ORIGINAL ARTICLE

Inhibition of Progesterone Secretion by Oestradiol Administered in the Luteal Phase of Assisted Conception Cycles

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Summary

A prospective randomised study was done to assess the effect of supplemental oestradiol in addition to progesterone on the luteal steroid profiles and pregnancy outcome in stimulated cycles with and without pituitary down regulation. Women undergoing stimulated cycle IVF with GnRH-a and FSH (Group A, n = 63) or stimulated intrauterine insemination using CC and FSH (Group B, n = 55) were studied. These subjects were randomly allocated to receive either 400mg daily of vaginally administrated Cyclogest (progesterone) alone or in combination with 2mg daily of oral Oestradiol Valerate (E2V) during the luteal phase. Significant lower concentrations of plasma progesterone were observed in those subjects supplemented with both E2V and progesterone compared to those in whom progesterone only was given during the luteal phase ($P < 0.05$). Exogenous E2V had a minimal impact on plasma oestradiol concentrations and did not disguise the characterised mid luteal decline in oestradiol secretion. The suppressive effect of E2V on plasma progesterone was lost if implantation occurred normally because any small change in steroid concentrations was reversed by the rapidly increasing concentrations of HCG. Similar pregnancy rates were observed among subjects supplemented with or without oestradiol. The addition of oestradiol to the luteal supplement suppresses endogenous corpus luteum progesterone secretion irrespective of the type of assisted conception cycle and that its use is unlikely to be beneficial to the process of implantation

Key Words: Oestradiol, Progesterone, Luteal phase, Pregnancy, In-vitro fertilisation, Corpus luteum

Introduction

The role of supplementing progesterone during the luteal phase in stimulated in-vitro fertilisation (IVF) is the subject of numerous publications. However, there are relatively little attempts to clarify the significance of oestradiol following ovarian stimulation. Although the role of oestradiol in endometrial priming during the follicular phase is well recognised, the impact of oestradiol on the luteal phase in human remains controversial.

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A concomitant decline in plasma sex hormones concentrations was constantly observed in pituitary down regulated cycles [1]. Such precipitous decline in steroids may be harmful to receptive endometrium and implantation, hence luteal support was recommended [2]. A study by Sharara and McClamrock (1999) assessed 106 progesterone supplemented IVF cycles with pituitary down regulation [3]. Cycles were divided into groups according to the ratio of oestradiol concentrations on the day of HCG administration to the mid luteal phase. It was found that the pregnancy rate is significantly lower if the ratio exceeded 5.0 compared to those less than 5.0. They postulated that the magnitude of decline in oestradiol concentrations following ovarian stimulation is highly predictive of pregnancy outcome and the rapid fall in oestradiol following oocyte recovery may compromise endometrial integrity. This study has also raised the issue of incorporating oestradiol into the progesterone supplement.

Oestradiol supplementation has been extended into Clomiphene Citrate (CC)-stimulated IVF cycles. Studies of endometrium after exposure to CC have shown histological features consistent with a hypooestrogenic effect [4] and reduction in glandular density and an increase in the number of vacuolated cells [5]. The deleterious effect of CC on endometrium maturity can be reverse by 0.2 mg of ethinyl-oestradiol [4]. Hurd et al. (1996) have randomised 79 subjects to either receiving no luteal support or support with both oral oestradiol and vaginal progesterone in CC stimulated IVF cycles. They found that the latter group exhibited a significantly higher pregnancy rate [6].

This study was performed in an attempt to clarify the impact of oestradiol supplement on the corpus luteum function as well as the pregnancy outcome, following ovarian stimulation with (GnRH-a and r-FSH) or without (CC and r-FSH) pituitary down regulation.

Materials and Methods

118 subjects attending Sheffield Fertility Centre, Sheffield, United Kingdom between Jan 1998 to Jan 1999 were recruited into the study. None of the women had had previous infertility treatment and with a minimum duration of infertility of 2 years. The mean age of the subjects was 32.2 $\pm$ 4.5 years (range from 22 to 39) and all of them exhibited basal follicle stimulating hormone (FSH) of <10 IU/l.

Subjects were allocated to one of the two treatment groups, A and B. Subjects in Group A (n=63) were stimulated with long protocol down regulated IVF regime (GnRH-a/r-FSH). Forty-percent of the subjects in this group had tubal disease, 29% had male factor infertility, 10% had endometriosis, 19% had ovulatory disorder and 2% of the cases were unexplained infertility. Some of these subjects had more than one infertility factor.

All subjects in Group B (n=55) have undergone stimulated intrauterine insemination treatment using Clomiphene citrate and r-FSH (CC/r-FSH). All the subjects this group had unexplained infertility. They had ovulatory cycles (confirmed by a recent ovulatory mid luteal phase progesterone measurement), bilateral patent tubes (confirmed by either a recent laparoscopy or hysterosalpingogram) and normal semen analysis (semen concentrations $\geq$ $20x10^6$/ml, motility (grade I + II) (30% and normal forms $\geq$ 25%).

The local ethics review committee approved this study and all subjects gave informed consent.

Treatment protocols

Stimulated IVF (Group A; GnRH-a/r-FSH) 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). 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.

Stimulated intrauterine insemination (Group B; CC/r-FSH) involved using 100mg of CC per day for 5 days, starting on cycle day 4 (day 0 is the first day of the period). This was followed by 75 IU of FSH (Gonal-F, Ares-Serono Ltd., London W1N 1AF, UK), given daily, starting on cycle day 7. Treatment was monitored by daily measurement of oestradiol and LH, starting from day 9 of the cycle, together with alternate day vaginal ultrasound scans (Combison 310, Kretztechnik, AG, Austria). When two to four follicles measuring ≥ 18 mm were present and circulating E2 was ≥ 2000 pmol/l, 10000 IU of HCG was administered followed by intrauterine insemination 34-36 hours later.

Luteal support regimes

Subjects undergoing stimulated IVF (Group A; GnRH-a/r-FSH) and stimulated intrauterine insemination (Group B; CC/r-FSH) cycles were allocated randomly to the one of the two luteal support regimes on the day of embryo transfer or insemination.

1. Progesterone supplement only: 200 mg pessary (Cyclogest, Hoechst UK Ltd., Hoechst House, Salisbury Road, Hounslow, Middlesex) administered vaginally twice daily.
2. Progesterone supplement (as described above) together with 2mg per day of oral oestradiol valerate (E2V).

All luteal supplements were administered between 1800-2100 hours, commenced on luteal phase (LP) day 1, the day after egg collection or intrauterine insemination.

Definitions

Positive HCG is defined as the detection of plasma HCG concentrations of ≥ 10 IU/l by day 14 following egg collection. A rise in plasma HCG concentrations between 14 and 18 days after egg collection is also classed as positive HCG. *Clinical pregnancy* is defined as fetal heart activity that is visualised on ultrasound. *Length of luteal phase* is the number of days from the time of egg collection (in IVF cycles) or intrauterine insemination (in ovulation induction cycles) to the first day of menstrual bleeding.

Hormonal assays

Blood samples were collected between 0800-0900 hours on luteal days 8 and 12 only. All blood samples were collected and stored at -20° C until the day of assay. Serum oestradiol (E2) and progesterone (P) were measured and the samples belonging to the same patient were assayed in the same run to reduce inter-assay variability.

Plasma oestradiol was measured using a commercially available micro-particle enzyme immunoassay (MEIA) system [IMx assay technology, Abbott Laboratories, UK]. The sensitivity of this system is 91.8pmol/l. The intra- and inter-assay variation was 3.8-10.4% and 4.3-16% respectively. 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]. The 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%.

Statistic analysis

Hormonal data were first logarithmically transformed to normalise the distributions and then geometric means and 95% confidence intervals calculated. Statistical analysis such as χ^2 and Students' t-test were employed where appropriate. This was performed with SPSS (Version 10) for Windows. *P* value of less than 0.05 indicates statistical significance.

Results

Individual variation to ovarian stimulation in both Groups A and B resulted in a wide range of confidence intervals for plasma oestradiol concentrations on luteal day 8. Despite the addition of E2V, no significant difference was seen in plasma oestradiol concentrations on luteal day 8 when compared to those without any E2V supplementation in both stimulated IVF and insemination groups (Figure 1). The presence of corpora lutea, consequence to ovarian stimulation, led to a supra-physiological amount of oestradiol in the circulation. Hence, the small contribution of E2V was masked by the enormous oestradiol production in the mid luteal phase. However, as the endogenous oestradiol secretion fell during the late luteal phase (Day 12) in non-conception cycles, plasma oestradiol concentrations became significantly higher for the group receiving E2V ($P < 0.05$) (Figure 1).

The inclusion of E2V suppressed endogenous progesterone secretion in patients in both Groups A and B, leading to lower concentrations on luteal days 8 and 12. Statistical analysis between the groups supplemented with or without E2V reached significant value ($P < 0.05$) (Figure 1).

Interestingly, the negative impact of E2V can be overcome by achieving a successful implantation. Exponentially rising HCG derived from the embryo(s) promptly rescued the corpora lutea and no further difference was evidenced between plasma progesterone concentrations on day 12 (Figure 2).

In the non-conception cycles, the length of the luteal phase was 14.9 ± 1.8 and 15.6 ± 1.5 in the Group A and 14.7 ± 1.7 and 15.4 ± 1.5 in Group B in subjects supplemented with Cyclogest alone or in combination with E2V respectively. There is no statistical difference in the duration between these groups.

Tables I and II show the clinical parameters and pregnancy rates in women supplemented with Cyclogest or E2V in Groups A and B respectively. No significant difference in positive HCG and clinical pregnancy rates between the two supplemented groups in subjects undergoing assisted conception treatment using CC or GnRH-a (χ^2, $P > 0.05$).

Table I: Comparison between different parameters and pregnancy rate for women supplemented with Cyclogest alone or in combination with E2V in stimulated IVF cycles.

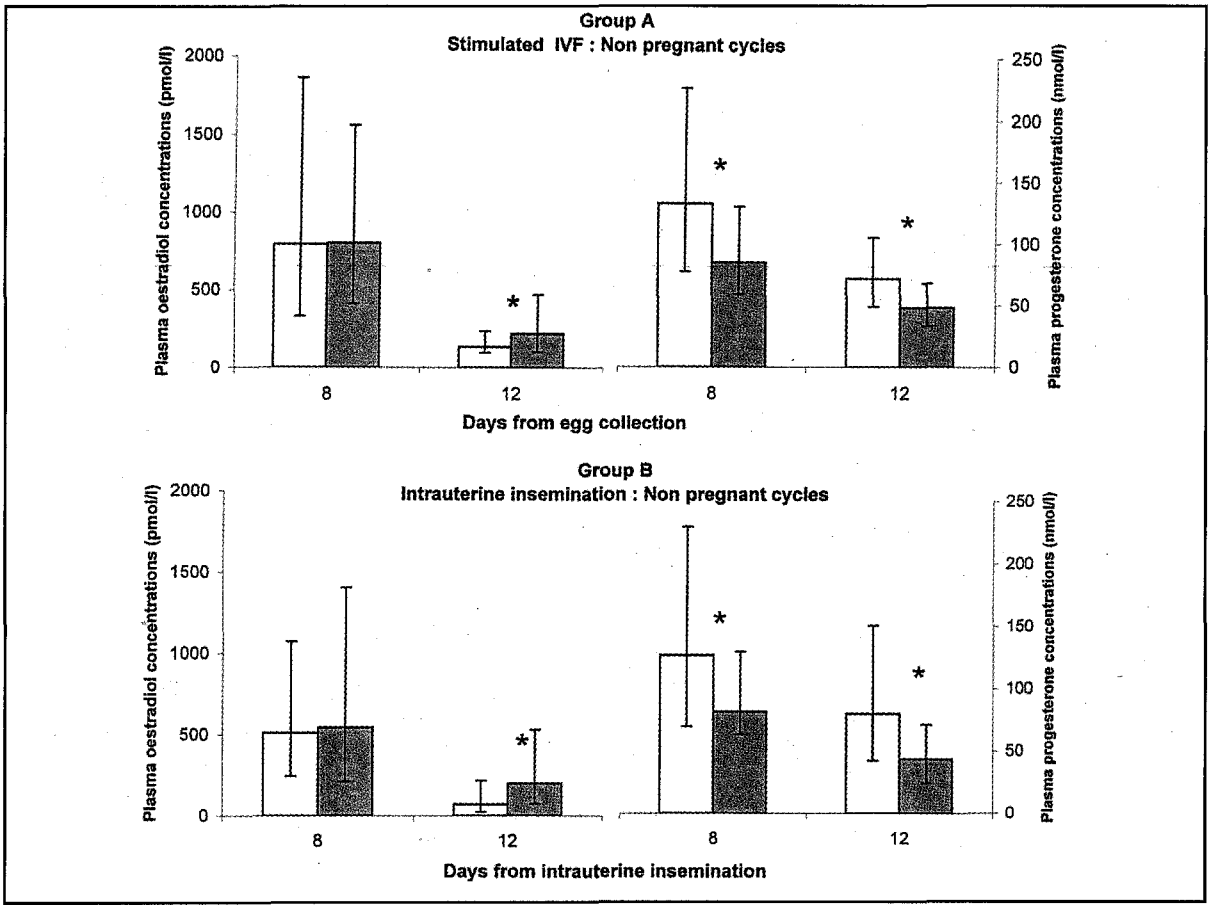
	Cyclogest	Cyclogest and E2V
No. of cycles	35	28
Age (years)	33 ± 6	35 ± 4
Gonadotrophin used (Amps)	25.9 ± 8.1	22 ± 10.3
Duration of stimulation (days)	12.5 ± 3.4	11.3 ± 2.0
Peak oestradiol (pmol/ml) *	7174 (4681-10974)	5089 (2491-8730)
No. oocytes /patient	10.2 ± 6.5	9.1 ± 7.2
Fertilisation rate (%)	69 ± 20	77 ± 16
No. embryos transfer	2.6 ± 0.4	2.3 ± 0.5
Positive HCG (%)	10/35 (28)	7/28 (25)
Clinical pregnancy (%)	7/35 (20)	5/28 (18)

All values are means ± SD except * denoting geometric mean and 95% confident intervals.

Table II: Comparison between the clinical parameters and pregnancy rate for women supplemented with Cyclogest alone or in combination with E2V in stimulated insemination cycles.

	Cyclogest	Cyclogest and E2V
No. of cycles	30	25
Age (years)	29 ± 2.4	32 ± 3.4
Gonadotrophin used (Amps)	5.2 ± 3.4	4.2 ± 3.2
Peak oestradiol (pmol/ml) *	1759 (890-4871)	2083 (1179-5781)
No. follicles ≥18 mm	2.4 ± 1.7	2.8 ± 1.3
Positive HCG (%)	7/30 (23)	5/25 (20)
Clinical pregnancy (%)	5/30 (17)	3/25 (12)

All values are means ± SD except * denoting geometric mean and 95% confident intervals.



Legends

Fig. 1: Plasma oestradiol and progesterone concentrations (Geometric means ± 95% confidence intervals) on days 8 and 12 following egg collection or intrauterine insemination in stimulated *non-conception cycles* supplemented with progesterone only (□) or both progesterone and Oestradiol Valerate (▩). Pairs that are significantly different (P < 0.05) are shown by asterisks.

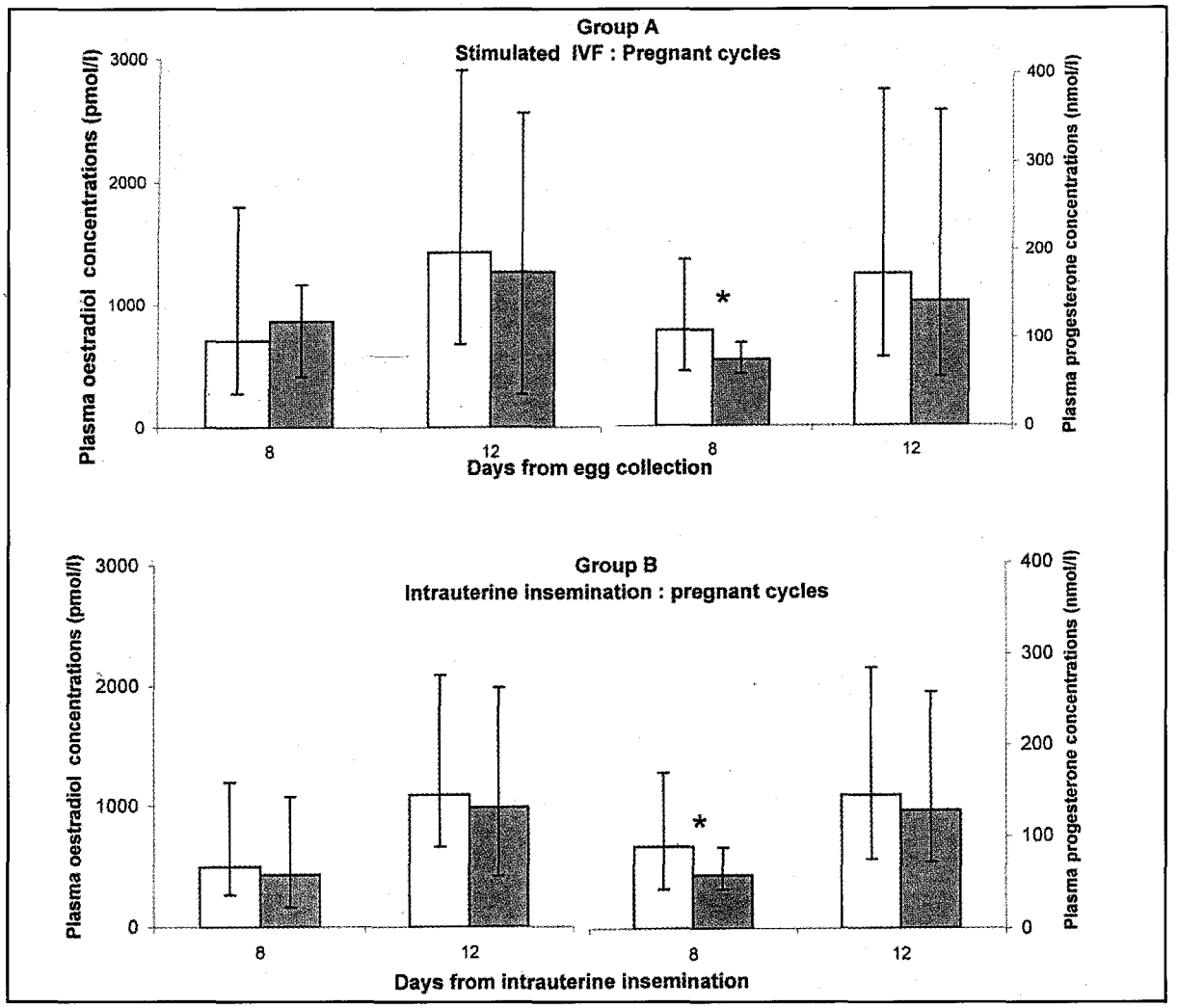


Fig. 2: Plasma oestradiol and progesterone concentrations (Geometric means ± 95% confidence intervals) on days 8 and 12 following egg collection or intrauterine insemination in stimulated *conception cycles* supplemented with progesterone only (□) or both progesterone and Oestradiol Valerate (▩). Pairs that are significantly different (P < 0.05) are shown by asterisks.

Discussion

Progesterone is the most important sex hormone for human implantation, while oestradiol may play a permissive, but not an essential role [7]. Therefore, when the luteal support is used in ovarian stimulation, progesterone is frequently used alone and oestrogen is usually omitted. This approach fails to recognize a potential role for oestrogen during the peri-implantation period. A recent study by Sharara and McClamrock (1999) has recognised the significance of luteal oestradiol in pregnancy outcome [3]. A greater degree of decline in oestradiol levels during the mid luteal phase following GnRH-a/HMG cycles was shown to

significantly associate with higher pregnancy losses and lower implantation rate. Therefore, further studies into the role of luteal oestradiol supplementation are warranted.

With the supplement of progesterone in the spontaneous cycles, a 2 to 3 fold rise in concentration was observed [1]. However, the addition of oestradiol valerate into the supplemental regime immediately induced a fall in progesterone concentrations. This observation suggests that a physiological quantity of exogenous oestradiol can adversely affect corpus luteum function. The mechanism of oestradiol-related inhibition of progesterone secretion is not entirely clear and the sites at which oestradiol act needs clarification. One possible mechanism is that exogenous oestradiol acts indirectly upon the hypothalamo-hypophyseal axis to inhibit LH secretion [8], thus denying the corpus luteum of necessary luteotrophic support. Evidence for such an effect is provided by Schoonmaker *et al.* (1981) [9]. They demonstrated that a small increase in peripheral oestradiol produced by a subcutaneous oestradiol implant in rhesus monkeys could lead to LH suppression and premature luteolysis in the absence of any increase in oestradiol within the corpus luteum.

In subjects possessing an intact hypothalamic-pituitary ovarian feedback system, it is probable that the oestradiol-related suppression in pituitary LH secretion might lead to abnormal corpus luteum function. However, similar mechanism cannot be applied to down regulated IVF cycles whereby very little LH is present in the circulation throughout the entire luteal phase[10]. In an environment with low LH concentrations, single ovulatory dose of HCG given prior to egg collection is the only stimulus for supporting corpora lutea function [11]. The levels of HCG are not influenced by exogenous oestradiol. From these observations, it seems likely that oestradiol has a systemic as well as a local effect on the ovaries. Injection of oestradiol into the ovary containing the corpus luteum induces premature luteal regression in rhesus monkeys [11]. Under in vitro experimental conditions, oestradiol can act directly upon human [12] and monkey granulosa and theca cells 13 to inhibit both basal and gonadotrophin stimulated progesterone production. It has been suggested that oestradiol may act through a short loop feedback paracrine action and play a vital organizing role in androgen and progesterone production [12].

Several studies have suggested a luteolytic role for exogenous oestradiol on the corpus luteum, as evidence by a reduction in progesterone secretion and the onset of premature menstruation [11]. Because of the supplementation with progesterone, most of the subjects in this study did not experience premature menstruation. The progesterone supplement seems to be the key that ensures optimal priming and maintenance the endometrium in its ' functional state'. Moreover, in this study, the corpus luteum retained its full 'functional capacity' as it was readily rescued by human chorionic gonadotrophin (HCG) after embryo implantation with production of appropriate amounts of progesterone (data not shown). This indicates that, although oestradiol can render the corpus luteum less active, it does not induce a functional destruction of the corpus luteum.

Relatively high amounts of oestradiol seen on day 8 mask the presence of exogenous E2V in both stimulated and natural cycles and reflect the small amount of E2V that was used in this study. Not surprisingly, the exogenous oestradiol had a minimal impact and failed to prevent the decline in oestradiol levels during the mid luteal phase. One way of correcting this decline is to increase the doses of E2V, but this would not seem wise because it may further exacerbate the suppressive effect of oestradiol on the function of corpus luteum. Another approach to correct the defect is to use human chorionic gonadotrophin (HCG) as this restores the concentrations of both oestrogen and progesterone in the mid luteal phase [14, 15]. The argument against the routine use of HCG is the higher incidence of ovarian hyperstimulation syndrome. Shoham and Schachter (1996) have

suggested that the actual concentrations of plasma oestradiol do not affect the quality of endometrial receptivity, providing that a certain threshold level is exceeded [8].

Hurd *et al.* (1996) have analysed 79 CC stimulated IVF cycles and have found that luteal supplement of oestradiol and vaginal progesterone improved pregnancy outcome in comparison to those subjects receiving no luteal support [6]. However, their data have to be interpreted with caution, as the question of whether the oestradiol, or the progesterone or a combination of both is responsible for the increase pregnancy rate remains unanswered. In this study, it is worth noting that there is no statistical difference in the pregnancy rates between the groups supplemented with or without E2V. This finding is in agreement with previous studies on CC [16] and GnRH-a cycles [17]. An important point to be stressed is that the small number of subjects in this study may lead to the insufficient power of the study. Therefore, the possibility remains that the results of these trials, including the current one, are false negative and a meta-analysis in the future may overcome this problem. However, judgment of meta-analysis too has to be interpreted with caution, as publication bias can distort the end result [18].

In conclusion, the inclusion of exogenous oestradiol in the form of oestradiol valerate to a luteal supplement for assisted conception does not dismiss nor induce a functional destruction of the corpus luteum, but suppresses secretion of progesterone by the corpus luteum and is unlikely to be beneficial to the process of implantation.

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