

Comparison between different routes of progesterone administration as luteal phase support in infertility treatments

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Different routes of natural progesterone supplementation have been tried as luteal phase support in infertility treatments. Orally administered progesterone is rapidly metabolized in the gastrointestinal tract and its use has proved to be inferior to i.m. and vaginal routes. Progesterone i.m. achieves serum progesterone values that are within the range of luteal phase and results in sufficient secretory transformation of the endometrium and satisfactory pregnancy rates. The comparison between i.m. and vaginal progesterone has led to controversial results as regards the superiority of one or the other in inducing secretory endometrial transformation. However, there is increasing evidence in the literature to favour the use of vaginal progesterone. Vaginally administered progesterone achieves adequate endometrial secretory transformation but its pharmacokinetic properties are greatly dependent on the formulation used. After vaginal progesterone application, discrepancies have been detected between serum progesterone values and histological endometrial features. Vaginally administered progesterone results in adequate secretory endometrial transformation, despite serum progesterone values lower than those observed after i.m. administration, even if they are lower than those observed during the luteal phase of the natural cycle. This discrepancy is indicative of the first uterine pass effect and therefore of a better bioavailability of progesterone in the uterus, with minimal systematic undesirable effects.

Key words: In-vitro fertilization/luteal phase/oocyte donation/progesterone supplementation

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Introduction

Adequate secretory transformation of the endometrium is essential for embryonic implantation during the so-called 'implantation window'. Synchronization between embryonic development and endometrial receptivity is of main concern in infertility treatments, namely in IVF, in oocyte donation programmes and in frozen-thawed cycles. Although it has been several years since the first report of a delivery after IVF (Stephoe and Edwards, 1978) and oocyte donation (Lutjen *et al.*, 1984), the mechanisms that govern endometrial receptivity and embryo implantation have not yet been

fully elucidated. Secretory endometrial transformation and receptivity depend solely on the duration of exposure to adequate progesterone concentrations, provided that sufficient oestrogen priming has already occurred during the follicular phase (de Ziegler *et al.*, 1992).

Human chorionic gonadotrophin (HCG) and progesterone are ordinarily used as luteal phase support in IVF. It has been suggested that HCG might be superior to progesterone in gonadotrophin-releasing hormone (GnRH) agonist cycles for IVF (Soliman *et al.*, 1994), but its application has also been associated with ovarian hyperstimulation syndrome (OHSS) (Herman *et al.*, 1990; Smitz *et al.*, 1990; Araujo *et al.*, 1994). Since natural progesterone is rapidly metabolized after oral administration, synthetic progestins have been designed which resist enzymatic degradation. However, synthetic progesterone derivatives have been associated with a number of undesirable effects, notably on lipids (Hirvonen *et al.*, 1981; Ottosson *et al.*, 1985) or with psychological effects that may be severe enough to limit their use (Dennerstein *et al.*, 1979; Sherwin and Gelfand, 1989). In addition, synthetic progestogens, mainly those with androgenic properties, have been connected with an increased risk of fetal congenital malformations (Revesz *et al.*, 1960; Wilkins, 1960; Nora and Nora, 1975; Aarskog, 1979). Natural progesterone has no

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adverse effects on high density lipoproteins (HDL) (Ottoosson *et al.*, 1985), no known teratogenicity (Chez, 1978; Rock *et al.*, 1985; Check *et al.*, 1986) and is more effective in inducing secretory endometrial features than progesterone derivatives, e.g. dehydrogesterone (Pellicer *et al.*, 1989).

Different routes of progesterone administration have been analysed, such as the intranasal (Steege *et al.*, 1986; Cicinelli *et al.*, 1993; Cicinelli *et al.*, 1994), sublingual (Chakmakjian and Zacharian, 1987; Stovall *et al.*, 1996) and rectal routes (Nillius and Johansson, 1971; Johansson 1972; Chakmakjian and Zacharian, 1987). However, oral (Whitehead *et al.*, 1980; Maxson and Hargrove, 1985; Simon *et al.*, 1993), i.m. (Nillius and Johansson, 1971; Johansson 1972; Devroey *et al.*, 1989; Bourgain *et al.*, 1990) and vaginal (Nillius and Johansson, 1971; Villanueva *et al.*, 1981; Devroey *et al.*, 1989; Archer *et al.*, 1995; Fanchin *et al.*, 1997) routes have been the most frequently investigated.

As natural progesterone is rapidly metabolized after oral ingestion, a number of techniques have been developed in order to improve its pharmacokinetic properties (Kincl *et al.*, 1978). Micronization of progesterone reduces particle size and increases progesterone absorption and bioavailability (Maxson and Hargrove, 1985; Kimzey *et al.*, 1991). Furthermore the combination of micronized progesterone with polycarophil gel results in a sustained-release vaginal formulation (Casanas-Roux *et al.*, 1996; Ross *et al.*, 1997; Fanchin *et al.*, 1997; Gibbons *et al.*, 1998).

Many studies have reported on the different effects that different natural progesterone formulations exert on endometrial secretory transformation. Orally administered natural progesterone has been shown to be ineffective in inducing an in-phase secretory endometrium (Lane *et al.*, 1983; Dehou *et al.*, 1987; Devroey *et al.*, 1988; Bourgain *et al.*, 1990; Moyer *et al.*, 1993). Progesterone i.m. results in high serum progesterone concentrations, adequate endometrial secretory features (Navot *et al.*, 1986; Devroey *et al.*, 1989; Davies *et al.*, 1990; Sauer *et al.*, 1991) and satisfactory pregnancy rates (Navot *et al.*, 1986; Younis *et al.*, 1992; Smitz *et al.*, 1992; Pados *et al.*, 1992; Artini *et al.*, 1995). However, daily injections may be uncomfortable, especially for long-term treatments, and endometrial histological characteristics may be inferior to those observed after vaginal progesterone application (Devroey *et al.*, 1989; Bourgain *et al.*, 1990). Vaginal progesterone results in adequate endometrial secretory transformation, despite serum progesterone concentrations that may be lower than those observed during the luteal phase (Salat-Baroux *et al.*, 1988; Cicinelli *et al.*, 1996; Fanchin *et al.*, 1997). These discrepancies between serum progesterone values and histological findings after vaginal progesterone administration are indicative of the so-called first uterine pass effect, i.e. the direct effect that might be generated on the endometrium after vaginal progesterone application (Miles *et al.*, 1994; Balasch *et al.*, 1996; Fanchin *et al.*, 1997).

The aim of this paper is to provide a review of published data regarding the different routes of progesterone supplementation as luteal phase support in infertility treatments, mainly in ovulation induction for IVF and oocyte donation programmes. Pharmacokinetic properties and histological findings after different routes of progesterone supplementation are reviewed. Particular emphasis is placed on the existing evidence from the literature for the so-called first uterine pass effect after vaginal progesterone administration.

Pharmacological properties

Oral progesterone

Although the convenience of orally administered progesterone is indisputable, its use has been associated with systematic adverse effects, e.g. drowsiness, flushing and nausea (Maxson and Hargrove, 1985; Kimzey *et al.*, 1991; Pouly *et al.*, 1996). Sedative and hypnotic effects or fluid retention have also been attributed to progesterone or its metabolites after oral ingestion (Arafat *et al.*, 1988). Pharmacokinetic properties of orally administered progesterone are further influenced by food uptake (Simon *et al.*, 1993) or by the characteristics of progesterone preparation such as vehicle and particle size (Hargrove *et al.*, 1989).

After oral digestion, progesterone is rapidly absorbed, rapidly metabolized from the intestines and, during the first hepatic pass, cleared from the circulation (Whitehead *et al.*, 1980; Nahoul *et al.*, 1993). Maximal plasma progesterone concentrations are reached simultaneously with progesterone metabolites within 4 h (Whitehead *et al.*, 1980; Padwick *et al.*, 1986). Micronization of natural progesterone improves its absorption and bioavailability (Chakmakjian and Zacharian, 1987; Norman *et al.*, 1991). After ingestion of 200 mg of micronized progesterone, mean serum progesterone concentrations (within the range of luteal phase) are attained in 2–4 h and remain significantly elevated for the next 6–7 h (Maxson and Hargrove, 1985; Padwick *et al.*, 1986; Norman *et al.*, 1991). Nevertheless, even higher doses of oral micronized progesterone (200 or 300 mg/day) have failed to induce uniform secretory endometrial features in menopausal women (Lane *et al.*, 1983; Moyer *et al.*, 1993). It has been indicated that interference from high concentrations of progesterone metabolites produced during the first liver pass, might provide serum progesterone concentrations that are erroneously high, thus accounting for discrepancies between biopsies and serum progesterone concentrations (Nahoul *et al.*, 1987; Nahoul and de Ziegler, 1994). These falsely elevated progesterone values are caused by cross-reaction of metabolites with the anti-progesterone polyclonal antibodies in the direct immunoassays used in routine laboratories. However, part of this interference can be eliminated by sample pretreatments such as extraction with organic solvents followed by chromatography or by using selected specific monoclonal antibodies (Nahoul *et al.*, 1987; Nahoul and de Ziegler, 1994).

Progesterone i.m.

The i.m. application of progesterone is uncomfortable, as it requires daily injections to maintain appropriate serum concentrations. This may be of main concern for patients with ovarian failure in oocyte donation programmes, who are in need of a long-term treatment for pregnancy support. Furthermore, i.m. progesterone administration may lead to marked inflammation at the injection site, resulting in redness, pain and even sterile abscess formation.

Progesterone is rapidly absorbed after i.m. administration. High plasma concentrations are achieved within 2 h and peak concentrations are reached within 8 h (Nillius and Johansson, 1971; Johansson, 1972; Simon *et al.*, 1993). Serum concentrations equivalent to those seen during the luteal phase have been attained after the injection of 25 mg progesterone (Nillius and Johansson, 1971; Johansson, 1972). It has been also indicated that the i.m. site of injection might function as a depot, by progesterone accumulation within fat tissue, thus resulting in more sustained

serum progesterone concentrations after i.m. injection than after vaginal or rectal application (Nillius and Johansson, 1971). However, this was not confirmed by other studies using different progesterone formulations, where better steady-state concentrations were detected with the vaginal route (Devroey *et al.*, 1989; Artini *et al.*, 1995). In a randomized study (Simon *et al.*, 1993), comparing oral micronized progesterone (200 mg) with i.m. progesterone (50 mg), it was found that maximum serum progesterone concentrations (C_{max}) were higher (14.3 versus 4.3 ng/ml) and the time (T_{max}) taken to achieve these concentrations was longer (8.7 versus 2.5 h) after i.m. progesterone administration. Furthermore, the relative bioavailability of oral progesterone was significantly lower, only 10% of that observed after i.m. progesterone indicating, according to the authors, a delayed rate of progesterone absorption after i.m. administration, but with a greater availability of this dosage form.

Vaginal progesterone

Vaginal mucosa epithelium readily absorbs proteins and lipids (Hafez, 1977; Forsberg, 1995). It has been indicated that the vagina might have a reservoir effect and vaginal mucosa might function as a rate-limiting membrane allowing only a finite amount of progesterone to be absorbed (Archer *et al.*, 1995). Transvaginal application of progesterone has been correlated with some side-effects, e.g. discharge, irritation or local warmth (Kimzey *et al.*, 1991; Archer *et al.*, 1995; Pouly *et al.*, 1996). Progesterone absorption from the vaginal epithelium is enhanced after vaginal oestrogenization (Villanueva *et al.*, 1981). Progesterone absorption is further influenced by the formulation used, whether tablets, suppositories, creams, oil-based solutions or the recently released polycarbophil gel (Price *et al.*, 1983; Fulper *et al.*, 1987; Kimzey *et al.*, 1991; Cicinelli *et al.*, 1996; Fanchin *et al.*, 1997).

Vaginal application results in avoidance of first-pass metabolism in the gastrointestinal tract and liver, and in sustained plasma concentrations (Nillius and Johansson, 1971; Norman *et al.*, 1991; Kimzey *et al.*, 1991; Archer *et al.*, 1995). After vaginal administration of progesterone, plasma progesterone levels reach maximal concentrations within 3–8 h, depending on the formulation used (Nillius and Johansson, 1971; Norman *et al.*, 1991; Archer *et al.*, 1995; Artini *et al.*, 1995) and gradually fall during the next 8 h (Nillius and Johansson, 1971). Vaginally administered progesterone disappears more rapidly from the circulation than the i.m. Furthermore, higher doses are necessary of vaginal progesterone (100 mg) than of i.m. progesterone (25 mg), in order to achieve serum progesterone concentrations of the luteal phase range (Nillius and Johansson, 1971; Johansson, 1972). The comparison of the same doses (300 mg) of micronized progesterone in a non-liquefying cream vaginally and capsules of micronized progesterone orally favoured the use of the vaginal formulation, since all the patients achieved luteal phase progesterone concentrations in the vaginal group compared with only two out of five patients in the oral group (Kimzey *et al.*, 1991). The number of daily doses necessary to achieve sustained serum progesterone concentrations is dependent on the formulation used. Most frequently, 300–600 mg of progesterone is administered daily, spread over two or three dosages (Devroey *et al.*, 1989; Critchley *et al.*, 1990; de Ziegler *et al.*, 1992). However, it has been suggested that lower doses (45–90 mg) applied once a day or even once every other day, might be effective with sustained-release formulations

(Pouly *et al.*, 1996; Fanchin *et al.*, 1997; Ross *et al.*, 1997; Warren *et al.*, 1999).

Different routes of progesterone support in IVF

The introduction of GnRH analogues has led to fewer cycle cancellations in IVF, by the prevention of a premature LH surge. In cycles using GnRH agonists, a luteal phase defect has been described, thus making luteal phase support necessary (Wildt *et al.*, 1986; Smitz *et al.*, 1987). However, the need for luteal phase supplementation was not confirmed in other ovarian stimulation protocols not using GnRH analogues (Daya, 1988). HCG (i.m.) and progesterone (in various routes) are used as luteal phase support in ovulation induction, but the superiority of one form over the other has not yet been established. In a meta-analysis of randomized trials comparing different types of luteal support, it was suggested that HCG might be superior to progesterone in GnRH agonist cycles (Soliman *et al.*, 1994).

Oral, vaginal and i.m. routes of progesterone administration have been used as luteal phase supplementation in ovarian stimulation for IVF. Orally administered progesterone has proved to be inefficient in comparison with i.m. HCG (Buvat *et al.*, 1990a). The comparison of i.m. HCG with i.m. or intravaginal progesterone has provided controversial results. Some of the studies have found no difference in pregnancy rates between i.m. progesterone and i.m. HCG (Smitz *et al.*, 1988; Claman *et al.*, 1992; Araujo *et al.*, 1994; Artini *et al.*, 1995) or between vaginal progesterone and i.m. HCG (Buvat *et al.*, 1990b; Artini *et al.*, 1995) and others have indicated the superiority of i.m. HCG to i.m. progesterone (Golan *et al.*, 1993).

There are few prospective randomized studies in the literature specifically comparing different routes of progesterone supplementation as luteal phase support in IVF cycles (Table I). No differences in pregnancy rates were detected in a prospective randomized comparison of i.m. HCG, i.m. progesterone and micronized progesterone vaginally as luteal phase supplementation in GnRH agonist cycles. However, better steady-state serum progesterone concentrations were achieved with the vaginal formulation (Artini *et al.*, 1995). The comparison of oral micronized progesterone to i.m. progesterone supported the use of the latter, as it resulted in significantly higher implantation rates (18.1% oral versus 40.9% i.m.) (Licciardi *et al.*, 1999). In addition, significantly higher implantation rates were detected in GnRH agonist cycles using vaginal micronized progesterone as luteal phase support compared with cycles using the same dose (400 mg/day) of micronized progesterone orally (Buvat *et al.*, 1990b). On the other hand, no difference in pregnancy rates was detected between oral micronized progesterone and micronized progesterone in sustained-release carbophilic gel as luteal phase support in GnRH agonist cycles (Pouly *et al.*, 1996).

In another prospective randomized study comparing i.m. natural progesterone (50 mg/day) with vaginal micronized progesterone (600 mg/day), in GnRH agonist cycles for IVF, significantly lower early miscarriage rate as well as a trend towards higher implantation rates, despite lower serum progesterone concentrations, was detected in the vaginal group (Smitz *et al.*, 1992). This might be correlated with improved endometrial features after vaginal, rather than after i.m. progesterone application, despite lower plasma concentrations, thus suggesting a better local progesterone

Table I. Summary of prospective randomized studies comparing different routes of progesterone support in GnRH agonist cycles for IVF

| Author | Stimulation protocol | No of cycles | Luteal phase support | Pregnancy rates (%) (implantation rates) (%) |
|--------------------------------|-----------------------------|--------------|--|--|
| Buvat <i>et al.</i> (1990) | Triptorelin/HMG (short) | 32 | HCG 3 × 1500 IU ^a | 62.5 ^b |
| | | 19 | Utrogestan 400 mg/day orally | 5 |
| | | 20 | Utrogestan 400 mg/day vaginally | 55 ^c |
| | Triptorelin/HMG (long) | 47 | HCG 3 × 1500 IU ^a | 40 |
| | | 41 | Utrogestan 400 mg/day orally | 27 |
| | | 35 | Utrogestan 400 mg/day vaginally | 40 |
| Smits <i>et al.</i> (1992) | Buserelin/HMG (long) | 131 | Progesterone 50 mg i.m. + oestradiol valerate 6 mg/day | 30.5 (11.6)* |
| | | 131 | Utrogestan 600 mg vaginally + oestradiol valerate 6 mg/day | 35.1 (16.3)* |
| Artini <i>et al.</i> (1995) | Buserelin/pFSH + HMG (long) | 44 | HCG 3 × 2000 IU | 13.6 |
| | | 44 | Progesterone 50 mg i.m./day | 13.6 |
| | | 44 | Micronized progesterone in vaginal cream 100 mg/day | 15.6 |
| | | 44 | No support | 9.1 |
| Pouly <i>et al.</i> (1996) | Decapeptyl/HMG (long) | 139 | Crinone 90 mg/day vaginally | 28.8 (35.3) |
| | | 144 | Utrogestan 300 mg/day orally | 25 (29.9) |
| Licciardi <i>et al.</i> (1999) | GnRH/FSH/HMG (long) | 19 | Progesterone 50 mg i.m. | 57.9 (40.9)** |
| | | 24 | Micronized progesterone 600 mg/day orally | 45.8 (18.1)** |

Utrogestan = natural micronized progesterone capsules; Crinone = natural progesterone in vaginal gel.

^aNo HCG was given as luteal phase support if the concentration of oestradiol was >2700 pg/ml.

^b*P* < 0.01 versus oral; ^c*P* < 0.01 versus oral.

P* = 0.07; *P* = 0.004.

bioavailability in the uterus (Bourgain *et al.*, 1992; Bourgain *et al.*, 1994). Endometrial biopsies obtained in the mid-luteal phase in GnRH/human menopausal gonadotrophin (HMG) cycles for IVF revealed a higher proportion of increased dissociated maturation in the i.m. group, while most endometria were in phase after supplementation with vaginal micronized progesterone (Bourgain *et al.*, 1994). Maturation delay with a mean value of 2.3 days was also detected in mid-luteal biopsies after similar ovarian stimulation and luteal phase supplementation with i.m. progesterone (50 mg/day) and oestradiol valerate (6 mg/day) (Van Steirteghem *et al.*, 1988). On other hand, 18 of 22 mid-luteal biopsies performed in GnRH/HMG cycles were in phase after supplementation with vaginal micronized progesterone (600 mg/day) (Smits *et al.*, 1993).

Different routes of progesterone support in oocyte donation

Adequate secretory endometrial transformation and therefore endometrial receptivity is the goal in oocyte donation programmes. Oocyte donation protocols provide a useful tool in studying endometrial receptivity. Progesterone supplementation must induce secretory endometrial transformation and lead to endometrial features that permit embryo implantation during the so-called 'implantation window'. Furthermore, the need for long-term

progesterone treatment in women with deprived ovarian function points to the importance of detecting the optimal progesterone formulation. Many studies have therefore been performed in order to investigate the various effects that different routes and doses of progesterone supplementation exert on the endometrium.

The majority of oocyte donation programmes in the last decade have used the i.m. (Navot *et al.*, 1986; Younis *et al.*, 1992; Pados *et al.*, 1992; Potter *et al.*, 1998) or the vaginal route (Lutjen *et al.*, 1986; Salat-Baroux *et al.*, 1988; Frydman *et al.*, 1990; Pados *et al.*, 1992) and both of these have succeeded in providing favourable pregnancy results. Different studies have analysed the hormonal and histological parameters after various routes and doses of progesterone supplementation in oocyte donation programmes (Table II). Devroey *et al.* (1989) studied women with missing ovaries for oocyte donation. The authors compared the histological endometrial features in four groups of patients. After endometrial priming with oestrogen, the patients received either 100 mg of natural progesterone i.m. or 300 mg of micronized progesterone orally or micronized progesterone vaginally in two different doses (300 or 600 mg/day). Progesterone supplementation was commenced on day 14 and endometrial biopsies were performed on day 21. Serum progesterone concentrations five times higher in the i.m. group than in the intravaginal group were detected, although better steady-state concentrations with fewer fluctuations in serum

progesterone concentrations were observed in the vaginal group. Among the four groups, serum progesterone values were lowest after oral progesterone administration. Additionally, none of the biopsies obtained from this group provided evidence of adequate progesterone effect. On the contrary, classical endometrial secretory morphology was induced both in i.m. and intravaginal groups but better endometrial maturation was achieved in the vaginal group.

The insufficiency of orally administered progesterone to induce adequate endometrial secretory transformation in oocyte donation programmes has been confirmed by other studies (Dehou *et al.*, 1987; Bourgain *et al.*, 1990; Critchley *et al.*, 1990). Dehou *et al.* (1987) reported on endometrial maturation delays and abnormal endometrial features, as assessed both with light and electron microscopy, in biopsies from artificial cycles replaced during the secretory phase with 300–600 mg oral micronized progesterone/day. The control group that was composed of women treated with 50–100 i.m. natural progesterone/day revealed normal glandular maturation. In another study, it was demonstrated that only one out of five mid-luteal biopsies during mock cycles in patients with premature ovarian failure were in phase after daily ingestion of 300 mg oral micronized progesterone. In contrast, five out of six biopsies obtained after daily treatment with 300 mg vaginal micronized progesterone were in phase (Critchley *et al.*, 1990).

The ability of natural vaginal progesterone to induce secretory transformation of the endometrium has been repeatedly demonstrated in the literature (Salat-Baroux *et al.*, 1988; Hung *et al.*, 1989; Devroey *et al.*, 1989; Bourgain *et al.*, 1990; de Ziegler *et al.*, 1992; Cicinelli *et al.*, 1996). De Ziegler *et al.* (1992) performed endometrial biopsies on days 20 and 24 of the cycle in women with deprived ovarian function after application of 300 mg micronized progesterone vaginally/day and prior endometrial priming with oestrogen for 14 days. In most of the cases, the endometrium was in phase and the distribution of oestrogen and progesterone receptors was typical of that expected for this day of the cycle. Similarly, all late luteal phase biopsies were in phase in artificial cycles after substitution with vaginal progesterone (Hung *et al.*, 1989).

Progesterone administered i.m. induces adequate endometrial transformation and satisfactory pregnancy rates in oocyte donation protocols. Of late luteal endometrial biopsies, 14% were out of phase after i.m. progesterone (50–100 mg), but this was limited to patients aged >40 years and was corrected by higher progesterone dosages (Potter *et al.*, 1998). Furthermore, endometrial biopsy specimens obtained on days 18 and 22 of artificial cycles supplemented with 25–50 mg i.m. progesterone/day corresponded to the expected days of the cycle (17 ± 0.57 and 21 ± 1.4 respectively) (Navot *et al.*, 1986).

The comparison of i.m. and vaginal routes has produced conflicting results. Bourgain *et al.* (1990) performed mid-luteal biopsies during trial cycles in patients undergoing oocyte donation because of primary ovarian failure. A range of abnormal endometrial features in the group of patients receiving a dose of 100 mg i.m. progesterone/day were detected, e.g. endometrial maturation delay in 43.5% of patients and asynchrony between endometrial glands and stroma in 9%, with the maturation delay observed in the glandular endometrial compartment. On the other hand, all endometrial features studied both with electron and light microscopy, were markedly improved in the vaginal groups which

were supplemented with 300 or 600 mg of micronized progesterone daily. Asynchronous maturation between endometrial glands and stroma with the stroma being fibrocystic or dense in the first replacement cycle have also been reported after i.m. progesterone treatment (Dehou *et al.*, 1987). Furthermore, maturation delay in the endometrial glands compared with stroma, have been detected after i.m. progesterone treatment (Miles *et al.*, 1994) and, although not statistically significant, such dissociated maturation was not found after supplementation with vaginal progesterone. As clinical pregnancies were achieved only in patients with synchronous endometrial maturation and no maturation delay of >2 days (Bourgain *et al.*, 1990), improved and synchronous endometrial development might provide a better chance of implantation.

Although overall endometrial delay was improved in the vaginal groups, significantly higher serum progesterone concentrations were observed in the i.m. group and no difference was detected between the two vaginal groups (progesterone dose of 300 or 600 mg) with respect to morphology or serum hormone concentrations (Bourgain *et al.*, 1990). The observation that, despite significantly lower serum progesterone concentrations, vaginally administered progesterone results in adequate secretory endometrial transformation and satisfactory pregnancy and implantation rates was also confirmed by a recent randomized study comparing i.m. progesterone with vaginal micronized progesterone in a sustained-release polycarbophil gel, in trial cycles for oocyte donation (Gibbons *et al.*, 1998). Endometrial histology assessed with late endometrial biopsies (days 25–27), showed normal endometrial development in both groups, although serum progesterone values were significantly higher in the group receiving i.m. progesterone. However, steady-state progesterone concentrations were attained earlier after vaginal progesterone application.

On the other hand, different results have been obtained in other studies comparing i.m. with vaginal routes of progesterone supplementation in preceding cycles of oocyte donation. Sauer *et al.* (1991) reported on better histological results and higher progesterone concentrations after i.m. progesterone supplementation (100 mg/day), than after vaginal progesterone suppositories (200 mg) in ovarian failure patients. Since better endometrial scoring with less discrepancy with the chronological date expectations (–1.5 days versus –2.9 days) and higher serum progesterone concentrations have been reported after daily i.m. progesterone administration than after vaginal administration in patients with primary ovarian failure (Davies *et al.*, 1990), it has been suggested that higher luteal serum progesterone concentrations might be correlated with improved endometrial histology. It was also found that five-fold higher i.m. progesterone dosage than the standard had no effect on endometrial glandular maturation in artificial cycles; however, according to the authors, it might have some effect on the stroma (Li *et al.*, 1992). Furthermore, luteal phase progesterone concentrations were not detected to be a significant factor affecting pregnancy or implantation rates in a retrospective analysis in an oocyte donation programme (Younis *et al.*, 1992).

There is increasing evidence in the literature that serum progesterone concentrations after vaginal progesterone application are not indicative of the effect that vaginally administered progesterone has on the endometrium. The daily application of micronized progesterone in an oil-based solution (100 mg) to

Table II. Summary of studies comparing hormonal and histological parameters between different routes of progesterone administration as luteal phase support in artificial cycles for oocyte donation and after pretreatment with various types of oestrogen during the follicular phase

| Author | Aetiology | Subjects | Daily progesterone dose | Luteal progesterone values | Day of biopsy | Biopsy results |
|--------------------------------|---|----------|---|--|---------------|---|
| Dehou <i>et al.</i> (1987) | Primary ovarian failure | 8 | Utrogestan 300–600 mg p.o | | 16–24 | Maturation delay |
| | | 10 | Progesterone 50–100 mg i.m. | | 16–24 | Normal glandular maturation. Fibrocystic stroma in first cycle, improved in next cycles |
| Devroey <i>et al.</i> (1989) | Absent ovaries | 11 | Utrogestan 300 mg p.o. (100 mg on day 14) | Lowest serum progesterone between all four groups | 21 | 100% out of phase |
| Critchley <i>et al.</i> (1990) | Premature ovarian failure | 31 | Progesterone 100 mg i.m. (50 mg on day 14) | Serum progesterone five times higher than the vaginal groups | 21 | 2/31 out of phase |
| | | 18 | Utrogestan 300 mg p.v (100 mg on day 14) | | 21 | 18/18 in phase |
| | | 10 | Utrogestan 600 mg p.v. (200 mg on day 14) | | 21 | 10/10 in phase |
| | | 5 | Micronized progesterone 300 mg p.o. | 14 ± 2 (mean ± SE) nmol/l ^a | 21 | 1/5 normal. |
| Bourgain <i>et al.</i> (1990) | Primary ovarian failure | 4 | 100 mg micronized progesterone (p.v. pessary) | 30 ± 16 (mean ± SE) nmol/l ^a | 21 | 2/4 normal. |
| | | 6 | 300 mg micronized progesterone p.v. | 45 ± 5 (mean ± SE) nmol/l ^a | 21 | 5/6 normal |
| | | 12 | Utrogestan 300 mg p.o. | 2.79 ± 0.6 (mean ± SEM) µg/l | 21 | 1/12 in phase |
| | | 34 | Progesterone 100 mg i.m. | 43.4 ± 0.60 (mean ± SEM) µg/l ^c | 21 | 16/34 in phase |
| | | 21 | Utrogestan 300 mg p.v | 6.79 ± 1.28 (mean ± SEM) µg/l ^c | 21. | 16/21 in phase. |
| | | 8 | Utrogestan 600 mg p.v. | 8.09 ± 1.74 (mean ± SEM) µg/l ^c | 21 | 6/8 in phase |
| Davies <i>et al.</i> (1990) | Premature ovarian failure/gonadal dysgenesis/failure of IVF stimulation | 22 | Progesterone 25–50 mg i.m. | 60 ± 8 nmol/l ^b | 21 | Mean endometrial score: –1.5 days ^d |
| | | 11 | Cyclogest 200–400 mg p.v. | 29.8 ± 5.4 nmol/l ^b | 21 | Mean endometrial score: –2.9 days ^d |
| Sauer <i>et al.</i> (1991) | Idiopathic ovarian failure/post-surgery/radiation/chemotherapy | 19 | Progesterone 100 mg i.m. (50 mg on day 15) | 48.8 ± 10.4 ng/ml | 26 | 19/19 normal |
| Miles <i>et al.</i> (1994) | Functionally agonadal | 19 | Progesterone suppositories 200 mg p.v. (100 mg on day 15) | 29.4 ± 4.8 ng/ml | 26 | 7/19 excessive oestrogen effect |
| | | 5 | Progesterone 100 mg i.m. | 69.8 ± 5.9 (mean ± SE) ng/ml ^e | 21 | Mean dating of glands: 18.9 ± 0.1 days |
| Gibbons <i>et al.</i> (1998) | Primary ovarian failure/diminished ovarian reserve | 15 | Micronized progesterone capsules 800 mg p.v. | 11.9 ± 1.2 (mean ± SE) ng/ml ^e . | 21 | Mean dating of glands: 20.5 ± 1 days |
| | | 24 | Progesterone 100 mg i.m. | 89.3 ng/ml ^f | 25–27 | 23/24 in-phase |
| | | 55 | Crinone 180 mg p.v. | 19 ng/ml ^f | 25–27 | 55/55 in-phase |

Utrogestan = natural micronized progesterone capsules; Crinone = natural progesterone in vaginal gel; Cyclogest = natural progesterone pessaries; p.o. = orally; p.v. = vaginally
^a $P < 0.01$; ^b $P = 0.014$; ^c $P < 0.0001$; ^d $P = 0.03$; ^e $P < 0.05$; ^f $P < 0.00001$.

post-menopausal women after previous oestrogen priming, was able to induce endometrial secretory changes, despite maximal serum hormonal concentrations lower than those observed during the luteal phase (5.4 ± 0.92 ng/ml) (Cicinelli *et al.*, 1996). Similarly, 16 of 18 mid-luteal biopsies performed in trial cycles for oocyte donation, were in phase after endometrial preparation with oestrogen and vaginal micronized progesterone (100–200 mg), resulting in pregnancy rates of 31%, despite serum progesterone concentrations lower than those observed during the luteal phase of the natural cycle (Salat-Baroux *et al.*, 1988). Low mid-luteal progesterone concentrations but a rate of 75% in-phase late (day 28) endometrial biopsies were also reported by Balasch *et al.* (1996) in infertile patients undergoing artificial cycles and supplemented with vaginal micronized progesterone.

Successful secretory endometrial transformation was also observed in post-menopausal women or women with secondary amenorrhoea after application of micronized progesterone in carbophilic gel, with low doses ranging from 45 to 90 mg every other day (Casanas-Roux *et al.*, 1996; Ross *et al.*, 1997; Fanchin *et al.*, 1997; Warren *et al.*, 1999), despite low mean serum progesterone concentrations (3.6 ± 0.2 ng/ml) (Fanchin *et al.*, 1997). The fact that serum progesterone concentrations are not indicative of the local bioavailability that vaginally administered progesterone exerts on the endometrium was further confirmed by Miles *et al.* (1994). After 6 days of progesterone replacement in 20 functionally agonadal women who were candidates for oocyte donation, serum progesterone concentrations were almost seven times lower as a result of the vaginal route (800 mg/day) than as a result of the i.m. route (100 mg/day), whereas endometrial progesterone concentrations were almost 10 times higher after vaginal progesterone than after i.m. progesterone.

First uterine pass effect

Vaginal progesterone administered as luteal phase supplementation in oocyte donation programmes and in stimulated cycles results in adequate endometrial secretory transformation and satisfactory pregnancy rates (Lutjen *et al.*, 1986; Salat-Baroux *et al.*, 1988; Devroey *et al.*, 1989; Smitz *et al.*, 1992; Smitz *et al.*, 1993; Artini *et al.*, 1995; Gibbons *et al.*, 1998). In the majority of the studies, orally administered progesterone was found to be inferior to vaginal (Devroey *et al.*, 1989; Bourgain *et al.*, 1990; Critchley *et al.*, 1990) or to i.m. administration (Dehou *et al.*, 1987; Devroey *et al.*, 1988; Licciardi *et al.*, 1999) and it has been suggested that its use might be limited to preparatory cycles (Devroey *et al.*, 1989; Pados *et al.*, 1992).

The comparison between vaginal and i.m. progesterone provided contradictory results. Some of the studies advocated the superiority of the vaginal route due to higher implantation and lower early miscarriage rates (Smitz *et al.*, 1992) or due to better histological findings (Bourgain *et al.*, 1990, 1994) or because of better steady-state progesterone concentrations (Artini *et al.*, 1995; Gibbons *et al.*, 1998) and other trials supported the superiority of the i.m. route (Davies *et al.*, 1990; Sauer *et al.*, 1991).

Although some studies have demonstrated better steady-state concentrations after vaginal formulation with less intra- and inter-individual variations (Devroey *et al.*, 1989; Artini *et al.*, 1995), lower serum progesterone values were constantly observed after

vaginal application than after i.m. application (Devroey *et al.*, 1989; Davies *et al.*, 1990; Sauer *et al.*, 1991; Smitz *et al.*, 1992; Miles *et al.*, 1994). In some trials, although the observed serum progesterone concentrations were lower than those observed during the luteal phase of the natural cycle, adequate secretory endometrial transformation was achieved (Balasch *et al.*, 1996; Casanas-Roux *et al.*, 1996; Ross *et al.*, 1997; Fanchin *et al.*, 1997; Warren *et al.*, 1999). This apparent incompatibility between low serum progesterone concentrations and normal histological findings suggests that vaginally administered progesterone exerts a pronounced local effect on the endometrium, the so-called first uterine pass effect; i.e. a fraction of the regimen might have on the endometrium a direct impact, without entering at first the systematic circulation. Consequently, this better local bioavailability of vaginally administered progesterone in the uterus might result in a maximal local endometrial effect and minimal undesirable systematic effects.

There is increasing evidence in the literature from experimental models that drugs administered vaginally have a preferential distribution in the uterus. Miles *et al.* (1994) have elegantly shown in their experiment that, despite lower serum progesterone concentrations, endometrial progesterone concentrations are higher after vaginal progesterone application than after i.m.. Significantly higher progesterone concentrations were detected in the uterine artery than in the radial artery in post-menopausal women undergoing hysterectomy who received micronized progesterone in an oil-based solution before the operation, providing further evidence of the preferential drug distribution to the uterus after vaginal application (Cicinelli *et al.*, 1998). High endometrial progesterone concentrations were detected in an ex-vivo uterine perfusion model after application of radio-labelled progesterone in the vaginal cuff after hysterectomy, suggesting that progesterone migrates progressively into the uterus and reaches high concentrations in endometrium and myometrium (Bulletti *et al.*, 1997). Similar results have also been obtained after the vaginal application of other compounds, e.g. terbutaline, misoprostol, danazol (Kullander and Svanberg, 1985; El-Refaey *et al.*, 1995; Mizutani *et al.*, 1995).

Parallel to the benefit of achieving high endometrial progesterone concentrations with the vaginal route, it has been suggested that such high concentrations might exert an unfavourable effect by influencing the secretion of endometrial progesterone-dependent peptides such as insulin-like growth factor binding protein-1 (IGFBP-1). This was indicated by a prospective, randomized study comparing orally (300 mg/day) and vaginally (300 mg/day) administered progesterone in non-IVF, clomiphene citrate-induced cycles, where lower pregnancy rates and higher serum IGFBP-1 concentrations were observed with the vaginal formulation (Wang and Soong, 1996). Nevertheless, it has also been demonstrated that clomiphene treatment increases serum concentrations of IGFBP-1 (Pekonen *et al.*, 1992) and the need of luteal phase support in cycles using clomiphene citrate has not yet been confirmed (Daya, 1988; Agarwal and Buyalos, 1995; Shalev *et al.*, 1995; Deaton *et al.*, 1997). Furthermore, no difference in endometrial histological features were detected between two (300 and 600 mg/day) (Bourgain *et al.*, 1990) and three (45, 90, 180 mg every other day) (Fanchin *et al.*, 1997) different vaginal progesterone dosages as luteal phase support in patients with ovarian failure. However, it

could be intriguing to investigate the possible favourable or adverse effects of high endometrial progesterone concentrations, on all the possible paracrine or autocrine mechanisms that are involved in embryo implantation and in all different forms of ovulation induction and luteal phase support.

Although there is evidence for the first uterine pass effect after vaginal drug application, the mechanism of this has not yet been elucidated. Whether this is due to absorption into the rich venous or lymphatic vaginal system and/or possibly countercurrent transfer between uterovaginal lymph vessels or veins and arteries, or due to direct drug diffusion through tissues or due to intraluminal transfer from the uterus to vagina similar to sperm transport, has not yet been clarified. Nevertheless, the vaginal route might, as a result of the first uterine pass effect, be proved to be a valuable route for drug delivery, not only in infertility treatments but also in daily practice in general obstetrics and gynaecology.

Conclusions

There is increasing evidence in the literature suggesting that vaginal progesterone might be superior to other routes, mainly due to the postulated first uterine pass effect, which results in a better local progesterone bioavailability in the uterus. However, large prospective randomized studies are necessary in order to confirm this superiority and to detect the optimal dose and formulation. Furthermore, the development of experimental models is necessary in order to investigate the importance and mechanism of the postulated first uterine pass effect.

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