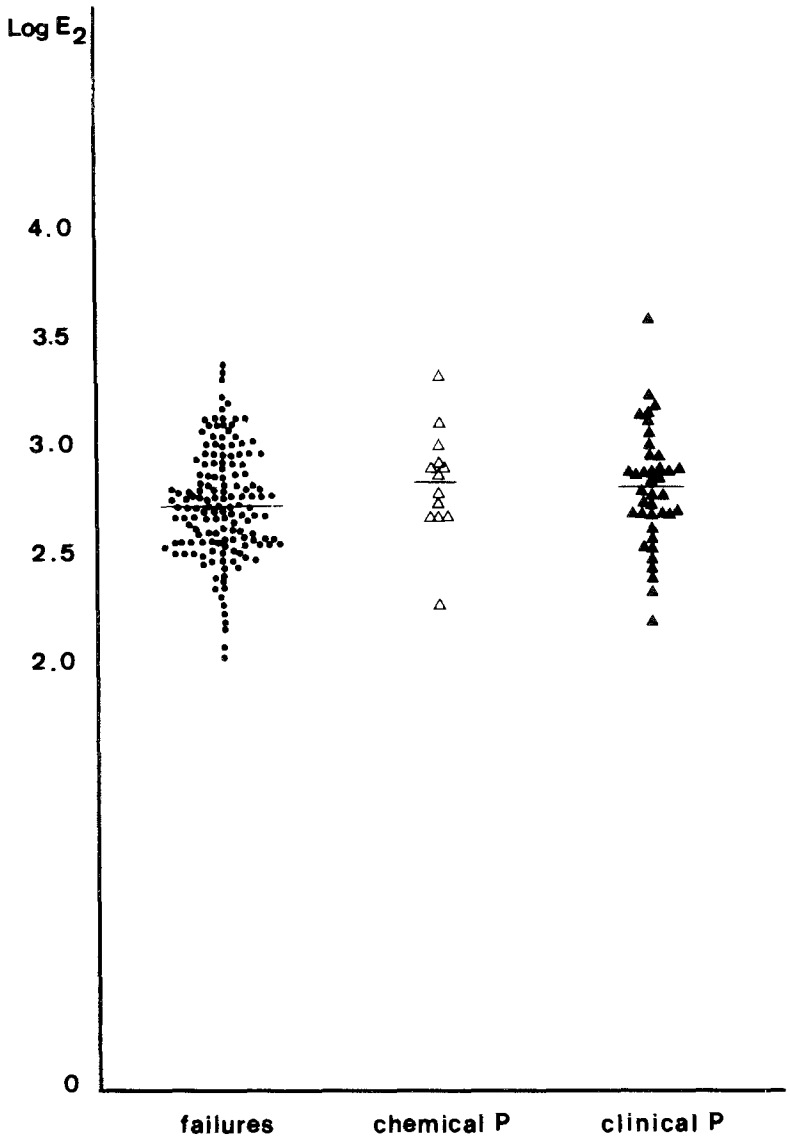

Fig. 3. Comparison of E_2 levels among failures, chemical pregnancies, and clinical pregnancies on day 3 of the luteal phase.

below 10 ng/ml (12) and a luteal-phase duration shorter than 12 days (13,14) are indicative of hormonal disorder and are frequently associated with infertility, recurrent miscarriage, and chemical pregnancy (15–17). Moreover, the comparison between spontaneous conception and sterile cycles has demonstrated that higher P levels are detected as early as day 3 of the luteal phase of conception cycles (18). In IVF programs, few detailed studies of the luteal phase have been made so far. Yovich *et al.* (19) observed significantly higher serum concentrations of P in the early luteal phase of pregnancy cycles such as we have found in our own material (20) and confirmed in the present study. The mean duration of the luteal phase that we observed in IVF cycles (13 ± 2 days) was within the normal range and only 10% of the cases showed midluteal P levels below 10 ng/ml. It is difficult to envisage that absolute P levels in the luteal phase of stimulated IVF cycles are of critical importance since values observed in failures as well as in pregnancy cycles are much higher than after spontaneous ovulation.

Although the difference does not appear significant, elevated E_2 levels such as we have observed in relation to chemical pregnancy might be detri-

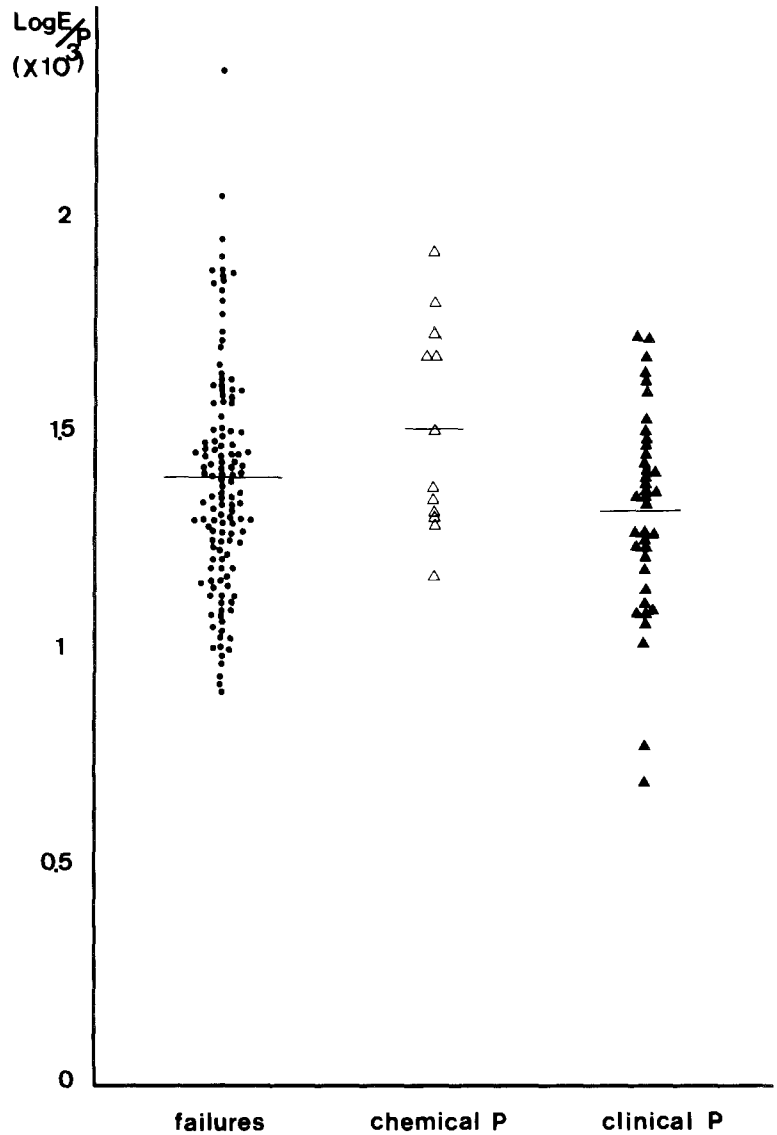

Fig. 4. Comparison of the E_2/P ratio among failures, chemical pregnancies, and clinical pregnancies on day 3 of the luteal phase.

Table I. Endocrine Parameters on Day 3 of the Luteal Phase in IVF

	Clinical pregnancies	Chemical pregnancies	Failures
log progesterone (P)			
(mean ± SD)	1.44 ± 0.23	1.31 ± 0.27 (NS)	1.31 ± 0.27 ($P < 0.05$)
No. of cases	38	12	134
log estradiol (E_2)			
(mean ± SD)	2.77 ± 0.27	2.81 ± 0.25 (NS)	2.72 ± 0.26 (NS)
No. of cases	40	13	141
log $E_2/P \times 10^3$			
(mean ± SD)	1.33 ± 0.24	1.52 ± 0.24 ($P < 0.05$)	1.41 ± 0.27 (NS)
No. of cases	38	12	133

[a] Differences between groups have been assessed by analysis of variance and Tukey test and statistical significances are given versus clinical pregnancies.

mental to the implantation process in humans as has been demonstrated in animal models (21).

We observed a significant trend toward lower values of the E_2/P ratio in relation to clinical pregnancy. Indeed, the E_2/P ratio on day 3 in clinical IVF pregnancies ($25.10^{-3} \pm 12.10^{-3}$) lies mostly within the range of values observed in spontaneous cycles ((24.10^{-3} to 7.10^{-3})), while it is largely higher than the latter in failures and chemical pregnancies (Table III).

The presence of a large subgroup in which arrest of embryonic development was the limiting factor in failed cycles without hCG may account for the lack of a significant difference in E_2/P values between this group and cycles leading to clinical pregnancy. However, when comparing chemical pregnancies to clinical conceptions, a statistically significant difference in the E_2/P ratio on day 3 was found. Since all embryos from both these group proved vigorous enough to secrete detectable amounts of hCG, it is possible that abnormally high values of the E_2/P ratio at the beginning of the luteal phase could prevent the development of proper uterine receptiveness, thereby resulting in faulty implantation.

As reported in this paper, the correlation between levels of the E_2 peak or the number of developing follicles in the follicular phase and P concentrations in the early luteal phase confirms the relationship between the characteristics of the luteal phase and the amplitude of the ovarian response to stimulation. The higher the follicular E_2 values, the more numerous are oocytes and embryos, but also the early luteal phase is improved in terms of P levels and, hence, of the E_2/P ratio. Increased pregnancy rates observed in high responders (22) may thus be related not only to the number of embryos transferred but also to the quality of the luteal phase.

Table II. Comparison of Cycles Ending in Failure and Cycles Ending in Clinical Pregnancy in the Same Patients ($N = 23$)[a]

	Clinical pregnancy	Failure	P
Follicular E_2 peak	1837 pg/ml	1671 pg/ml	NS
Luteal day 3 P	40 ng/ml	29.4 ng/ml	<0.01
Luteal day 3 E_2	821 pg/ml	801 pg/ml	NS
Luteal day 3 E_2/P	21.3	30.6	<0.01

[a] Statistical evaluation effected by Student's t test for paired samples.

As suggested by Lenton *et al.* (18), the early rise of P in conception cycles might be related to a luteotrophic factor secreted by the unimplanted embryo. In our program embryos were replaced about 48 hr after oocyte retrieval and could not have exerted a luteotrophic effect before that time. They were thus not responsible for the higher P levels observed in pregnancy cycles as soon as the first day after oocyte retrieval (19,20). Another explanation would be that implantation preferentially occurs in cycles in which the P concentration rises more quickly.

No negative correlation between luteal E_2 and midluteal P ($r = 0.02$) or luteal-phase duration ($r = -0.12$) was observed. Edwards (23) found an inverse correlation between the average daily estrogen output during the follicular phase of IVF cycles and the luteal-phase duration. We did not confirm such relationship in terms of the amplitude of the follicular estradiol peak, although the latter is bound to reflect the average estrogen secretion in the preceding days. This indicates that, at concentrations observed in the luteal phase of IVF trials, E_2 has no luteolytic effect, although such a mechanism has been demonstrated in monkeys (24). Our data suggest that improvement of success rates in

Table III. Endocrine Parameters of the Luteal phase (Mean ± SE) in Clinical Pregnancies (Cl), Chemical Pregnancies (Ch), and Failures (F)

	Day					
	0	1	2	3	8	12
E_2 (pg/ml)						
Cl	668 ± 52	282 ± 30	490 ± 54	643 ± 53	565 ± 60	520 ± 45
Ch	808 ± 111	344 ± 92	537 ± 161	752 ± 128	750 ± 120	320 ± 55
F	765 ± 34	321 ± 21	450 ± 24	621 ± 33	525 ± 55	140 ± 25
P (ng/ml)						
Cl	4.1 ± 0.4	9.7 ± 0.9	17.7 ± 1.8	30.6 ± 2.4	19.4 ± 2.1	41.2 ± 8.4
Ch	3.5 ± 0.4	7.1 ± 1.1	12.8 ± 1.4	20.7 ± 3.1	19.4 ± 4.0	13.2 ± 7.0
F	3.9 ± 0.2	7.5 ± 0.4	15.2 ± 0.8	23.8 ± 1.1	22.6 ± 2.2	5.2 ± 0.8
$E_2/P \times 10^3$						
Cl	187 ± 18	33 ± 3	31 ± 3	24 ± 2	36 ± 4	15 ± 4
Ch	247 ± 36	51 ± 9	43 ± 7	41 ± 6	41 ± 6	24 ± 6
F	224 ± 13	64 ± 11	36 ± 3	32 ± 2	37 ± 4	26 ± 3

IVF might depend on (i) stimulation aimed at shifting more patients into the high-responder group, since the quality of the early luteal phase seems to be related to the height of the follicular E_2 peak, and (ii) early luteal supplementation with P, which should start soon after oocyte retrieval.

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