ORIGINAL ARTICLE

The Impact of Luteal Supplement on Pregnancy Outcome Following Stimulated IVF Cycles

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

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

Summary

This is a prospective randomised study designed to clarify the impact of various luteal support regimes (HCG and progesterone) on progesterone profiles and pregnancy outcomes. This study involved subjects undergone down regulated stimulated IVF cycles using various types of luteal support, namely: Cyclogest (n = 35), Crinone gel (n = 36), various doses of Utrogestan (n = 55) and HCG (n = 35). Various doses of Utrogestan (administered vaginally), Crinone gel (progesterone administered vaginally) and Cyclogest (progesterone administered rectally) supplementation induced similar end plasma progesterone concentrations ranging from 26 to 32 mmol/l. These progesterone regimes produced no significant differences. Hence, the impact of exogenous progesterone supplement was relatively trivial and did not 'stabilise' the sub-optimal luteal phase. In contrast, two small HCG injections during the early and mid-luteal phase possessed a much greater ability to 'stabilise' progesterone profiles. Despite this additional advantage, implantation and pregnancy rates with either HCG or progesterone supplements were similar. Although none of these forms of luteal support adequately 'normalised' luteal progesterone profiles, this did not appear to be detrimental to the process of implantation.

Key Words: HCG, In-vitro fertilisation, Luteal phase, Progesterone, Pregnancy

Introduction

Abnormal progesterone profiles were frequently observed in the luteal phase following IVF cycles treated with GnRH-agonist (GnRH-a)[1]. The impact of these hormonal changes on pregnancy outcome is unknown. Recently, there is evidence to suggest that a large magnitude of decline in luteal oestradiol concentrations following stimulated IVF is associated with a significantly lower ongoing implantation and pregnancy rates[2]. Since progesterone appears to be necessary for implantation and maintenance of an early intrauterine pregnancy[3], one would extend the above findings and speculate that a rapid decline in plasma progesterone in the mid luteal phase could also induce a similar insult to the process of implantation.

To overcome the sub-optimal steroid environment, luteal supplements such as progesterone[4] or human chorionic gonadotrophin (HCG)[4] have been recommended. There is no consensus as to the best luteal support regime. Vaginal[5,6], oral[7] and intramuscular[8] administration of progesterone are physiologically effective and commonly used. Further, a variety of preparations and dosages of progesterone are employed. Similarly, HCG supplementation is diverse, ranging from a single injection of 5000IU given 2 days after oocyte recovery[9] to multiple 1500IU injections given alternate days from day 5 to 15 after oocyte recovery[10]. Although there are numerous publications correlating the impact of various luteal supplements on pregnancy outcome, there are relatively few attempts to clarify the effectiveness of luteal supplement in correcting luteal steroid profiles.

This article was accepted: 30 August 2004

Corresponding Author: Paul Tay Yee Siang, Department of Obstetrics and Gynaecology, University Malaya Medical Centre, 50603 Kuala Lumpur

This study hypothesised that the rapid decline in progesterone during the mid luteal phase, occurring as a consequence of ovarian stimulation, compromised implantation. To test this hypothesis, attempts were made to study the impact of various luteal support regimes by addressing two questions. First, do various luteal supplements (either progesterone or HCG) deliver different amounts of progesterone and differentially affect pregnancy outcome? Second, how effective are these luteal support regimes in correcting abnormal progesterone profiles following ovarian stimulation?

Materials and Methods

One hundred and sixty eight subjects undergoing stimulated IVF treatment at the Sheffield Fertility Centre were prospectively recruited from 1st April 1999 till 31st March 2000. The local ethics review committee approved this study and all subjects gave informed consent. These subjects were aged 21-41 years (mean 32.4 years) and 57.5% of them had primary infertility. All subjects had basal FSH of less than 12 IU/l and body mass index between 19 and 30. 44% of these subjects had tubal disease, 34% had male infertility, 9% had ovulatory dysfunction, 8% had endometriosis and 5% of the cases had unexplained infertility. Some of them had multiple infertility factors. Subjects with a pre-ovulatory oestradiol concentration ≥ 15000pmol/l and/or total oocyte number of ≥ 15 were excluded from this study in order to reduce the potential for ovarian hyperstimulation syndrome (OHSS).

Five altruistic egg donors (stimulated cycle-control) and 5 subjects undergoing natural cycle frozen embryo replacement treatment (natural cycle-control) were also recruited to act as controls. All the control subjects did not conceive and none were given any form of luteal supplement.

Types of treatment
Long protocol stimulated IVF regime and natural cycle frozen embryo replacement treatment were used in this study (see Chapters 1 and 2 for details).

Luteal support regimes
Subjects were randomised on the day of embryo transfer to one of the following groups:

1. Cyclogest (n = 35) (Shire Pharmaceuticals Ltd., Hants SP10 5RG): natural progesterone prescribed rectally at the dose of 200mg twice daily.
2. Eight percent Crinone gel (n = 36) (Wyeth Lab, Maidenhead, Berks SL6 0PH): 90mg of natural progesterone administered vaginally once daily.
3. Utrogestan (n = 55) (Laboratories Besins-Iscovesco, 75003 Paris): 200, 400 and 600 mg of natural progesterone administered vaginally with divided doses given 2 to 3 times daily. (All progesterone supplements were administered between 1800-2100 hours, starting on the 4th day and continued daily until the 14th day after egg collection).
4. HCG injection (n=35) (Pregnyl, Organon Lab Ltd., Cambridge CB4 4FL): given subcutaneously at a dose of 1500IU between 1800-2100 hours on days 4 and 7 after egg collection.

Reference points and plasma sampling
In stimulated IVF cycles, day 0 indicates the day of egg collection (2 days after an ovulatory dose of HCG). Since the natural cycles exhibited spontaneous LH surge rather than artificially induced with HCG, a different reference point in the luteal phase is used. Day 0 in natural cycles represents the day of spontaneous ovulation, 2 days after the beginning of the LH surge.

Plasma samples were collected between 0800-0900 hours on luteal days 10 and 14 in all subjects. To enable a more in-depth comparison, additional blood samplings were obtained on a daily basis in 3 groups of study subjects, namely: stimulated cycle-controls, HCG and Cyclogest groups.

Progesterone assay
Plasma samples were tested for progesterone concentrations. Plasma progesterone concentrations were measured using a high affinity monoclonal antibody in a competitive enzyme immunoassay system with magnetic phase separation [Serozyme immuno-assay system, BIODATA Diagnostics, Italy]. This system has a sensitivity of less than 0.48 nmol/l. Its intra-assay variation was 3.7-3.9% and inter-assay variation was 6.4-10.8%.

Statistical analysis
Hormonal data were presented in the form of geometric means with 95% confidence limits. Statistical analysis employed one-way analysis of variance (ANOVA), χ^2 analysis and Students't-test. This was performed using SPSS (Version 10) for Windows. P value of less than 0.05 indicated statistical significance.

Results

As seen in Table I, plasma progesterone concentrations in the stimulated cycle controls were significantly lower than the natural cycle controls on luteal days 10 and 14 ($P < 0.05$). The mean progesterone concentration in the

stimulated group was negligible on day 14. Subjects in the stimulated group also tend to experience early menstruation as demonstrated by significantly shortened luteal phase duration of 11.1 days compared to a normal duration of 14.2 days ($P < 0.05$). It should be noted again that these controls did not conceive and were not supplemented with any luteal support.

In order to study the steady state concentrations of various luteal supplements in stimulated IVF cycles, plasma progesterone concentrations were measured on luteal day 10 and 14. These days were chosen because they typically represent a period of stable and low progesterone secretion during the late luteal phase where the endogenous production of progesterone by the corpus luteum is minimal.

Fifty-five subjects allocated to the Utrogestan group were divided into three sub-groups, receiving 200, 400 and 600 mg administrated vaginally (Table II). However, only data from the 29 non-pregnant subjects were used to determine terminal plasma progesterone concentrations. Day 14 plasma progesterone concentrations are a direct consequence of Utrogestan. There was a small dose related response that was not statistically significant ($P > 0.05$). Hence, all Utrogestan data were pooled to give geometric means of 49.6 and 30.9 nmol/l on luteal days 10 and 14 respectively (Table III).

In Table III, non-pregnant subjects undergoing ovarian stimulation receiving Cyclogest (administered rectally) were compared with subjects supplemented with Utrogestan and Crinone (both administered vaginally). Plasma measurements following the administration of progesterone were remarkably similar despite various routes and preparations of progesterone. Statistical analysis revealed no significant difference in progesterone levels and length of luteal phase between these regimes ($P > 0.05$).

Unlike progesterone supplement, HCG is an indirect form of luteal support. It stimulates corpora lutea to secrete supra-physiological amounts of endogenous progesterone that is still clearly evident on day 10, 3 days after the last HCG injection. Plasma progesterone concentrations measured in the HCG group was statistically higher than in those subjects using progesterone supplements ($P > 0.001$). However, as the stimulatory effect of HCG was short-lived, the day 14 progesterone concentrations were lower than those seen with progesterone supplement. Despite this, the luteal phase duration in subjects using HCG was extended to 15.9 days (Table III).

Luteal supplement, irrespective of the preparation, is successful in maintaining the plasma progesterone concentrations at or above the physiological level throughout the late luteal phase. Furthermore, the length of luteal phase was 'normalised' in supplemented subjects undergoing ovarian stimulation.

Progesterone supplements were administered in a constant dosage and daily manner. Hence, single measurement of plasma progesterone concentration on luteal day 14 in a non-conception cycle would clearly reflect the steady state concentration of the administered luteal supplement. However, monitoring of the luteal phase on a daily basis is required for other luteal supplements such as HCG as the resulting plasma progesterone concentrations would depend on the timing and frequency of injections. In order to assess the distinctive efficacy of progesterone and HCG in "correcting" abnormal progesterone profiles, daily measurement of plasma progesterone were carried out in the luteal phase (Figure 1).

In Figure 1, plasma progesterone concentrations following Cyclogest supplement were only marginally higher than the unsupplemented subjects. The levels became significantly different from day 6 ($P < 0.01$), two days following the first use of the Cyclogest suppositories. A similar mid luteal decline in plasma progesterone concentrations still exist, indicating that the impact of progesterone supplement is very small.

On the contrary, two small injections of 1500IU of HCG administered during the early and mid luteal phase maintained plasma progesterone well above the physiological concentrations between days 5 to 13 ($P < 0.01$), reversing the sub-optimal hormonal environment during the entire luteal phase (Fig. 1b). Therefore, there is a distinctive difference in the impact of progesterone and HCG supplement on corpus luteum function, whereby HCG possessed a greater ability in correcting abnormal luteal phase than progesterone.

Pregnancy outcome

Clinical characteristics of the subjects, number of ampoules of gonadotrophin used, duration of stimulation, follicle responses to stimulation, together with the implantation and pregnancy rates in different luteal supplement regimes are presented in Table IV. The overall implantation and pregnancy rates in different luteal regimes were similar and exhibited no statistical significance ($P > 0.05$).

Table I: Geometric mean (95 % confidence interval) plasma progesterone concentration (nmol/l) and length of luteal phase (days) in the control groups (all subjects did not conceive and none of them received any luteal supplement).

Control	Day 10	Day 14	Luteal phase duration
Spontaneous cycle (n = 5)	33.9 (25-46)	7.8 (3-18)	14.2 ± 0.7
Stimulated cycle (n = 5)	12.0 (5-28)[a]	2.3 (0.9-6)[a]	11.1 ± 1.1[b]

[a] Significantly lower than spontaneous cycle-control, P < 0.05 (Students' t-test)
[b] Significantly lower than spontaneous cycle-control, P < 0.01 (Students' t-test)

Table II: Geometric mean (95 % confidence interval) plasma progesterone concentration (nmol/l) and length of luteal phase (days) in groups given Utrogestan vaginally (data from non-pregnant subjects only).

Utrogestan	Day 10	Day 14	Luteal phase duration
200 mg (n=10)	45.3 (32-65)	26.1 (18-37)	14.6 ± 2.0
400 mg (n=12)	49.4 (31-79)	30.6 (19-50)	14.0 ± 0.8
600 mg (n=11)	53.4 (40-71)	35.5 (25-50)	14.8 ± 1.4

No significant differences was observed among these groups (P > 0.05, ANOVA)

Table III: Geometric mean (95% confidence interval) of plasma progesterone concentration (nmol/l) and length of luteal phase (days) for each luteal support (data from non-pregnant subjects only).

Various regimes	Day 10	Day 14	Luteal phase duration
Cyclogest (n = 21)	51.5 (30-86)	37.0 (22-62)[b]	13.6 ± 2.0
Crinone (n = 22)	42.4 (33-54)	27.0 (20-36)[b]	13.3 ± 1.2
Utrogestan (n = 33)	49.6 (34-71)	30.9 (20-46)[b]	14.6 ± 1.5
HCG (n = 22)	316.6 (190-528)[a]	11.5 (5-28)	15.9 ± 1.9[b]

[a] Significantly higher than Crinone, Utrogestan, Cyclogest and both the control groups; (P < 0.001, ANOVA)
[b] Significantly higher than spontaneous cycle-control; (P < 0.05, Students' t-test)
No significant difference was observed between Crinone, Utrogestan and Cyclogest groups; (P > 0.05, ANOVA)

Table IV: Comparison between clinical parameters and pregnancy rates for women supplemented with various luteal support regimes in down regulated IVF cycles.

	Cyclogest	Crinone	Utrogestan	HCG
No. of cycles	35	36	55	35
Age (years)	32.2 ± 4.1	33.5 ± 3.2	31.6 ± 5.6	31.0 ± 3.4
Basal FSH (iu/l)	6.8 ± 1.8	8.1 ± 2.5	7.6 ± 2.6	5.9 ± 1.0
Gonadotrophins (iu)	24.2 ± 5.7	25.4 ± 4.2	19.0 ± 6.3	27 ± 5.3
Duration of stimulation (days)	10.6 ± 2.4	11.2 ± 2.1	9.5 ± 2.2	11.7 ± 1.8
Peak oestradiol (pmol/ml)	8249 (4292-11782)	9357 (5134-15535)	9975 (6350-13202)	5096 (3562-7293)
No. oocytes/patient	9.9 ± 3.8	7.1 ± 4.4	9.3 ± 4.4	8.6 ± 3.4
Fertilisation rate (%)	76	79	83	84
No. embryos transfer	2.5 ± 0.5	2.3 ± 0.6	2.3 ± 0.7	2.3 ± 0.5
Implantation rate (%)	23	20	23	21
Expected live birth rate (%)	35% (12/35)	36% (13/36)	35% (19/55)	34% (12/35)

Implantation rate is defined as the number of fetal hearts over the total number of embryos replaced. Biochemical and ectopic pregnancies are classed as single implantation.
On going pregnancy is defined as a pregnancy of more than 14 weeks gestation.

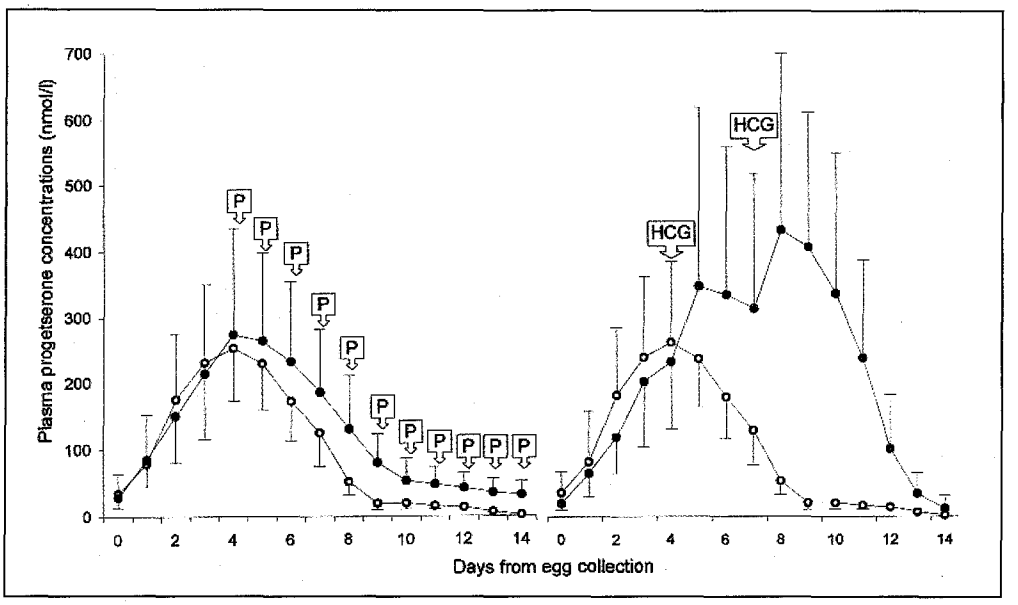

Fig. 1: Geometric means and 95% confidence intervals of plasma progesterone concentrations (nmol/l) in stimulated IVF cycles given additional (a) Cyclogest (□, n = 16) (b) HCG (□, n = 18) are contrasted with the unsupplemented stimulated cycles (□, n = 5) throughout the entire luteal phase. Cyclogest or HCG administrations are shown in the box.

Discussion

Abnormal hormonal profiles in luteal phase following stimulated cycles using GnRH-a have been clearly demonstrated[1]. Strategies to overcome these problems led to the use of luteal support. There are numerous types of supplemental regimes used by many IVF units, but little data exists in the literature to demonstrate the efficacy of these regimes on plasma concentrations and their impact in normalising hormonal profiles.

Segal and Casper (1992)[11] have assessed plasma progesterone concentrations following exogenous supplement administered vaginally in stimulated IVF cycles. Concentrations measured during the mid luteal phase were 140 and 50nmol/l in supplemented and unsupplemented groups respectively. The authors implied that vaginal suppositories could deliver approximately 90nmol/l of progesterone to the circulation. Data in Tables II and III clearly contradict their results. There are two reasons to account for such discrepancy. First, progesterone concentrations in their study were measured at a time when corpora lutea are still actively secreting supra-normal amount of progesterone. Second, concentrations were measured over a wide period of time, between days 7 and 9 after embryo transfer, potentially introducing errors into their results. To measure the amounts of progesterone delivered by various luteal supplements accurately, we believe that it is best performed in non-pregnant cycles during the late luteal phase, in a situation where endogenous contribution to progesterone secretion by the corpus luteum is minimal.

Natural progesterone, administered vaginally and rectally, deliver approximately 30-40 nmol/l of progesterone to the circulation in this study. Perhaps not surprisingly, the addition of luteal progesterone was grossly inadequate to either prevent or correct the mid luteal decline in plasma progesterone concentrations. The impact of progesterone supplement was relatively trivial in comparison to the supra-normal concentrations (in the region of 200-300nmol/l) secreted endogenously during the early luteal phase. Under these circumstances, it may seem sensible to overcome these problems by increasing the dose of progesterone administrated. However, although dose proportionality was demonstrated, as shown by the increase in plasma

progesterone concentrations after the administration of increasing doses of 200, 400 and 600mg of Utrogestan, these concentrations did not reach statistical significance (Table II). This dose-independent phenomenon indicates that the pharmacokinetic processes of absorption, distribution, and elimination for micronised progesterone given vaginally is not altered with the changes in the administered dose [12].

Oral and intramuscular forms of progesterone are intentionally omitted from this study. Although orally administrated micronised progesterone might seem more convenient and the preferred method of choice for many patients, it produced poorly sustained plasma concentrations and is subjected to considerable first-pass pre-hepatic and hepatic metabolism [7, 13]. Further conversion to 3a-reduced metabolites yields compounds that potentially elicit hypnotic effects [14]. Intramuscular progesterone was not assessed because this route of administration is not well tolerated by the patients with reported side effects such as multiple painful injections and allergic site reactions [15, 16].

Progesterone, a corpus luteum substitute, is a direct form of luteal support. It delivers a constant amount of 'exogenous' progesterone to the circulation and is completely independence of corpus luteum function. In comparison, HCG, a corpus luteum stimulant, is an indirect form of luteal support. It binds to the LH/HCG receptors on corpus luteum to produce 'endogenous' progesterone. The action of HCG depends heavily on the number and functional ability of corpus luteum. Liu et al. (1995) [17] have confirmed that early luteal phase corpus luteal activity correlates with the degree of ovarian stimulation and folliculogenesis. Multiple stimulated follicles will eventually transform into corpora lutea. Hence, following the occupation of all the LH/HCG receptors found on the corpora lutea by HCG supplement, a surge in steroidogenic activity took place. Two 1500iu injections of HCG given during the early and mid luteal phase managed to rescue corpora lutea and maintain progesterone well above the physiological range (in the region of 200 to 400nmol/l) between luteal days 9 to 11, around the time of expected embryo implantation. Furthermore, HCG stabilises progesterone profiles in this study up to 10 days after egg collection.

It is now clear that the routine use of progesterone supplements following stimulated IVF is not effective in stabilising the progesterone profile. Interestingly, despite the greater ability of HCG supplement to stabilise the abnormal progesterone decline, similar implantation and pregnancy rates were observed in all groups. If the rapid decline in mid luteal progesterone is implicated in failure to implant, stabilising these hormonal defects using HCG luteal support would be expected to create the best environment and so should produce the highest pregnancy rates. In fact this was not the case. Therefore, this observation did not substantiate the original hypothesis that the premature and rapid decline in progesterone during the mid luteal phase was detrimental to the integrity of the endometrium.

Although plasma and saliva progesterone measurement is a commonly used tool in assessing luteal phase defects, a recent publication has questioned the validity of these methods. Miles *et al.* (1997) [18] have studied the plasma and endometrial levels of progesterone following administration of intramuscular and vaginal progesterone on agonadal women. Significantly higher progesterone concentrations were found in endometrium in subjects using vaginal progesterone, in comparison to those subjects using intramuscular progesterone, despite the plasma progesterone concentrations demonstrating the reverse. This progesterone concentration difference in the uterine cavity has probably to do with a "uterine first-pass effect" [19]. A distribution mechanism could be involved with diffusion from the vaginal/uterine vein to the artery, as progesterone concentrations in the uterine artery were found to be higher than in the radial artery following vaginal administration [20].

In conclusion, natural progesterone, administered either rectally or vaginally, delivers a relatively small amount of progesterone (30 to 40 nmol/l) to the circulation and is independent of the dose and preparations. HCG, despite possessing a greater ability in optimising hormonal environment than progesterone supplement, did not improve the pregnancy outcome. Mid luteal decline in progesterone concentrations did not appear to be detrimental to the process of implantation.

References

1. Tay Pys, Lenton EA. Progesterone profiles in pregnant, non pregnant natural and stimulated IVF cycles with and without luteal support. Med. J. Malaysia. 2002; 57: 178-87.
2. Sharara, FL, McClamrock HD. Ratio of oestradiol concentration on the day of human chorionic gonadotrophin administration to mid-luteal oestradiol concentration is predictive of in-vitro fertilization outcome. Hum. Reprod. 1999; 11: 2777-82.
3. Speroff L, Glass RH, Kase NG. Regulation of the menstrual cycle. In Speroff, L., Glass, R.H., Kase, N.G. (eds), Clinical Gynaecologic Endocrinology and Infertility. 5th edition, Williams and Wilkins, Baltimore, MA, USA, 1994; 1183-230.
4. Penzias AS. Luteal phase support. Fertil. Steril. 2002; 77: 318-23.
5. Penzias AS, Alper MM. Luteal support with vaginal micronized progesterone gel in assisted reproduction. Reprod. Biomed. Onl. 2003; 6: 287-95.
6. Ludwig M, Schwartz P, Babahan B, Katalinic A, Weiss JM, Felberbaum R, Al-Hasani S, Diedrich K. Luteal phase support using either Crinone 8% or Utrogest: results of a prospective, randomized study. Eur. J. Obstet. Gynecol. Reprod. Bio. 2002; 103: 48-52.
7. Nahoul K, Dehennin L, Jondet M, Roger M. Profiles of plasma estrogens, progesterone and their metabolites after oral or vaginal administration of oestradiol or progesterone. Maturitas; 1993; 16: 185-201.
8. Gurbuz B, Yalti S, Ficicioglu C, Delikara N, Alpay Z. Bleeding patterns in women using intramuscular progesterone for luteal support in in-vitro fertilisation cycles. J. Obstet. Gynaecol. 2003; 23: 267-70.
9. Trounson A, Howlett D, Rogers P, Hoppen H. The effect of progesterone supplementation around the time of oocyte recovery in patients superovulated for in-vitro fertilisation. Fertil. Steril. 1986; 45: 532-35.
10. Fulimoto A, Osuga Y, Fujiwara T, Yano T. Human chorionic gonadotropin combined with progesterone for luteal support improves pregnancy rate in patients with low late-mid luteal estradiol levels in IVF cycles. J.Asst. Reprod. Genet. 2002; 19: 550-4.
11. Segal S, Casper RF. Progesterone supplementation increases luteal phase endometrial thickness and oestradiol levels in in-vitro fertilisation. Hum Reprod. 1992; 9: 1210-13.
12. Simon JA, Robinson DE, Andrews MC, Hildebrand JR, Rocci ML, Blake RE, Hodgen GD. The absorption of oral micronised progesterone: the effect of food, dose proportionality, and comparison with intramuscular progesterone. Fertil. Steril. 1993; 60: 26-33.
13. Nahoul K, Dehennin L, Jondet M, Roger M. Profiles of plasma estrogens, progesterone and their metabolites after oral or vaginal administration of oestradiol or progesterone. Maturitas 1993; 16: 185-201.
14. Arafat ES, Hargrove JT, Maxson WS. Sedative and hypnotic effects of oral administration of micronised progesterone may be mediated through its metabolites. Am. J. Obstet. Gynecol., 1988; 159: 1203-209.
15. Damario MA, Goudas VT, Session DR, Hammitt DG, Dumesio DA. Crinone 8% vaginal progesterone gel results in lower embryonic implantation efficiency after in vitro fertilization-embryo transfer. Fertil. Steril.; 1999; 72: 830-6.
16. 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.
17. Liu HC, Pyrgiotis E, Davis O, Rosenwaks Z. Active corpus luteum function at pre-, peri- and post-implantation is essential for a viable pregnancy. Ear. Preg: Bio. & Med. 1995; 281-87.
18. Miles RA, Paulson RJ, Lobo RA, Press MF, Dahmoush L, Sauer MV. Pharmacokinetics and endometrial tissue levels of progesterone after administration by intramuscular and vaginal routes: a comparative study. Fertil. Steril.1994; 62: 485-90.
19. Fanchin R, De Ziegler D, Bergeron C, Righini C, Torrisi C, Frydman R. Transvaginal administration of progesterone. Obstet Gynecol 1997; 90: 396-401.
20. Cicinelli E, De Ziegler D. Transvaginal progesterone: evidence for a new functional "portal system" flowing from the vagina to the uterus. Hum Reprod Update 1999; 5: 365-72.