ORIGINAL ARTICLE

Progesterone Profiles in Pregnant, Non-pregnant, Natural and Stimulated IVF Cycles with and without Luteal Support

P Y S Tay, MRCOG*, E A Lenton, PhD.**

*Department of Obstetrics and Gynaecology, University of Malaya, 50603 Kuala Lumpur, ** Sheffield Fertility Centre, 26, Glen Road, Sheffield S7 1RA, United Kingdom

Summary

Objectives: (1) To describe the progesterone profiles following pituitary down regulation in stimulated IVF cycles with the use of GnRH-a (2) To assess the impact of progesterone supplement and pregnancy on the subsequent luteal phase.

Study design: A prospective observational study performed in a specialist infertility clinic based at a tertiary centre in the north of England. Subjects were divided into cohorts depending on their treatment (natural or stimulated IVF cycles), the type of luteal support (nil or progesterone) and eventual outcome (successful pregnancy or failure to conceive). Saliva progesterone concentrations were the only measuring outcome.

Results: *Natural versus stimulated cycle (SIVF):* As expected saliva progesterone concentrations were significantly higher in subjects undergoing SIVF than in the natural cycle from day 1 to day 6 of the cycle ($P < 0.001$) but thereafter stimulated cycle concentrations declined prematurely to fall below those of the natural cycle group by day 7, becoming significantly lower than natural cycle concentrations by days 9 and 10 ($P < 0.01$).

With and without progesterone supplementation: Saliva progesterone concentrations in subjects undergoing NIVF and receiving progesterone supplement were 2.5-3 times greater than those concentrations seen in the unsupplemented natural cycle ($P < 0.001$). Similarly in the SIVF-progesterone supplemented group, saliva concentrations remained significantly higher ($P < 0.001$) than in the unsupplemented cycle throughout the luteal phase. Despite this, luteal supplementation did not prevent nor reverse the acute mid luteal (day 7) decline in progesterone seen in all stimulated cycles.

Conclusions: Luteal phase following pituitary down regulation is grossly abnormal. The timing and degree of luteal support routinely provided following stimulated IVF is not effective in 'correcting' the progesterone profile.

Key Words: Luteal support, Natural cycle, Non-pregnant, Pregnant, Saliva progesterone profiles, Stimulated cycle

This article was accepted: 5 February 2002

Corresponding Author: P Y S Tay, Department of Obstetrics & Gynaecology, University Malaya, 50603 Kuala Lumpur

Introduction

Achieving a successful pregnancy that reaches full term is the goal of all assisted conception units. Despite fertilisation rates of between 70 to 90%[1] and the introduction of techniques such as intracytoplasmic sperm injection (ICSI), the average live birth rate (UK) remains low at 19% per cycle started [2]. The pathophysiology of this discrepancy may involve endometrium/embryo interactions and the endocrinology of the luteal phase.

Concerns that controlled ovarian hyperstimulation regimes could result in endometrial retardation [3] or other abnormality in the progesterone profile[4] have led to the widespread empirical use of luteal support, such as the administration of exogenous progesterone. However, there is little evidence that progesterone supplementation can reverse abnormal luteal phase or that it is imperative for implantation. Similarly it is not known whether luteal support is necessary to achieve a pregnancy following egg collection in the natural cycle.

It has been known for some time that a proportion of circulating steroids are excreted in saliva and there is a direct correlation between unbound plasma and saliva progesterone levels[5]. The total concentration of progesterone in saliva is two orders of magnitude lower than that seen in plasma[5]. Further, progesterone concentrations in saliva do not vary with saliva flow rate [6].

With these facts in mind, the following study was undertaken to characterise endogenous progesterone profiles in both stimulated and natural IVF cycles, with and without luteal support, utilising saliva samples as a convenient and less invasive alternative to daily blood sampling. The primary aim of the study was to describe the impact of progesterone supplementation on endogenous progesterone profiles. A secondary objective was to assess the magnitude and timing of the endogenous progesterone response to implantation using those cycles where pregnancy occurred.

Materials and Methods

At the time of this study it was normal practice in our clinic for all patients undergoing IVF, whether natural or stimulated, to be monitored during the luteal phase. Natural cycle IVF was the treatment of first choice and stimulated IVF was only offered if the patient was unsuitable or did not wish to have natural cycle IVF. Luteal monitoring was only performed in those patients who had embryos transferred. This study utilises the results from 180 subjects who were treated sequentially during a 10-month period. As this was intended to be a simple observational analysis, the women were not randomised to luteal support or no luteal support; the choice of treatment was made by the managing clinician after discussion with the individual concerned.

IVF treatments

Treatment regimes included both natural cycle and stimulated IVF.

Beginning on cycle day 9, all subjects undergoing natural cycle IVF (NIVF, n=136) were tested for plasma oestradiol and luteinizing hormone (LH) daily to ascertain the onset of the LH surge. An oocyte was collected by means of ultrasonically guided transvaginal aspiration approximately 36 hours after LH surge

Stimulated IVF (SIVF, n=44) consisted of down regulation with gonadotrophin releasing hormone agonist (GnRH-a) to achieve complete pituitary desensitisation before starting ovarian stimulation. GnRH-a (Suprefact, Shire Pharmaceuticals Ltd., Hants SP10 5RG, UK) was administered daily starting 7 days before the expected period and continued for 10-14 days in total. The subjects were then given 150 or 225 IU/day of recombinant FSH (Gonal-F, Ares-Serono Ltd., London W1N 1AF, UK), according to the patient's age, previous follicular response and early follicular phase FSH levels. Follicle development was monitored by oestradiol measurement and serial trans-vaginal ultrasonography (US) (Combison 310, Kretztechnik, AG, Austria). HCG

10,000IU (Profasi; Ares-Serono Ltd., London W1N 1AF, UK) was administered subcutaneously when ≥ 2 follicles measuring ≥ 18mm in diameter were present. Transvaginal egg collection under ultrasonic guidance took place 35-36 hours later. GnRH-a was discontinued on the day of HCG administration.

Oocyte(s) were inseminated and cultured according to conventional IVF technique. When fertilisation and cleavage occurred, a maximum of three embryos were transferred in-utero 2 to 3 days after egg collection. The excess embryos were cryopreserved.

Luteal support

Luteal support was given in the form of Cyclogest suppositories (200 mg progesterone, Hoechst UK Ltd., Hoechst House, Salisbury Road, Hounslow, Middlesex), twice daily, commencing the day after egg collection (luteal day 1). It should be noted that luteal day 0 represents the day of egg collection, not the day of the LH surge as is commonly used.

Subject groups (Figure 1)

The flow chart in Figure 1 demonstrated the distribution of patients in this study. The non-conception cycles were divided into four groups according to their treatment (natural cycle or stimulation) and the type of luteal support (nil or exogenous progesterone), forming NIVF-progesterone (a, n=29), NIVF-nil (b, n=95), SIVF-progesterone (c, n=21) and SIVF-nil (d, n=17) groups.

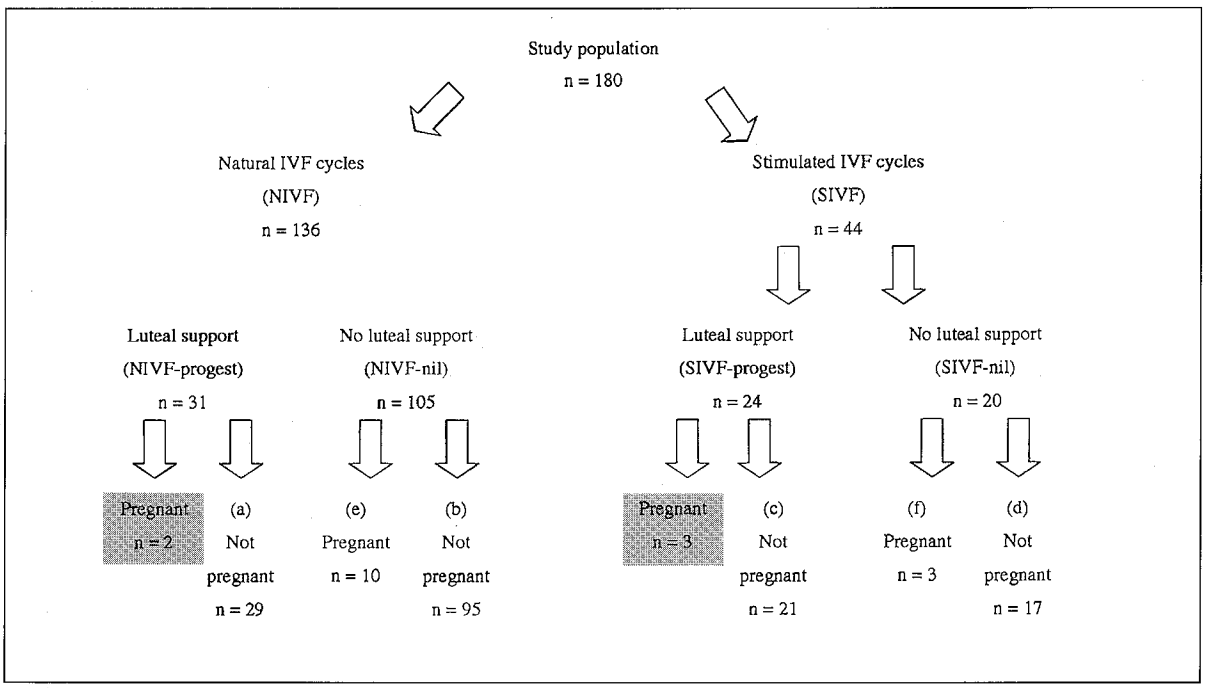

Figure 1: Flow chart demonstrating the distribution of the study population.

NIVF-progest = Natural IVF cycles supplemented with progesterone
NIVF-nil = Natural IVF cycles with no supplementation
SIVF-progest = Stimulated IVF cycles supplemented with progesterone
NIVF-nil = Stimulated IVF cycles with no supplementation
Hormonal data of the pregnant cycles supplemented with progesterone (grey-coloured boxes) were excluded for comparison.

Clinical pregnancies occurred in all groups as follows: NIVF-progesterone (n=2), NIVF-nil (e, n=10), SIVF-progesterone (n=3) and SIVF-nil (f, n=3). To study the distinct impact of pregnancy on the luteal phase, progesterone profiles from pregnant subjects *receiving* luteal support (represented by the grey coloured boxes) have not been analysed further.

Saliva samples

Subjects entering the study were provided with a box containing numbered tubes and were asked to collect 2ml samples of saliva at the same time each day throughout the luteal phase, beginning on luteal day 0, the day of egg collection. The labelled samples were stored in the freezer compartment of the subjects' refrigerator until the end of the menstrual cycle or until pregnancy was confirmed after which time the samples were transferred to the laboratory and kept frozen (-20°C) until analysed en bloc.

Saliva progesterone assay

Samples from each individual were assayed together. The saliva progesterone radio-immunoassay was a simple, direct steroid assay, employing reagents available from Steranti Laboratories (Steranti Research Ltd, St.Albans. UK). These comprised progesterone-^{125}I-tyramine glucoronide and an antibody against progesterone glucoronide 11β hemisuccinate BSA, which was covalently bound to a solid phase support. Solutions of progesterone, initially dissolved in ethanol and then progressively diluted in assay buffer (phosphate/gelatin, pH 7.0) were used as standards (giving a range of 10 to 2600 pmol/l). Quality controls were prepared from male saliva by the addition of progesterone (high QC=732 pmol/l, low QC=380 pmol/l). Prior to analysis, all samples of saliva were thawed and centrifuged at 900 *g* to precipitate mucins. Reagent volumes employed in the assay were: antibody, 50µl; ^{125}I-progesterone, 50µl; saliva or standard solution, 150 µl, giving a total of 250µl. Assay tubes were incubated overnight at room temperature, and then centrifuged at 1400 *g* for 20 min. The supernatants were decanted and the precipitate (containing the bound fraction) was counted in a gamma-counter for 1 min. Sensitivity was 20 pmol/l and inter-assay coefficients of variation of high and low quality controls were 8.0% and 7.5% respectively.

Statistical analysis

Data were first logarithmically transformed to normalise the distributions before geometric means and 95% confidence intervals were calculated. Further analysis was by Student's t-test applied to log transformed data. This was performed with SPSS (Version 10) for Windows. A probability of 0.05 was used to indicate statistical significance.

Results

Non-pregnant (b) NIVF-nil versus (d) SIVF-nil (Figure 2)

In unsupplemented natural cycles, saliva progesterone concentrations rose sharply during the first 6 days after single egg collection, plateaued for 2 to 3 days and declined thereafter. Progesterone concentrations in the stimulated cycles rose rapidly following egg collection to reach concentrations 4-5 times *higher* than the natural cycle by day 4 and remained significantly higher until day 6 ($P < 0.001$). Progesterone concentrations began to decline 'prematurely' from day 6 and continued falling to become significantly *lower* than in natural cycles by days 9 and 10 ($P < 0.001$). Progesterone concentrations remain sub-optimal for the rest of the luteal phase ($P < 0.001$).

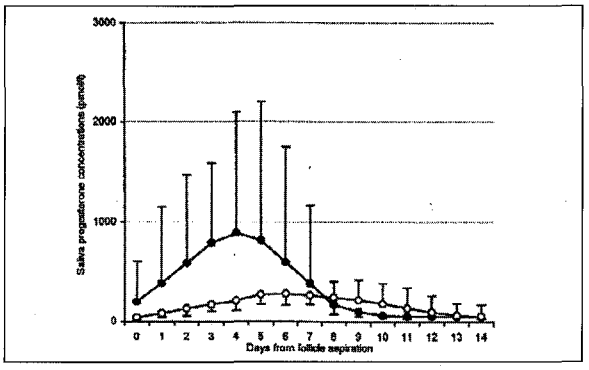

Figure 2: Geometric means and 95% confidence intervals for saliva progesterone (pmol/l) in SIVF-nil (•, n=17) versus NIVF-nil (o, n=95). Progesterone levels were significantly higher in SIVF-nil group from day 1 until 6 (P < 0.001) but became lower than the NIVF-control group by day 9 and 10 (P < 0.01).

Non-pregnant (a) NIVF-progesterone versus (b) NIVF-nil (Figure 3)

Saliva progesterone concentrations in NIVF-progesterone were significantly higher (P < 0.001) and 2.5-3 times greater than NIVF-nil from day 2, the day following the first use of the Cyclogest suppositories.

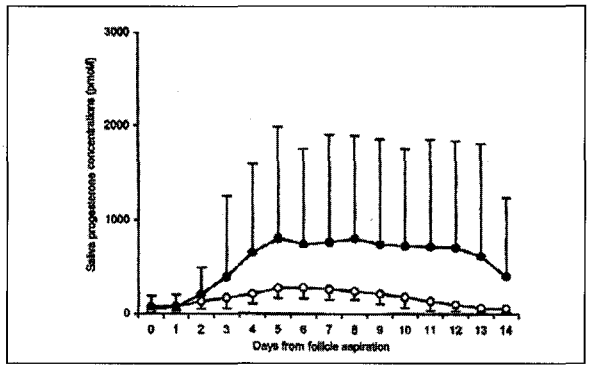

Figure 3: Geometric means and 95% confidence intervals for saliva progesterone (pmol/l) in NIVF-progesterone ((, n=29) versus NIVF- nil (o, n=95). Exogenous progesterone administration commenced on day 1; differences in saliva progesterone became significant from day 2 onwards (P < 0.001).

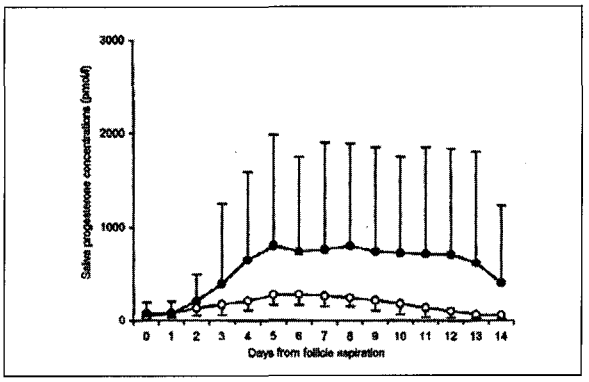

Figure 4: Geometric means and 95% confidence intervals for saliva progesterone (pmol/l) in SIVF-progesterone ((, n=21) versus SIVF-nil (o, n=17). Exogenous progesterone administration commenced on day 1; differences in saliva progesterone became significant from day 2 onwards (P < 0.001).

Non-pregnant (c) SIVF-progesterone versus (d) SIVF-nil (Figure 4)

Saliva progesterone concentrations in SIVF-progesterone increased rapidly from day 0 to reach a peak in both groups by day 4 but overall concentrations almost doubled with the administration of progesterone (P < 0.001). The previously described rapid decline in progesterone (Fig. 1) from luteal day 4 was not remedied by the luteal support regime. However, the significantly low late luteal progesterone concentrations seen in unsupplemented cycles were effectively masked by the addition of progesterone.

Pregnant (e) versus (b) non-pregnant NIVF (Figure 5)

During the first half of the luteal phase progesterone concentrations were identical in both pregnant and non-pregnant subjects. Following implantation in the pregnant group, concentrations diverged from day 8. These differences became significant by day 10 (P < 0.001) following egg collection.

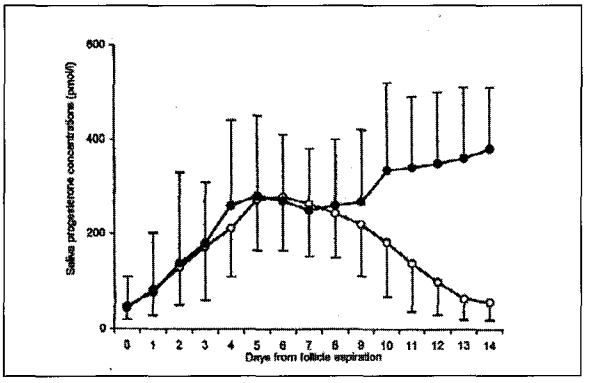

Figure 5: Geometric means and 95% confidence intervals for saliva progesterone (pmol/l) in NIVF pregnant ((, n=10) versus NIVF non-pregnant (o, n= 95). No luteal support was given to any of the subjects shown. Endogenous progesterone differences were significant from day 10 (P < 0.001). Note the change of scale from the other figures.

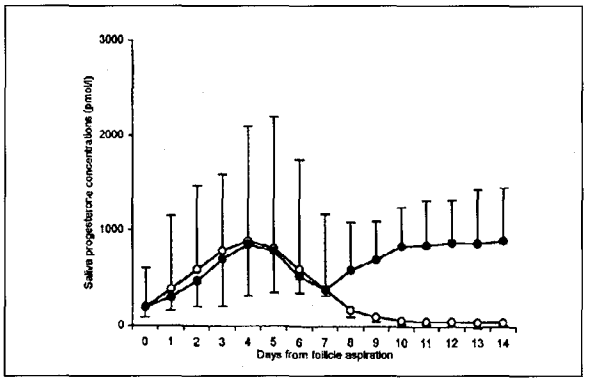

Figure 6: Geometric means and 95% confidence intervals for saliva progesterone (pmol/l) in SIVF pregnant ((, n=3) versus SIVF non-pregnant (o, n=17). No luteal support was given to any of these subjects. Endogenous progesterone differences were significant from day 10 (P < 0.001).

Pregnant (f) versus (d) non-pregnant SIVF (Figure 6)
Again saliva progesterone concentrations were not different until day 7 when those in the pregnant group increased sharply becoming significant from day 10 onwards (P < 0.001).

Pre- and post- implantation stages in various groups (Table I)
To enable a more direct comparison between the regimes, mean saliva progesterone concentrations were calculated for each subject over the intervals day 3-5 and 8-10 respectively. These periods represent a fairly stable progesterone secretion during the pre- (day 3-5) and post- (day 8-10) implantation stages. All the subject mean progesterone values over the 3-day period were presented as the geometric means with 95% confidence intervals.

Table I: Longitudinal concentrations of saliva progesterone (pmol/l) over days 3-5 and 8-10 of the luteal phase.

Groups	Days 3-5	Days 8-10	Luteal phase duration
NIVF-nil	210 (125-345)	205 (110-385)	13.9 ±0.4
SIVF- nil	830 (305-2265) [b]	110 (30-390) [e]	11.2 ±1.1 [e]
NIVF- progest	610 (260-1430) [b]	760 (330-1745) [b]	14.1 ±0.4
SIVF- progest	1669 (1055-2530) [b,c]	560 (285-1095) [b]	13.7 ±0.9
[α]NIVF- pregnant	240 (132-447) [a]	295 (160-530) [b]	-
[α]SIVF- pregnant	780(330-1845) [b]	710 (505-1113) [b]	-

Significantly higher than NIVF-nil, a P < 0.05, b P < 0.001
Significantly higher than SIVF-nil, c P < 0.01, d P < 0.001
Significantly lower than NIVF-nil, e P < 0.001

* Non-conception and conception cycles were analysed separately and none of the conception cycles shown here received any luteal supplementation. The data are given as geometric means ± 95% confidence intervals in parentheses whilst luteal phase duration is recorded in days.

Compared with natural IVF cycle (NIVF-nil), stimulated cycles without luteal support (SIVF-nil) were characterised by an acute drop in progesterone in the late luteal phase (days 8-10). Similarly, the mean length of luteal phase in these cycles was significantly shorter (Table I). Following luteal supplementation saliva progesterone concentrations were two to four-fold higher in both natural (NIVF-progest.) and stimulated cycles (SIVF-progest.) throughout out the luteal phase. The mid-luteal fall in progesterone concentrations was not eliminated but was slightly ameliorated. Luteal phase duration was however restored to 14 days.

Comparing the pregnant and non-pregnant natural cycles, mean saliva progesterone levels were slightly higher ($P < 0.05$) in the pregnant group over days 3-5, and significantly higher by days 8-10 ($P < 0.001$) due to rescue of the corpus luteum by the ongoing pregnancy. Progesterone profiles in pregnant and non-pregnant stimulated cycles were not significantly different over days 3-5 but again by days 8-10 pregnant SIVF cycle concentrations were approximately seven-fold higher than the equivalent non-pregnant concentrations ($P < 0.001$) and two-fold higher than in pregnant natural cycles ($P < 0.001$). Even though all the pregnancies were singletons (so concentrations of HCG would have been similar), the greater progesterone response in the stimulated group suggests that multiple corpora lutea resulting from the previous stimulation and multi-follicular development were being 'rescued' even though only one fetus was present.

Pregnancy outcome

Clinical pregnancies occurred in all groups as follows: NIVF-nil (n=10), NIVF-progesterone (n=2), SIVF-nil (n=3), and SIVF-progesterone (n=3) giving clinical pregnancy rates per embryo transfer cycle of 9.5%, 6.5%, 15.0% and 12.5% respectively. Despite the small number of patients, no statistical difference was seen in the rates of clinical pregnancy in NIVF or SIVF. All pregnancies in this study resulted in a single live birth.

Discussion

This is an observational study describing the saliva progesterone profiles following IVF in both natural and stimulated cycles. Within these two treatment types, the impact of pregnancy and luteal support on hormonal profiles is individually demonstrated. Oestradiol profiles have not been evaluated, as the concentrations in saliva are too low to be reliably quantified.

Lenton *et al.* (1988)[7] have shown that the concentration of progesterone in saliva can be used as an alternative to the measurement of the hormone in plasma and have defined reference values for the hormone during the luteal phase of spontaneous menstrual cycles. Although the mean saliva progesterone concentrations observed in the natural cycles in this study (Fig. 2) were slightly lower than in previous studies, the data were still within the 'normal' range of 300-800 pmol/l [7].

Saliva progesterone profiles became grossly distorted in stimulated cycles compared with natural cycles (Fig. 2). Following ovarian stimulation, concentrations rose sharply to a peak on luteal day 4, after which the levels declined precipitously over the mid-luteal phase. Maximum progesterone concentrations were 5 times higher during the early luteal phase whilst in the late luteal phase, concentrations were significantly lower than those seen in the natural cycle. Although the exact reason for this distorted profile is unclear, three possible mechanisms are proposed. First, the larger number of follicles in stimulated cycles will almost certainly mean greater asynchrony in follicle development. Some of the smaller, immature follicles may form corpora lutea that collapse prematurely during the luteal phase, leading to a decrease in progesterone secretion around the mid-luteal phase. Alternatively, the marked attenuation in plasma LH levels associated with the use of a gonadotrophin hormone releasing hormone-agonist could theoretically deprive the corpus luteum of trophic (LH) stimulation, leading to a

progressive deficiency in secreted progesterone[8]. Finally, the standard practice of administering a large dose of human chorionic gonadotrophin (HCG) to bring about follicular maturation in stimulated cycles may itself influence progesterone production during the luteal phase[9]. Note that HCG was not used to induce follicular maturation in any of the natural cycles studied.

The luteal phase can be effectively divided into 2 parts: days 0-7 and days 8-14 after egg collection. Over the first 7 days of the luteal phase the purpose and function of progesterone is to prepare the endometrium for implantation. In the second half of the luteal phase the function of progesterone changes; no longer is it required to actively prepare the endometrium for implantation but to hold it in a 'functional state' preventing menstrual shedding and loss of the embryo. This alteration in function has been demonstrated in morphometric and histological studies [10, 11] whereby cellular changes within the endometrium are more closely controlled over the first half of the luteal phase than over the second. Thus, the sub-optimal hormonal environment in stimulated cycles between luteal days 9 and 11 (Fig. 2), could affect on the integrity of endometrium. Certainly the majority of subjects in the unsupplemented stimulated group experienced premature menstrual bleeding (24% before 11 days and 59% between 11 and 13 days) and, whilst it is not known whether this actually influenced outcome, sufficient anxiety was generated amongst the patients for this arm of the study to be curtailed. Consequently this arm of the study (SIVF-nil) was terminated after 20 cycles; thus the number of conception cycles is too low to permit any definitive conclusions on the necessity or value of luteal support following controlled ovarian hyperstimulation. In spite of this, one of the three pregnancies that did occur was in a subject who experienced premature, but transient bleeding on day 9.

It is interesting to note that in the supplemented groups as well as in the unsupplemented natural cycle group, luteal phase length was constant at approximately 14 days even though exogenous progesterone had not been withdrawn (Table I). This suggests that rather more significant concentrations of progesterone (such as those seen by day 14 following rescue of the corpus luteum in the pregnant cycles) alone or in combination with oestradiol are required to postpone menstrual bleeding beyond 14/15 days.

Progesterone supplementation is employed on the assumption that it will overcome a potentially sub-optimal environment, prevent premature menstrual bleeding and possibly improve pregnancy rates. Although this study was not designed to validate the impact of luteal support on treatment outcome (pregnancy), it was sufficient to describe the effect of exogenous progesterone on hormone profiles (Figs. 3 and 4). When progesterone (Cyclogest) was administered from day 1 of natural cycles, a 2 to 3 fold increase in progesterone concentration was observed. This increase was large enough to grossly distort the endogenous progesterone profile, demonstrating that luteal support of this magnitude is unlikely to be necessary in the natural cycle (Fig. 3). Conversely, the same degree of luteal supplement in SIVF cycles was only marginally effective. In the early luteal phase, pre-existing supra-physiological amounts of endogenous progesterone indicate that further luteal supplementation is unnecessary. However, later in the luteal phase, progesterone administration failed to mask the mid luteal decline in progesterone levels (Fig. 4). Despite this, total progesterone levels were maintained above the equivalent natural cycle concentrations for the remainder of the luteal phase that was sufficient to normalise luteal phase duration (Table I).

The purpose of luteal support may be thought of as three fold; to ensure optimum priming of the endometrium, to prevent an early progesterone withdrawal bleed and to ensure adequate levels of progesterone around the time of implantation. Even with mild stimulation, progesterone concentrations appear more than adequate during the early luteal phase for normal endometrial

priming (Fig. 2) provided there has been no disruption of endometrial progesterone receptors. In its second role, luteal support as described here is part failure and part success. Administration of progesterone did generally prevent premature bleeding, but did little to alter the dynamics of rapid mid luteal decline in progesterone levels (Fig. 4). It would seem that steady lower levels of progesterone can block bleeding (endometrial shedding) but whether the endometrium is in anyway adversely affected by the rapid withdrawal of progesterone cannot be determined.

Saliva progesterone profiles in conception and non-conception natural IVF cycles (Fig. 5) were similar to those described earlier by Lenton *et al.* (1988) [7] in spontaneous menstrual cycles. A similar picture was seen in the stimulated conception cycles (Fig. 6) despite the small numbers. Again progesterone profiles diverged from day 7 compared with the non-conception situation and this difference was significant by day 10. In each case, when implantation occurred there was a prompt and sustained increase in endogenous progesterone secretion. This feature is well known. The interpretation is that the low progesterone concentrations in the late luteal phase of stimulated cycles are not a consequence of corpus luteum failure but simply due to a lack of trophic stimulation. This strongly suggests that the reason for the rapid mid-luteal decline is pituitary in origin and likely to be a result of inadequate LH secretion. More importantly should implantation occur, HCG from the trophoblast is sufficient to stimulate progesterone secretion, thus in general, those cycles where early bleeding is observed are the cycles where implantation has not occurred. In other words early bleeding is a consequence of failure to implant rather than a precipitating cause.

To conclude, progesterone secretion following ovarian stimulation is different compared to spontaneous cycles, with sub-optimal progesterone concentrations frequently observed during the 'implantation window'. The timing and degree of progesterone supplement routinely provided following ovarian stimulation is not effective in preventing the rapid decline in progesterone concentrations during the mid luteal phase, although it does suppress premature bleeding. If luteal support is to be used, we would recommend that it is not started until luteal day 4 or 5.

Acknowledgements

The authors would like to thank Infertility Research Trust for the financial support.

References

1. Peter AJ, Wentz AC. Endometrial receptivity and the luteal phase. Curr Opin Obstet Gynecol 1992; 4: 736-42.
2. The patients' guide to DI and IVF clinics; Human Fertilisation Embryology Authority (HFEA, 2000, 3rd edition) Praxton House, 30 Artillery Lane, London E1 7LS.
3. Bourgain C, Smitz J, Camus M, et al. Human endometrial maturation is markedly improved after luteal supplementation of gonadotrophin - releasing hormone analogue/ human menopausal gonadotrophin stimulated cycles. Hum Reprod 1994; 9: 32-40.
4. Smitz J, Devroey P, Braeckmans P, et al. Management of failed cycles in an IVF/GIFT program with the combination of a GnRH analogue and HMG. Hum Reprod 1987; 2: 309-14.
5. Wang DY, Knyba RE. Saliva progesterone: relation to total and non-protein bound blood levels. J Steroid Biochem Mol Biol 1986; 23: 975-79.
6. Vining RF, McGinley RA. Transport of steroids from blood to saliva. In Ninth Tenovus Workshop, Immunoassay of Steroids in Saliva (eds G.F. Read, D. Riad-Fahmy, R.F. Walker and K. Griffiths), 56-63. Alpha Omega Publishing Ltd., Cardiff, 1983.
7. Lenton EA, Woodward AJ. The endocrinology of conception cycles and implantation in women. J Reprod Fertil (Suppl) 1988; 36: 1-15.
8. Smitz J, Devroey P, Faguer B, Bourgain C, Camus M, Van Steirteghem AC. A prospective randomised comparison of intramuscular or intravaginal natural progesterone as a luteal phase and early pregnancy supplement. Hum Reprod 1992; 2: 168-75.
9. Valbuena D, Pellicer A, Guanes P, Remohi J, Simon C. Effect of disruption versus continuation of gonadotrophin - releasing agonist after human chorionic gonadotrophin administration on corpus luteum function in patients undergoing ovulation induction for in - vitro fertilisation. Hum Reprod 1997; 12: 2118-22.
10. Li TC, Lenton EA, Dockery P, Rogers AW, Cooke ID. The relation between daily saliva progesterone profile and endometrial development in the luteal phase of fertile and infertile women. Br J Obstet Gynecol 1989; 96: 445-53.
11. Dockery P, Li TC, Rogers AW, Cooke ID, Lenton EA, Warren MA. An examination of the variation in timed endometrial biopsies. Hum Reprod 1988; 3: 715-20.