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Hormonal profiles in the follicular phase, luteal phase and first trimester of pregnancies arising from in-vitro fertilization

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Summary. The hormonal profiles for oestradiol-17β, progesterone, prolactin and β-human chorionic gonadotrophin (β-hCG) are documented for the first 24 pregnancies arising from in-vitro fertilization during a collaborative project between the University of Western Australia and PIVET Laboratory. All patients had ovarian follicle stimulation with clomiphene citrate, sometimes combined with human menopausal gonadotrophin and all had oocyte recovery undertaken 36 h after injection of 5000 i.u. hCG. The follicular phase profile indicated that patients were admitted for the hCG injection when oestradiol-17β levels were around 1500 pmol/l per follicle with a dimension of ⩾1·6 cm on ultrasound. Luteal phase data indicated that oestradiol-17β and progesterone levels were two to three times higher than that expected during spontaneous conception cycles and those pregnancies which subsequently aborted had significantly lower levels in the late luteal phase. During pregnancy elevated oestradiol-17β and progesterone levels were maintained through the early weeks during organogenesis while the β-hCG profile was similar to that reported for spontaneous pregnancies arising without ovarian stimulation. Six women aborted and the other 18 pregnancies have generated 22 infants.

The first pregnancies leading to viable offspring by in-vitro fertilization and embryo transfer were achieved without ovarian stimulation (Steptoe & Edwards 1978; Lopata *et al.* 1980; Edwards *et al.* 1980). Greater efficiency and improved pregnancy rates have followed oocyte recovery in stimulated cycles (Trounson *et al.* 1981; Speirs *et al.* 1984) and most current reports concern the use of clomiphene citrate, human menopausal gonadotrophin (hMG) or a combination of both (Speirs *et al.* 1984; Garcia *et al.* 1983; Yovich *et al.* 1984*a*,*b*). Oocyte recovery is generally undertaken 34 to 36 h after an injection of human chorionic gonadotrophin (hCG) or following a spontaneous LH surge (26–32 h after onset detected in urine and 32–36 h after detection in serum). To date, the luteal phase has remained largely unsupported by any therapy although some reports indicate that hCG, progesterone and progestogens have been used (Feichtinger *et al.* 1982; Yovich *et al.* 1982, 1984*c*). This report covers the hormonal details for the first 24 pregnancies generated in the University of Western Australia/PIVET Laboratory in-vitro fertilization programme and provides a unique opportunity to assess the follicular and luteal phase characteristics for in-vitro fertilization cycles during which pregnancy was

achieved. It also allows a comparison of prospective hormonal data for ongoing and aborting pregnancies.

Patients and methods

The first pregnancy from the University of Western Australia/PIVET Laboratory in-vitro fertilization project arose from a small pilot study and led to the birth of a child in July 1982 (Yovich *et al.* 1982). Subsequently a larger programme combining both service and research was developed (Yovich *et al.* 1984*b*) and the additional 23 pregnancies were generated over 12 months between July 1982 and July 1983.

The clinical details of the 24 patients is the subject of a separate report. The majority were included in the in-vitro fertilization programme because of irreparable tubal disease, but some had been included with endometriosis, oligospermia (Yovich & Stanger 1984), heterologous sperm antibodies (Yovich *et al.* 1984*a*), unexplained infertility and one who failed to conceive despite successful suppression of hyperprolactinaemia using bromocriptine. All patients were stimulated from day 2 of the cycle, 15 with clomiphene citrate alone and nine with clomiphene citrate/hMG (C/hMG). Follicle development was monitored by daily ultrasonography, cervical scores and a serum hormonal profile (oestradiol-17β, progesterone and luteinizing hormone [LH]) as previously described (Yovich *et al.* 1984*b*). Each was given hCG about 36 h before oocyte recovery. Generally, those patients who had initiated an LH surge before the hCG injection were not scheduled for ovum aspiration, hence none of the 24 patients who conceived had ova collected following a spontaneous LH surge. The oocyte recovery, embryo culture and embryo transfer techniques have been fully described (Yovich *et al.* 1984*b*).

Sampling and assay methods

Hormonal assays were undertaken on early morning serum samples. The follicular phase was monitored daily for 5–8 days before oocyte recovery which was designated as Day 14 (fixed reference point) with the follicular and luteal days adjusted accordingly. Patients admitted for the hCG trigger injection had a blood sample collected immediately before the injection for LH assay in order to determine retrospectively whether a spontaneous surge was underway. In each case a mid-luteal blood sample was taken on day 21 for serum oestradiol-17β, progesterone, and prolactin and thereafter every third day for oestradiol-17β, progesterone and β-hCG until pregnancy was diagnosed by an elevated β-hCG level of ≥30 i.u./l on or after day 30 and rising on subsequent samples. Day 30 was selected to avoid possible confusion in those cases given hCG luteal support therapy. Thereafter a weekly serum sample was collected and assessed for oestradiol-17β, progesterone and β-hCG until 12 weeks gestation with a final sample at 16 weeks.

Oestradiol-17β and LH were assayed by a non-extraction double-antibody radioimmunoassay (Mallenkrodt, Melbourne, Australia), progesterone by Coat-a-Count solid phase radioimmunoassay (Diagnostic Products, Los Angeles, California) and both quantitative β-hCG and prolactin by double-antibody immunoassay (Amerlex, Amersham, England). The 95% confidence limit of sensitivity for β-hCG is 4 i.u./l.

Luteal support

Some patients conceived during a randomized study of luteal support comparing intramuscular hCG (1000 i.u. on days 18, 21, 24 and 27) and oral medroxyprogesterone acetate (MPA) (40 mg/day from day 15) with no therapy. That study has been reported (Yovich *et al.* 1984*c*) noting no difference in the pregnancy rates for the three groups, but an apparently better outcome for those pregnancies given hCG support. Blood samples for hormone assays were always taken before the hCG injections.

Early pregnancy support

Patients who presented with any vaginal bleeding after pregnancy was diagnosed were given oral MPA (80 mg to 120 mg/day) until 16 weeks gestation in an attempt to prevent abortion. In patients whose β-hCG levels continued to fall and ultrasound confirmed a blighted ovum or missed abortion, MPA was stopped and curettage undertaken. Histology on the evacuated uterine contents of the six patients with spontaneous abortion confirmed trophoblastic tissue on each occasion. Three patients developed poor gestational sacs on ultrasound and aborted in the early weeks whilst three developed advanced anembryonic sacs, aborting between 10 and 12 weeks gestation.

All data are recorded with arithmetic means and standard error (SEM) limits. The hormonal data were converted to logarithmic values before being analysed by single analysis of variance (Zar 1974) except for the luteal hormone levels which were compared by Students *t*-test.

Results

By day 13 the 15 patients stimulated with clomiphene citrate alone generated 46 ovarian follicles with dimensions of ≥1·6 cm on ultrasound with an overall mean diameter of 1·89 (SEM 0·06) cm to yield an average of 3·1 follicles per patient. Oocytes were recovered from 41 follicles (89%) with 35 (85%) developing to the stage of cleavage. However only 34 embryos were transferred as one contained three pronuclei at 16 h after insemination. Of the nine patients given hMG, 51 large follicles developed, with an average of 5·6 per patient. Forty-one oocytes were recovered (81%) with 34 cleaving embryos generated (83%), all of which were transferred.

Follicular phase

The follicular phase lengths measured from the onset of the cycle (first day of menstrual bleeding) until the day of aspiration was similar in the clomiphene citrate (14·4, SEM 0·32 days) and C/hMG groups (14·4, SEM 0·45). The follicular phase oestradiol-17β levels are shown in Fig. 1 along with changes in the cervical score. The mean oestradiol-17β level per follicle of ≥1·6 cm was 1586 (SEM 154·0) pmol/l in the clomiphene citrate alone group and 1379 (SEM 139·5) pmol/l in the C/hMG group on day 12 when the decision to give the hCG trigger was made. The level of rise of oestradiol-17β was significantly greater for those with added hMG throughout the follicular growth phase up to day 12, ($0·001<P<0·01$). A significant rise in oestradiol-17β levels was also noted between day 12 and the day after hCG (day 13) in the C/hMG group ($0·01<P<0·05$) but not confirmed in the clomiphene citrate group. A concomitant rise in the cervical mucus score (Insler *et al.* 1972) was often used as an additional index for the timing of hCG when the oestradiol-17β levels were rising. Although a slightly higher level was noted on day 12 for the C/hMG group, the cervical scores were similar on the day after, with both groups scoring 9 points out of a possible maximum of 12. A small proportion of patients failed to demonstrate a rise in cervical score despite rising oestradiol-17β levels and enlarging follicles. The LH levels are shown in Fig. 2 along with progesterone which was used as a supplementary index of luteinization. The LH levels were not significantly different between the clomiphene citrate and the C/hMG group. From day 8 to day 12, LH levels appeared to fall but the drop was not statistically significant ($0·05<P<0·10$). From

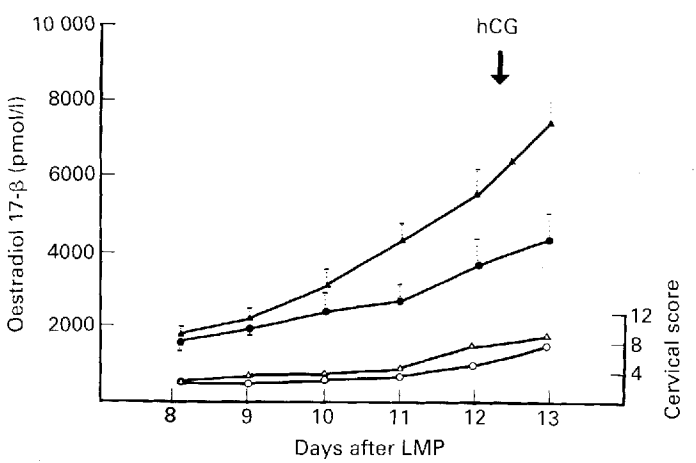

Fig. 1. Change in follicular phase serum oestradiol-17β (solid symbols) concentration and cervical score (open symbols) with time before and after hCG administration (arrow) in clomiphene citrate (● ○) and clomiphene citrate/hMG (▲ △) stimulated in-vitro fertilization conception cycles. LMP, Last menstrual period.

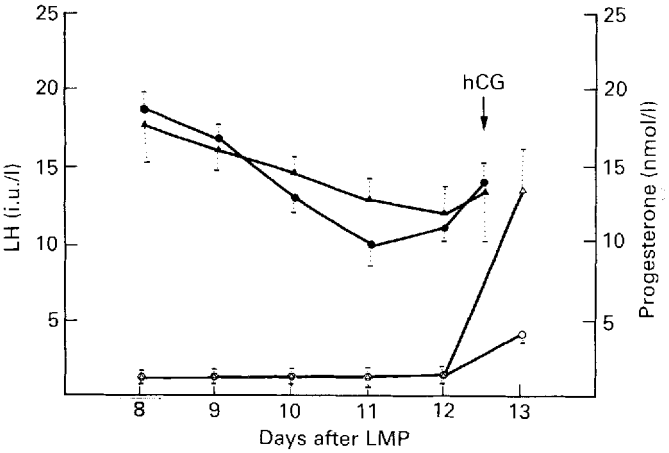

Fig. 2. Change in follicular phase serum LH (solid symbols) and progesterone (open symbols) concentrations with time before and after hCG administration (arrow) in clomiphene citrate (● ○) and clomiphene citrate/hMG (▲ △) stimulated in-vitro fertilization conception cycles. LMP, Last menstrual period.

day 12, LH values were increased slightly in the evening blood sample before the hCG injection although in no case was the value >2SD above the follicular phase mean for the patient and progesterone levels had not risen implying that the patient had not yet started an LH surge. Progesterone levels did not change from day 8 to day 12 but rose significantly after the hCG injection ($P<0{\cdot}001$), the rise being greater in the C/hMG group ($P<0{\cdot}05$).

Luteal phase

Luteal phase oestradiol-17β levels are shown in Fig. 3 with ongoing pregnancies charted according to their follicular phase stimulation regimen,

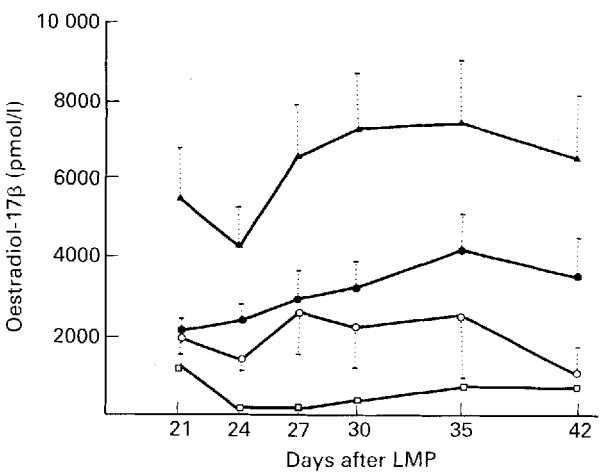

Fig. 3. Serum oestradiol-17β concentration in the mid- and late luteal and early pregnancy phase of successful (solid symbols) and unsuccessful (open symbols) in-vitro fertilization conceptions as a function of the regimen of follicle stimulation (●, clomiphene citrate; ▲, clomiphene citrate/hMG; ○, combined regimen MPA; □, clomiphene citrate/MPA in luteal phase). LMP, Last menstrual period.

and the aborting group categorized separately. The luteal phase data demonstrates high oestradiol-17β levels (mean 3329, SEM 555 pmol/l) at the mid-luteal point with persisting elevation throughout the luteal phase and into early pregnancy. It can be seen that the C/hMG group had higher oestradiol-17β levels throughout the luteal phase. Ten patients had hCG support injections and three had been given MPA for luteal support, but no significant difference was noted in oestradiol-17β levels for ongoing pregnancies given luteal support. There was a plateau effect from the luteal phase into the first weeks of pregnancy. Those patients who subsequently aborted did not show significantly reduced oestradiol-17β levels when compared with the ongoing clomiphene citrate group until after day 30 of the cycle, unless they had been given MPA support therapy when oestradiol-17β levels were markedly reduced from day 24 (two observations only). Similar findings were noted for progesterone (Fig. 4) where it can be seen that overall progesterone rose significantly from the beginning to the end of the luteal phase ($0{\cdot}01<P<0{\cdot}05$) when levels also plateaued into early pregnancy. This rise was more pronounced in the C/hMG group ($0{\cdot}001<P<0{\cdot}01$) than the clomiphene citrate only group ($0{\cdot}05<P<0{\cdot}10$). The mean mid-luteal progesterone level was 155 (SEM 92·4) nmol/l on day 21 with persisting elevation into early pregnancy. Again it can be seen that the aborting group had lower progesterone levels with a gradual reduction from day 30. Those having MPA support were also low throughout the luteal phase. These findings predated the abortion process by 2 to 8 weeks although the diagnosis was undoubtedly delayed by the use of MPA in some patients. Advanced anembryonic sacs were demonstrated in three patients at between 10 and 13 weeks gestation.

Quantitative β-hCG levels are shown in Fig. 5. Ongoing pregnancies had β-hCG levels of ≥30 i.u./l by day 27 and no difference was noted in the clomiphene citrate and C/hMG groups. Aborting pregnancies failed to reach diagnostic levels of hCG until day 30 and values were significantly lower than in ongoing pregnancies ($0{\cdot}01<P<0{\cdot}001$). Subsequently, apart from one patient in the C/hMG group with a normal high value on day 35, hCG levels in those destined to abort continued to demonstrate widening disparity from the ongoing group. Patients stimulated with clomiphene citrate and given either MPA or hCG support during the luteal phase showed no difference in β-hCG levels from unsupported cycles. Mid-luteal (day 21) prolactin estimates revealed similar levels for the clomiphene citrate (mean 353, SEM 82·5 nmol/l) and C/hMG groups (292, SEM 81·2 nmol/l) for ongoing pregnancies but the

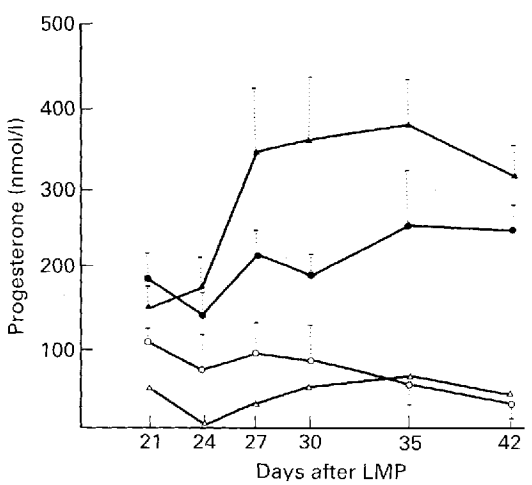

Fig. 4. Serum progesterone concentration in the mid- and late luteal and early pregnancy phase of successful (solid symbols) and unsuccessful (open symbols) in-vitro fertilization conceptions as a function of the regimen of follicle stimulation (●, clomiphene citrate; ▲ clomiphene citrate/hMG; ○, combined regimen MPA; △ clomiphene citrate/MPA in luteal phase). LMP, Last menstrual period.

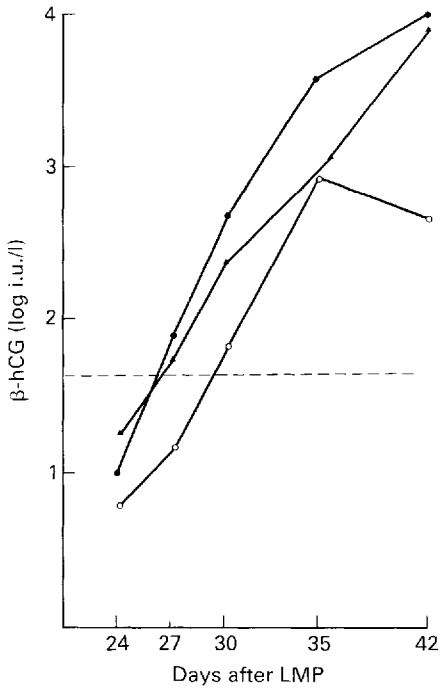

Fig. 5. Serum β-hCG concentrations in the late luteal and early pregnancy phase of unsuccessful (○) conceptions and successful clomiphene citrate (●) and clomiphene citrate/hMG (▲) stimulated in-vitro conceptions. Patients were considered pregnant when the concentration exceeded 30 i.u./l (dotted line).

patients who subsequently aborted (five clomiphene citrate and one C/hMG) tended to have lower values (mean 173, SEM 38·1 nmol/l) ($0{\cdot}05<P<0{\cdot}1$).

Pregnancy

The combined oestradiol-17β, progesterone and quantitative β-hCG information was recorded weekly to 16 weeks gestation for the 18 ongoing pregnancies in this study (Fig. 6 and Table 1). It can be seen that the luteal phase mean levels of progesterone gradually declined by week 7 of pregnancy to 196 (SEM 35) nmol/l with values maintained until week 11. An apparent decline thereafter was not confirmed at the 16 week observation. Oestradiol-17β levels were maintained at luteal values until week 11 when a significant rise was noted in the ensuing observations including week 16 ($0{\cdot}01<P<0{\cdot}05$). Quantitative β-hCG estimations continued rising rapidly with a peak of 70 296 (SEM 14 762) i.u./l centered around weeks 9 to 11, declining to plateau levels at 18 350 (SEM 4 917) i.u./l units from week 12.

From Table 1 relevant hormonal data has been documented with reference to the use of MPA during the luteal phase of the cycle and during the pregnancy. It can be seen that those patients who were given MPA during the luteal phase and continuing into pregnancy (MPA/MPA), demonstrated overall lower oestradiol-17β and progesterone levels throughout the first trimester when compared with those patients who had no support therapy and those who had MPA support after the pregnancy was diagnosed. Occasional low β-hCG levels in the MPA/MPA group are of doubtful significance given the exponential rise of hCG, the small number of observations and the fact that a single

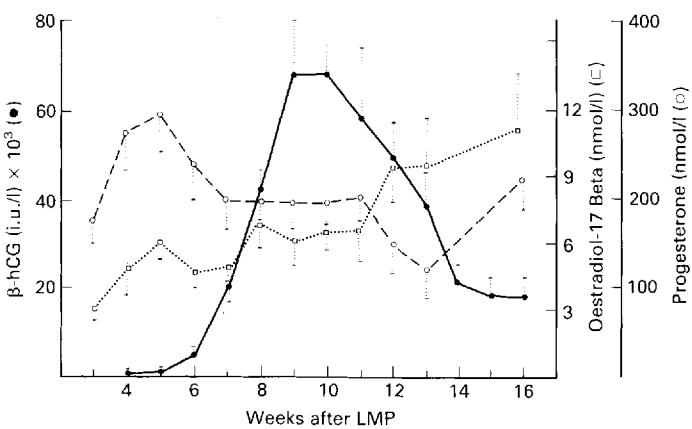

Fig. 6. The change in serum oestradiol-17β (□), progesterone (○) and hCG (●) over the first trimester of successful in-vitro fertilization conceptions arising from clomiphene citrate and clomiphene citrate/hMG stimulated cycles. LMP, Last menstrual period.

Table 1. Partition of weekly serum oestradiol-17β, progesterone and β-hCG concentrations during the first trimester of in-vitro fertilization conceptions as a function of exposure to medroxyprogesterone acetate (MPA) during the luteal phase or pregnancy

Hormone	MPA[a]	No. of patients	Gestation (weeks from last menstrual period)													
			Week 5		Week 6		Week 7		Week 8		Week 9		Week 10		Week 11		Week 12		Week 16	
Oestradiol-17β																				
(pmol/l)	−/−	6	5047	(954)	4285	(1013)	4873	(583)	8220	(2452)	5974	(1659)	7628	(1581)	7903	(2535)	8530	(2386)	13605	(1852)
	−/+	9	7045	(956)	6173	(988)	6151	(864)	7229	(631)	7328	(1374)	7624	(958)	8085	(2116)	11621	(3707)	17749	(7071)
	+/+	3	2196	(151)	1930	(290)	1922	(241)	2630	(359)	3635	(654)	3447	(847)	2762	(1122)	4810	(487)	11682	(2916)
Progesterone																				
(nmol/l)	−/−	6	311	(68)	178	(50)	112	(31)	132	(32)	166	(49)	135	(21)	123	(22)	118	(33)	189	(20)
	−/+	9	381	(60)	307	(51)	268	(48)	312	(45)	230	(30)	268	(48)	275	(40)	249	(45)	249	(39)
	+/+	3	150	(20)	168	(37)	123	(48)	60	(27)	59	(28)	89	(13)	127	(26)	101	(24)	140	(58)
β-hCG (i.u./l)	−/−	6	3205	(942)	10400	(3639)	34400	(8857)	75156	(27430)	68468	(12933)	60633	(7019)	40400	(5397)	36825	(6924)	22000	(6557)
	−/+	9	3535	(1112)	11279	(1717)	29500	(4683)	75121	(18021)	57875	(10937)	58314	(11188)	54571	(966)	47150	(11831)	18485	(5360)
	+/+	3	1364	(690)	4933	(348)	18733	(392)	23000	(5999)	69475	(6024)	58750	(5749)	37233	(8946)	42200	(14029)	22000	(8020)

[a] −/− denotes MPA not given; −/+ denotes MPA given during pregnancy; +/+ denotes MPA given during the luteal phase and pregnancy (see text).
Results are shown as mean (SEM).

Table 2. Luteal and early first trimester serum hormone levels of clomiphene citrate-stimulated ongoing in-vitro fertilization conceptions with or without luteal support

Hormone	Type of support	Number of patients	Days after last menstrual period											
			Day 21		Day 24		Day 27		Day 30		Day 35		Day 42	
Oestradiol-17β														
(pmol/l)	Nil	3	2510	(742)	2802	(920)	3128	(584)	3427	(103·9)	4752	(1373)	3190	(948)
	hCG	4	2383	(450)	2808	(410)	4240	(1370)	4215	(807)	5377	(1045)	5266	(1761)
	MPA	3	1312	(407)	1015	(155)*	1235	(217)*	975	(325)*	2196	(151)*	1930	(290)
	Total	10	2113	(359)	2358	(410)	2917	(709)	3207	(628)	4851	(837)	4653	(843)
Progesterone														
(nmol/l)	Nil	3	155	(92·4)	134	(32·6)	241	(78·4)	178	(57·3)	334	(106·1)	191	(69·3)
	hCG	4	204	(37·9)	181	(58·5)	228	(38·9)	208	(51·6)	248	(69·5)	322	(133·5)
	MPA	3	210	(61·0)	76	(5·5)*	173	(3·0)	183	(25·0)	150	(20·5)	168	(37·0)
	Total	10	189	(33·0)	142	(29·5)	220	(29·3)	192	(27·4)	255	(70·3)	247	(64·9)

* Denotes a hormone level significantly lower ($P<0·05$) than observed with nil support.
Results are mean (SEM).

reading was taken to represent 1 week. From Table 2, it can be seen that the reduced oestradiol-17β levels were apparent from day 21 and reduced progesterone levels from day 24 in those patients given MPA from day 15.

Discussion

In analysing the hormonal data for those patients who conceived after in-vitro fertilization, it was anticipated that an optimal range within the follicular phase could form the basis for selecting patients to proceed to ovum aspiration. Data from the luteal phase might detect those patients who were likely to have conceived and possibly define a sub-group for the consideration of luteal support therapy. An analysis of the early pregnancy hormonal changes is unique in having the fixed reference point of oocyte recovery ('ovulation'), and it was anticipated that the prognosis of each pregnancy might be shown from the data as well as providing an opportunity to analyse the effects of hormonal support therapy, both in the luteal phase and during the early weeks of pregnancy.

The follicular phase data demonstrate that satisfactory follicle development is reflected by steadily rising oestradiol-17β levels with a supportive rise in cervical score within the final 2 days before oocyte recovery. LH levels remained low and demonstrated an apparent decline over the 5 days before hCG injection while progesterone levels, remaining very low up to the time of hCG injection, subsequently demonstrate the rise expected with luteinization of the follicle. The rise was significantly greater with C/hMG, presumably due to the increased number of follicles present in that group.

It is relevant to regard the day of oocyte recovery to be equivalent to that of the day of 'ovulation' as this corresponded with the expected early luteinization effect (rising serum progesterone) and the subsequently successful development of embryos ensuing from oocytes inseminated a few hours after collection signifying that the oocytes had undergone appropriate maturational changes and had reached metaphase II of meiosis to be ready for successful fertilization.

The serum oestradiol-17β values recorded during the follicular phase reached levels four- to six-fold that recorded at the pre-ovulatory peak of spontaneous ovulatory cycles (Kerin 1982) and such a response would carry a significant risk for both multiple pregnancies and hyperstimulation syndrome of women undergoing ovulation induction without in-vitro fertilization. However, during in-vitro fertilization, the generation of several mature oocytes to achieve a number of embryos is considered of significant advantage in improving the pregnancy rate (Yovich *et al.* 1984*b*) as it appears that only 40% of embryos generated have the potential to implant successfully (Yovich *et al.* 1984*d*). No patients developed hyperstimulation syndrome during the series studied, but subsequently one patient has experienced moderate abdominal distension with ovarian cysts in the luteal phase and detectable ascites. She had previously experienced this during a routine ovulation induction using clomiphene citrate combined with hCG injections to trigger ovulation and support the luteal phase. It is assumed that the relatively low incidence of hyperstimulation relates to the aspiration of follicular contents at the time of oocyte recovery.

We have previously published luteal phase data showing that oestradiol-17β and progesterone declined significantly after day 24 (day 10 of the luteal phase) in non-conception in-vitro fertilization cycles (Yovich *et al.* 1984*c*). Those patients given hCG support during the luteal phase had higher oestradiol-17β and progesterone levels with the latter rising further in the late luteal phase. Patients destined to abort demonstrated falling oestradiol-17β and progesterone levels after day 30. The effect was most marked in those given MPA during the luteal phase as they also had very low oestradiol-17β and progesterone levels throughout that phase. We have previously discussed the significant luteotrophic effect noted with hCG support regimens and an apparent luteal suppressant action of MPA (Yovich *et al.* 1984*c*). The delayed and retarded rise of β-hCG in those patients who subsequently aborted, supports the view we proposed that blighted ova arising in this in-vitro fertilization programme may have come from embryos trying to implant within a poor luteal hormonal environment. However, the data is not conclusive and the possibility that a deficient embryo factor may fail to provide satisfactory stimulation of the corpus luteum should be considered. No difference was noted in the pregnancy rate with either hCG or MPA luteal support regimens but the outcome appeared better in that fewer abortions occurred with hCG support. A tentative argument is that

an inadequate steroidal environment limits trophoblast development by the embryo which then provides a less than optimal stimulus for further steroid output by the corpus luteum. A cycle is established which leads to failure of normal embryo development but which may be reversible by the timely use of hCG support injections to improve corpus luteal steroid output until the embryo is capable of stimulating the corpus luteum itself by endogenous hCG. We suspect that the markedly elevated progesterone values noted from the mid-luteal phase is an important feature for the successful implantation of pregnancies from in-vitro fertilization. Such values represent at least a three-fold increase from that noted with spontaneous, unstimulated non-in-vitro fertilization conceptions (Hull *et al.* 1982), but the earlier entry of embryos into the uterine cavity with in-vitro fertilization may create a special requirement for higher luteal progesterone levels. In this regard knowledge of the earlier luteal phase progesterone levels would be interesting to assess their possible relevance to successful embryo implantation.

During the first trimester of successful pregnancies, β-hCG levels continue to rise steadily, peaking at around 70 000 i.u./l between weeks 9 and 10. Thereafter values decline until week 14, plateauing at around 20 000 i.u./l. This pattern is not dissimilar to reports of pregnancies arising spontaneously (Kosasa 1981).

Those patients given MPA after pregnancy had been diagnosed (because of threatened abortion) did not demonstrate any significant differences in progesterone, oestradiol-17β or β-hCG levels if the pregnancy was progressing successfully (Table 1). In the first successful in-vitro fertilization pregnancy arising from this programme, MPA was given during the luteal phase and re-started after a short break during the early weeks of pregnancy when threatened abortion and declining progesterone levels were noted; it was felt that progestogen support therapy may well have salvaged that pregnancy. However, declining progesterone levels have not been seen in any of the subsequent ongoing pregnancies in this series. A useful therapeutic role for MPA support in early pregnancy cannot be concluded from this short series which tends to suggest that those pregnancies threatening to abort, with hormonal levels within the expected range for ongoing pregnancies, will continue to develop. The role of progestogen support therapy can only be assessed by a controlled study.

The trend to lower mid-luteal prolactin levels in the aborting group may be relevant in reflecting a failure of secretion from the decidual stromal cells which has been proposed as an important prerequisite for the successful implantation of the embryo (Maslar *et al.* 1980). An alternative explanation is that the observation may simply reflect inadequate decidualization of the endometrium which is progesterone-dependent as prolactin production can be increased progressively in decidual stroma by exogenous progesterone (Riddick *et al.* 1983).

From the end of the luteal phase oestradiol-17β and progesterone continued to rise for a further week, subsequently declining to mean plateau levels of 6488 (SEM 1066) pmol/l for oestradiol-17β and 196 (SEM 35) nmol/l for progesterone until week 12. At that stage oestradiol-17β levels begin to rise reaching mean levels of 17 357 (SEM 6148) pmol/l by week 16 and progesterone also rises after an initial decline to reach 231 (SEM 37) nmol/l by week 16. The rise in steroid output from the late first trimester into the mid-trimester is undoubtedly a reflection of placental development and the changeover from combined ovarian plus trophoblast secretion to essentially placental production (Csapo *et al.* 1972).

Previous reports have shown a steady increase in plasma progesterone values throughout the first trimester of normal pregnancies, rather than the plateau effect shown here. In a study by Radwanska *et al.* (1978), plasma progesterone levels ranged from 31·8 to 171·7 nmol/l (mean 81·1, SD 31·8 nmol/l) from 6 to 12 weeks gestation. The levels in our study were higher throughout the early weeks and may reflect continuing progesterone output from hyperstimulated ovaries. It is likely that a steady decrease in ovarian output was matched by trophoblast secretion masking the continuous rise which one might find if only a single corpus luteum were present in the ovary. The apparent decline in progesterone levels from weeks 12 to 14 before the mid-trimester rise may also reflect the end stage of ovarian secretion. It was not observed with oestradiol-17β, possibly because placental production was already occurring in large enough quantity to mask the effect.

The technique of in-vitro fertilization has provided a uniquely abnormal milieu for the generation of human pregnancies. The ovarian

stimulation programme leads to abnormally high levels of oestradiol-17β and progesterone (two- to three-fold that expected during spontaneous conceptions arising during unstimulated cycles). These high levels are carried through into the luteal phase and early weeks of pregnancy. It was postulated by Edwards *et al.* (1980) that hyperstimulation of the ovaries led to a markedly abnormal luteal phase which was foreshortened in proportion to the level of conjugated oestrogens excreted in the urine. That was one of the reasons cited for pursuing the techniques in unstimulated ovarian cycles and indeed their historic first successful pregnancy was achieved following the recovery of the single oocyte in a natural cycle (Steptoe & Edwards 1978). However, further experience with stimulated schedules using clomiphene citrate, hMG or combinations of the two have indeed shown that there is greater efficiency in generating in-vitro fertilization pregnancies following ovarian stimulation (Trounson *et al.* 1981; Speirs *et al.* 1984; Garcia *et al.* 1983). Abnormal luteal function has been well described following hMG/hCG induced ovulation (Olsen *et al.* 1983) but from the reports available to date, it remains uncertain if luteal phase support therapy improves the pregnancy rate, although it was noted in one study that the abortion rate was less following hCG support during the luteal phase (Yovich *et al.* 1984*c*). It has previously been noted in cycles stimulated with hMG or human pituitary gonadotrophin in non-in-vitro fertilization programmes for infertility therapy, that hCG support injections during the luteal phase improves the rate of successful pregnancies (Brown *et al.* 1980).

Concern has often been expressed that the techniques applied for in-vitro fertilization might generate abnormal embryos and cause fetal abnormalities. The exposure of the maturing oocyte to very high levels of sex steroids during the follicular phase and the implanting embryo during the luteal phase and early pregnancy requires consideration of the possibility of fetal abnormalities as such have been reported with the use of synthetic steroid and steroid-like drugs (Herbst *et al.* 1972; Levy *et al.* 1973; Janerich *et al.* 1974; Wilkins 1960; Aarskog 1979). The 22 infants delivered in this series were all perfectly normal and have been included in a long-term developmental assessment project which has so far revealed no abnormal features. Of 112 infants born following in-vitro fertilization in Australia to the end of July 1983 (Speirs *et al.* 1984), only one infant displayed an abnormality—that of a cardiac lesion in one twin (Wood *et al.* 1982). The vast majority of those pregnancies followed ovarian stimulation suggesting that high endogenous circulating steroid levels are unlikely to harm the developing embryo. Such conclusions are consistent with reported results of gonadotrophin therapy for ovulation induction which does not lead to increased congenital malformations or abnormal postnatal development even though hyperstimulation is reported in 3·6% to 7·8% of cases (Lunenfeld & Insler 1978*a*). Similarly, clomiphene citrate therapy for ovulation induction does not appear to cause fetal abnormalities (Lunenfeld & Insler 1978*b*) and the proportion exhibiting hyperstimulation is much less than for gonadotrophin.

In conclusion, this study reports the hormonal changes during the follicular phase, luteal phase and the first 16 weeks of pregnancy for conceptions arising from in-vitro fertilization where ovarian stimulation is a feature of the programme. Steroid hormone changes in response to luteal support therapy are noted, and it was found that pregnancies which subsequently aborted could be predicted by delayed and retarded β-hCG elevations combined with reduced oestradiol-17β and progesterone output in the late luteal and early pregnancy stages.

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