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Assessment and Hormonal Treatment of the Luteal Phase of In Vitro Fertilization Cycles

John L. Yovich, MRCOG, FRACOG

Department of Obstetrics and Gynaecology, University of Western Australia, King Edward Memorial Hospital, Perth, Western Australia

and

James D. Stanger B.Sc. (Hons), Jeanne M. Yovich B.Sc. and Ann I. Tuvik SRN

PIVET Laboratory, Cambridge Hospital, Wembley, Perth, Western Australia

Summary: Luteal phase lengths and hormonal profiles (progesterone, oestradiol-17 beta, beta HCG and prolactin) have been documented in 77 cases derived from a series of patients undergoing IVF. Nineteen pregnancies were generated during this series and 12 healthy infants have already been delivered. Luteal phase lengths were 14.5± 0.5 days with 14.3% demonstrating mid-luteal progesterone levels of less than 31 nmols/l, considered to be low for successful conception. A random study of luteal support regimens comparing HCG or medroxyprogesterone acetate (MPA) with nil therapy was studied in a series of 44 consecutive embryo transfers during which 10 pregnancies were achieved. No difference was noted in the pregnancy rates for the 3 groups but the pregnancy outcome was better in those who had HCG support and this was more apparent in the overall series of 19 pregnancies. A significant luteotrophic effect was noted with HCG support regimens whilst MPA appeared to have a luteal suppressant action. Six pregnancies which aborted with blighted ova were derived from cycles in which the luteal phase progesterone levels were low raising the possibility that a poor hormonal environment may predispose to blighted ova.

Western Australia's first in-vitro fertilization (IVF) pregnancy was achieved during a pilot study on 42 women with occlusive tubal disease whose cycles were stimulated with clomiphene(1). Luteal phase studies indicated normal luteal lengths but 22% revealed a serum progesterone level of less than 31 nmol/l which is below the conception range for stimulated cycles(2) indicating an inadequate luteal phase. Subsequently a fully developed programme of IVF has been established at PIVET Laboratory with further pregnancies generated and a total of 21 infants have now been delivered(3). A high spontaneous abortion rate of 31.6% was noted, similar to that reported by others(4,5,6). Although one phase of the programme generated a pregnancy rate of 22.7% per embryo transfer, the rates fluctuate and our overall success rate is 10%. The relatively low successful implantation rate may be due to embryonic abnormalities which are not obvious on morphological inspection, defective embryo transfer techniques or an unsatisfactory intrauterine environment. The latter possibility arising from hormonal inadequacy is the subject of this study which investigates hormonal profiles during the luteal phase of conception and non-conception IVF cycles and the effects of luteal support therapy.

Reprint Requests:
Dr. J. L. Yovich,
Department of Obstetrics & Gynaecology,
University of Western Australia,
King Edward Memorial Hospital,
Subiaco, Perth, Western Australia 6008

MATERIALS AND METHODS

The PIVET Laboratory IVF programme was initiated in June, 1982. Patients selected into this

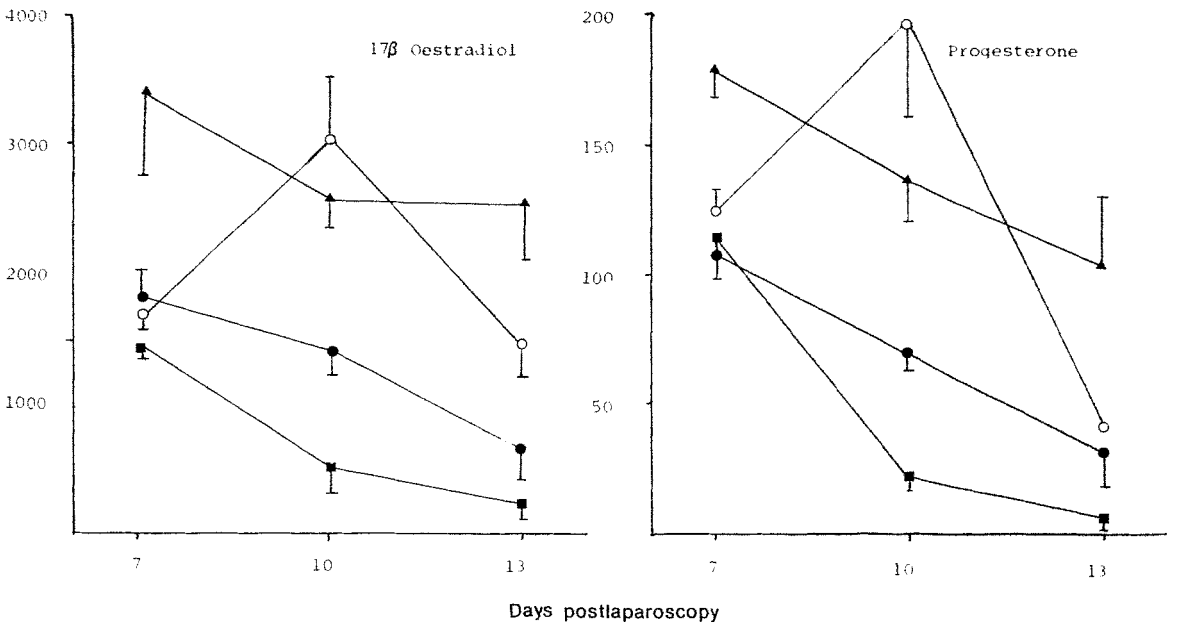

Figure 1. 17β Oestradiol (pmols/l) and progesterone (nmols/l) concentrations in non-conception IVF cycles on days 7, 10 and 13 postlaparoscopy under varying luteal support treatments.
● no support (n = 21); Δ HCG days 4, 7, 10, 13 (n = 32); ○ HCG days 7, 10 and 13 (n = 6); ■ MPA daily (n = 18).

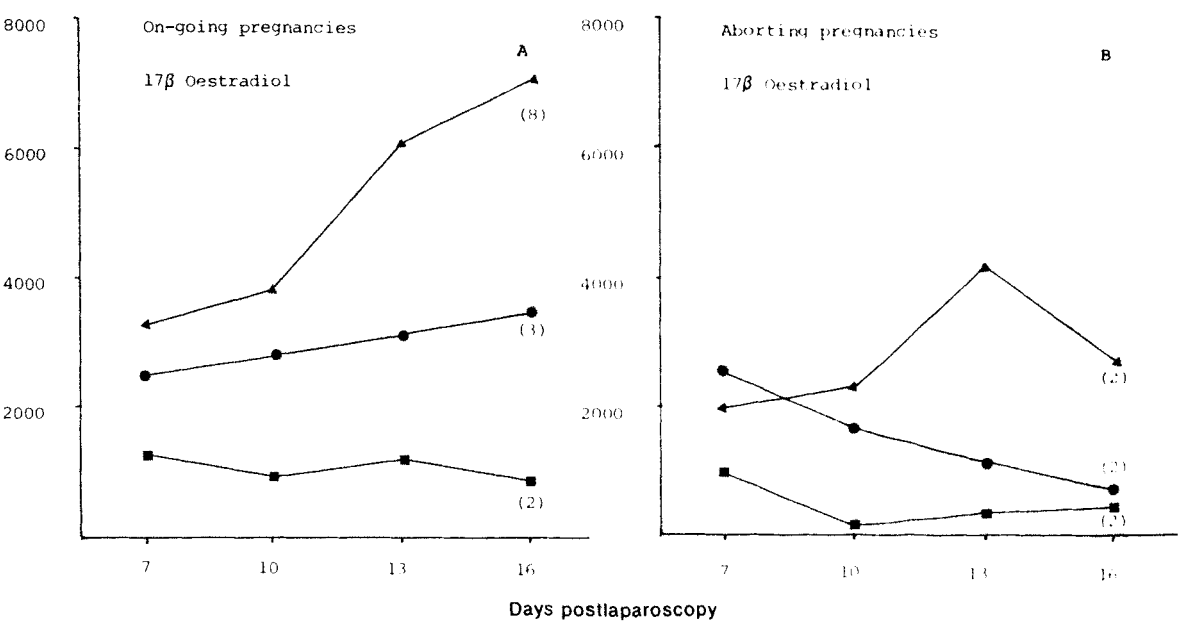

Figure 2. Serum 17β Oestradiol concentration (pmols/l) on days 7, 10, 13 and 16 postlaparoscopy in ongoing (n = 13) and unsuccessful (n = 6) IVF conception cycles under various luteal treatment regimens.
● no treatment; Δ HCG days 4, 7, 10, 13; ■ MPA daily. (n) = number of patients.

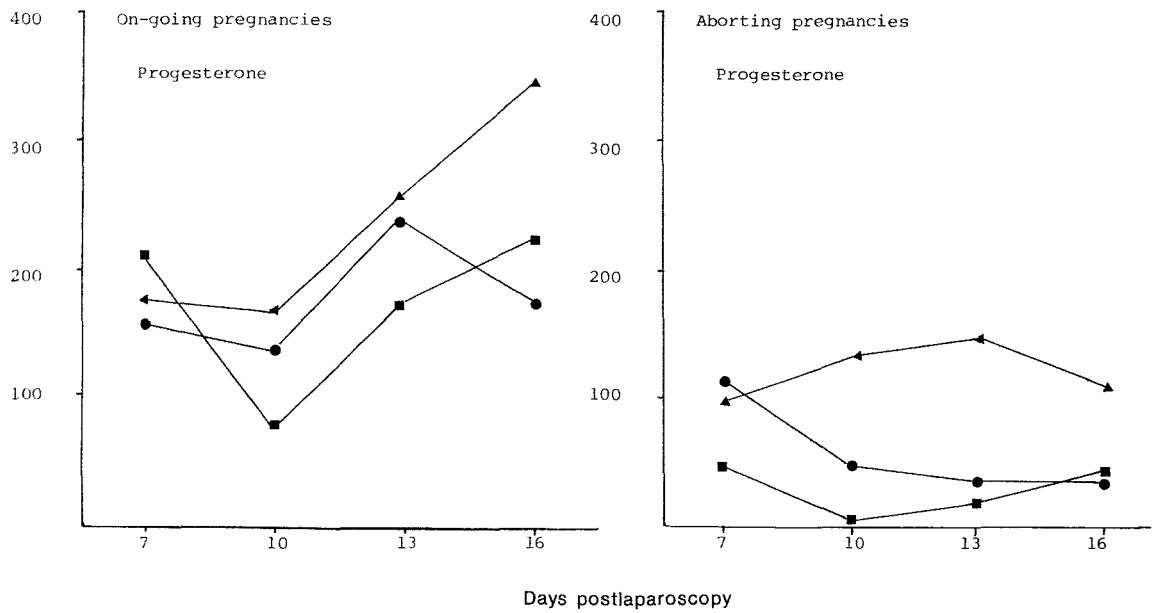

Figure 3. Serum progesterone concentrations (nmols/l) on days 7, 10, 13 and 16 postlaparoscopy of ongoing and aborting IVF conception cycles under varying luteal treatments.
Legend as in figure 2.

study have significant tubal disease underlying their infertility; all are stimulated with clomiphene alone or clomiphene with added human menopausal gonadotrophin (HMG). Oocyte recovery is undertaken at laparoscopy approximately 36 hours after an HCG injection or 28-30 hours after the beginning of a spontaneous LH surge. Ovarian follicles are aspirated using a 30 cm, double cannular needle which allows both suction and flushing to be applied simultaneously if required. The majority of follicles require 1 or 2 flushes with fertilizing solution containing added heparin to recover the oocyte. It is noted that repeated flushing tends to collect more of the membrana granulosa, especially from the larger follicles. The laparoscopy aspiration day is defined as day 0.

Patient Selection

The data reported in figure 1 is derived from 77 consecutive patients who had complete luteal phase analyses. Four regimens of luteal support therapy were studied — (A) nil; (B) HCG (Primogonyl, Schering, Berlin) 1,000 units on days 4, 7, 10 and 13 of the luteal phase; (C) medroxyprogesterone acetate (MPA), (Provera, Upjohn, U.S.A.) 10 mg qid beginning on day 1 until day 16 of the luteal phase and (D) HCG on days 7, 10 and 13. The results reported on conception cycles in figures 2, 3 and 4 are an analysis of 19 pregnancies arising from 206 patients proceeding to laparoscopy. The information in table 1 is derived from 44 consecutive patients proceeding through to embryo transfer who were randomly allocated into 3 groups for hormonal support therapy. The allocation was at the onset of the cycle and those who did not proceed to transfer were not replaced.

Assay Methods

Analyses were undertaken on early morning serum samples. Oestradiol-17 beta (E2) was assayed by a non-extraction double antibody radio immunoassay (Mallenkrodt, Australia), progesterone (P4) by Coat-a-Count solid phase radio immunoassay (Diagnostic Products, California) and both quantitative beta HCG and prolactin by double antibody immunoassay (Amerlex, Amersham, England). Data is presented with standard error limits.

The 95% confidence limit of sensitivity for beta HCG is 4 Iu/*l*. However as some patients were receiving HCG injections, pregnancy was diagnosed in this study only when a gestational sac was confirmed on ultrasound and the quantitative beta HCG level was greater than 30 units and rising after day 16.

RESULTS

Short luteal phases were not noted and those who had no luteal support therapy had luteal lengths of

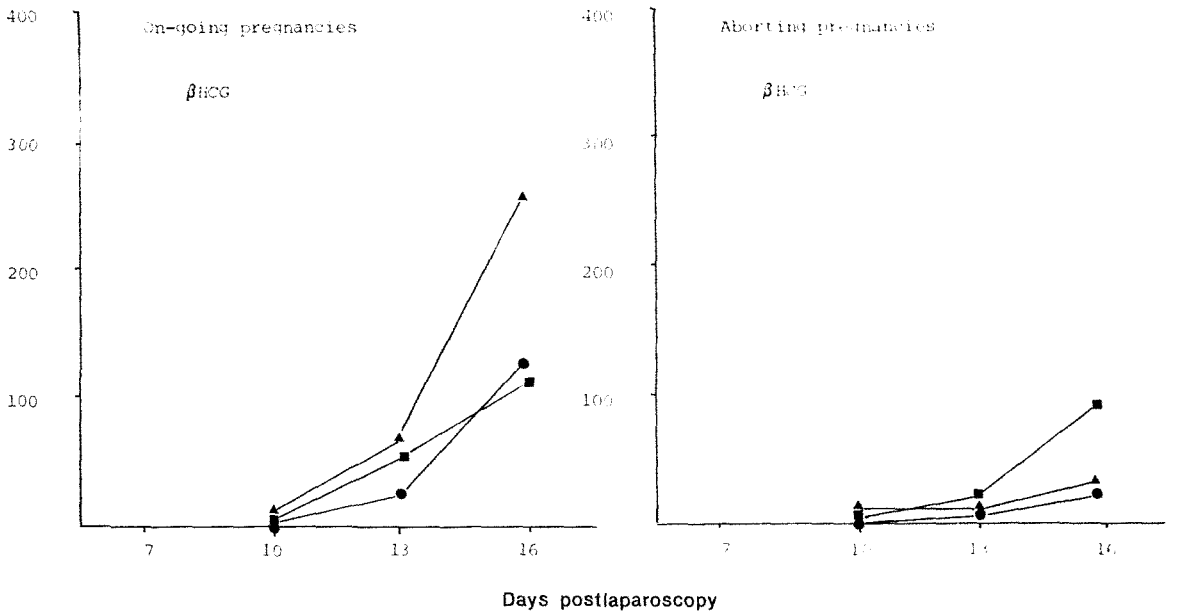

Figure 4. Serumβ HCG concentrations (Iu/l) on days 10, 13 and 16 postlaparoscopy of ongoing and aborting IVF conception cycles under varying luteal treatments. Legend as in figure 2.

14.5± 0.5 days and were significantly prolonged (P<0.05) when both HCG or MPA were given. The last injection of HCG was given on day 13 and the luteal lengths were 16.9± 0.5 days. MPA was ceased by day 16 and the luteal lengths were 17.6± 0.8 days. In figure 1 the luteal levels for E2 and P4 are recorded for the 4 regimens of support in non-conception cycles. In this series the average mid-luteal value of E2 observed was 2,408 pmols/l and P4 was 141.8 nmols/l. Of 21 patients on nil support, 3 (14.3%) demonstrated P4 levels less than 31 nmols/l in the mid-luteal range (days 7 and 10). Patients given HCG injections in regimen B had increased E2 and P4 levels (P<0.5) on day 7 compared with those on nil support. Both E2 and P4 levels were sustained past the mid-luteal phase and were significantly higher on day 13 (P<0.01).

Table 1. IVF Pregnancies with Luteal Support Therapy. Pregnancies and Abortions Arising from IVF with HCG (1,000 U days 4, 7, 10, and 13), MPA (40 mg daily) or Without Luteal Support from 49 Cases Randomly Allocated at the Beginning of the Treatment Cycle of which 44 Proceeded to Laparoscopy.

Luteal Support Treatment	Pregnant (No. aborting)	Not Pregnant	Total (pregnancy rate/ laparoscopy)
NIL	3 (2)	7	10 (30%)
HCG	3 (0)	14	17 (17%)
MPA	4 (2)	13	17 (31%)
TOTAL	10 (4)	34	44 (23%)

The 6 patients on regimen D were observed to have E2 and P4 levels similar to the nil support group on days 7 and 13, but on day 10 the 2 hormones were significantly greater (P<0.001). Those patients receiving MPA however (regimen C), whilst demonstrating hormonal levels on day 7 similar to nil support showed a significant reduction in both P4 and E2 by day 10 (P<0.001) compared with the nil treatment group. By day 13 the MPA and nil treatment groups were similarly low. An assessment of daily P4 levels from day 0 to 4 comparing nil therapy (n=14) and MPA (n=59) revealed no significant difference and it was noted that levels reached 71.48±/6.8 nmols/l by day 2.

The randomized allocation of 3 luteal support regimens presented in table 1 showed no difference in pregnancy rate for the 3 groups but the outcome was better in that less abortions occurred in Group B with HCG support. The numbers are too small for statistical validation.

Of the 19 conceptions generated, 6 have subsequently aborted. Figures 2, 3 and 4 provide a comparison of the E2, P4 and beta HCG levels for ongoing and unsustained pregnancies. Ten of the pregnancies arose in patients on HCG support injections given because of suspected luteal inadequacy demonstrated during the preliminary work-up. Ongoing pregnancies arising from the HCG support regimen showed apparently higher E2 levels on day 13 and higher E2, P4 and beta HCG levels

on day 16 in comparison to the nil therapy group. Similarly pregnancies arising on MPA therapy demonstrated lower E2 levels throughout the luteal phase including day 16. There was no difference with P4 and beta HCG levels. In conception cycles on MPA the levels of P4, generally lower than the other 2 groups on day 10 were nevertheless higher than in non-conception cycles. In pooled data, P4 was significantly lower ($P<0.05$) on days 7 and 10 for unsuccessful pregnancies and this was reflected in decreased beta HCG levels on days 13 and 16. Although the E2 levels were lower the variability was high and significance was not demonstrable until day 16.

Of the 6 abortions in this series, 3 developed poor gestational sacs on ultrasound whilst the other 3 developed advanced anembryonic sacs between 10 and 13 weeks' gestation. Of the 19 pregnancies 2 of 5 cases on nil support aborted, 2 of 4 cases on MPA aborted, but only 2 of 10 cases on HCG support aborted. Again the figures are small but a trend is inferred regarding the benefit of HCG support.

Completion of luteal phase assessments included mid-luteal prolactin estimations. The majority were well within the normal range (25-400 mIu/L) and only occasional high levels (400-900 mIu/L) were noted, more often in those who had conceived.

DISCUSSION

It has been reported that follicle aspiration for IVF is associated with disruption of the luteal phase(7) although significant changes were not detected in a study from Adelaide(8) with similar aspiration techniques. Our pilot study observations (22%) and this series (14.3%) with low mid-luteal progesterone levels suggest some disturbance of the luteal phase in a proportion of cases. We have achieved a high oocyte retrieval rate (98% per laparoscopy) using a double-lumen aspirator to allow ready flushing of the follicle but noted that repeated flushing tended to retrieve more of the membrana granulosa possibly creating a deficient corpus luteum. As several follicles are usually induced by the ovulation induction regimen, it is likely that the additive effect of several corpora lutea may maintain an adequate hormonal milieu despite poor steroid output from any single corpus luteum. The majority of patients analysed in this series did not demonstrate abnormally low steroid profiles in the mid-luteal phase but differences were noted in patients given luteal support therapy when compared with no treatment.

Boost injections of HCG appear to be luteotrophic in that E2 and P4 output increased and remained elevated longer during the luteal phase. High levels are still present on day 13 and the luteal phase was 2.5 days longer. The effect begins soon after the first injection and hence high levels can be demonstrated on day 7 in those who started on day 4. Although more pregnancies generated from our programme have had HCG support therapy, we were not able to demonstrate any benefit in terms of the pregnancy rate in a randomly allocated series. However, there was a better outcome for pregnancies following HCG support although the series is too small to validate. It is postulated that the high steroid levels on day 16 correlate with higher beta HCG levels noted in conception cycles following HCG therapy. The results suggest a bilateral recognition response between the implanted embryo and corpora lutea with each being trophic to the other, increasing their respective hormonal outputs. It may be that early endometrial exposure to the high steroid environment improves local factors for better embryo and trophoblastic development. It has been suggested(9) that high progesterone concentration during the early part of the luteal phase may constitute a pre-implantation component of the maternal recognition of pregnancy in women. Conversely the effects of MPA appear to be luteal suppressant in that E2 and P4 levels were significantly low on day 10. We had decided to utilize this progestogen since pure progesterone in a convenient short acting form is not available for human therapy in Australia. MPA rarely produces maternal side-effects and appears to have a high degree of safety regarding fetal side-effects(10). The reduced P4 output during the luteal phase has been reported with a number of protestogens, including MPA(11) and the effect is known to be reversed by HCG. P4 levels were higher on day 7 and rose by day 10 in successful pregnancies arising from the MPA schedule, but the E2 level showed no such response. We have observed this effect in patients outside this study, and in 2 patients given combined MPA and HCG such abnormally low progesterone levels were not seen (1 became pregnant), supporting the contention that MPA suppresses P4 production by enzyme inhibition(12) and this can be reversed by endogenous or exogenous HCG. It is useful to note however that the study by Shinada et al(12) was an in-vitro experiment and that progesterone itself was equally inhibitory to pregnenolone conversion. Serum P4 levels with MPA are maintained until somewhere between days 7 and 10 and this is of interest in that studies to induce luteolysis(13) indicate that the corpus luteum is highly resistant to luteolytic action during the first 5 days after the LH peak. One might expect therefore that embryos which develop more slowly to the expanded and hatched blastocyst stage might be attempting to implant when there is less reversibility of the luteolytic action of MPA.

The spontaneous abortions arising from this IVF series appear to be blighted ova. Whether the

phenomenon arises from abnormal embryos or from an abnormal hormonal milieu is unclear, but the latter is a possibility given the association with low steroid levels in the mid and late luteal phase of unsuccessful pregnancies although an intrinsic anomaly with the embryo may limit its ability to stimulate the corpora lutea. A comparison of hormone profiles for successful and unsuccessful pregnancies in our series reveals that the prognosis was evident before the pregnancy was clearly diagnosed.

Overall, our studies do not indicate a significant detectable disturbance in corpus luteal activity when assessed by luteal lengths and hormone profiles. However, we have revealed a luteotrophic response to HCG injections given in the luteal phase which may benefit the pregnancy outcome and a luteal suppressant action of MPA, which although not appearing to inhibit the pregnancy rate, did not provide benefit over the nil therapy group.

Acknowledgements

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