Intramuscular *versus* vaginal administration of progesterone for luteal phase support after in *vitro* fertilization and embryo transfer. A comparative randomized study

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Summary

A total of 156 patients were randomly treated with exogenous natural progesterone (intramuscularly, 50 mg/day) and vaginal gel (90 mg/day) P or nothing (Controls) from the day before embryo transfer (ET) for two weeks. In case of positive β-HCG, the treatment was continued for 12 weeks.

Plasma P and 17 β-Estradiol concentrations were estimated and compared with the control not supplemented group.

Both treatments were able to increase significantly the luteal plasmatic values of P versus controls.

The ongoing pregnancy and the living birth rates per transfer were significantly higher in the patients supplemented with intramuscular P than in those treated with vaginal gel P.

The intramuscular natural P appears the most suitable route of administration for luteal phase support in IVF-ET procedures.

Key words: Intramuscular progesterone; Vaginal gel progesterone; Luteal phase supplementation; IVF.

Introduction

Over the years, evidence has been accumulating that an impaired endometrial receptivity may play a contributory role in the low success rate of embryo implantation [1, 2]. It is well known that a considerable gap exists in embryo arrival time in the uterus between *in vivo* and *in vitro* fertilization with embryo transfer cycle.

In an effort to "accelerate" the endometrial maturation, exogenous natural progesterone could be successfully used in assisted reproductive techniques [3-6]. Progesterone stimulates endometrial gland maturation and decidual transformation of the endometrial stroma, thus providing the essential hormonal support for implantation and the maintenance of the pregnancy [7].

Moreover, progesterone might have an immunosuppressive influence during the implantation, helping the early pregnancy maintenance [8].

It may also modulate the negative effect of hyperestrogenism on endometrial maturation [9].

Based on the earlier observation that pregnancy rate is higher in IVF cycles with a significantly higher progesterone serum level [10], exogenous natural progesterone is routinely used for luteal phase support of standard IVF procedures or donor oocyte programs [11].

Nevertheless, it is still not clear which is the best route of administration [6].

The major choices for progesterone replacement include oral micronized pills, vaginal suppositories and intramuscular injections.

Oral administration of progesterone is associated with variable absorption and with metabolism by the liver and gut flora to inactive metabolites that may cause central nervous system sedation. In particular the α-reduced metabolites bind to a specific site on the GABA receptor neurones whose activation shares some of the tranquillizing properties of benzodiazepines [12].

Vaginal suppositories of progesterone have been shown to be effective for luteal phase support but these formulations can lead to variable absorption profiles [13].

Intramuscular progesterone is effective although the route of administration can cause discomfort [6].

The aim of this study was to compare the results in terms of pregnancy rates per transfer obtained *in vitro* fertilization and embryo transfer when natural progesterone is administered by intramuscular or vaginal routes for the support of the luteal phase.

Materials and Methods

Patients

Between February 1997 and March 1998, a total of 156 patients were enrolled in this study. Written informed consent was obtained from each patient. Inclusion criteria were 25-35 years of age, infertility of at least 2 years duration, tubal occlusion and a normal endometrial cavity as shown by hysterosalpingography.

They were randomly treated with intramuscular (50 mg a day in Group A) and vaginal (90 mg a day in Group B) progesterone or nothing (controls in Group C).

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Treatment protocol

All patients underwent pituitary desensitization by the intramuscular administration of GnRH-a on day+21 of the previous menstrual cycle.

After about 10 days of desensitization, purified Follicle Stimulating Hormone was administered to obtain ovarian hyperstimulation.

Plasmatic 17ß-Estradiol concentrations and ultrsonographic determination of follicular size and number were assessed on days +5, +7 and +12 of stimulated cycles.

The dosage of gonadotrophins was changed according to the individual response.

All patients were administered 10,000 I.U. of HCG intramuscularly when serum 17ß-Estradiol concentrations exceeded 200 pg per follicle and when there were at least three follicles with a minimum diameter of 18 mm.

Oocytes retrievel was performed 34-36 hrs after HCG administration with vaginal ultrasonography (day 0). Embryo transfer was performed at the 2- to 4-cells stage on day +2. A maximum of four embryos was placed.

The support of the luteal phase was performed as follows.

Starting the day before embryo-transfer (day +1), all patients were randomly enrolled in a double blind manner to one of the following three groups:

1. Group A, including 52 patients, underwent embryo transfer and were treated with intramuscular progesterone* at a dosage of 50 mg a day.

2. Group B, including 52 patients, underwent embryo transfer and were treated with vaginal gel progesterone** at a dosage of 90 mg a day.

3. Group C as controls, including 52 patients, underwent embryo transfer with luteal phase not supplemented and were treated with intramuscular saline solution every three days as placebo.

The mean number of embryos transferred in each group was the same (3-4 embryos per transfer). All groups were treated from the day before transfer (day+1) until ß-HCG evaluation (day+16). In cycles leading to a pregnancy the progesterone administration was continued for 10 weeks.

Laboratory determinations

Plasmatic concentrations of 17 ß-Estradiol and progesterone were determined on blood samples taken as follows: before starting the treatment; every 6 hr after the beginning of treatment on days +1 and +2; then, single determinations on days +9 and +16, respectively.

Moreover, single morning blood samples from the non-pregnant women were requested every 6 hours after ß-HCG evaluation for four times.

17 ß-Estradiol and progesterone serum levels were determined by radioimmunoassay (RIA).

Statistical analysis

Statistical differences were evaluated by applying the Chi square test; $p<0.05$ was considered significant.

Determination of pregnancy states

A biochemical pregnancy was defined as a small transitory increase in β-HCG levels followed by a decrease within a week.

Clinical pregnancies were defined by the visualization of a gestational sac at the first planned ultrasound (US) obtained at 6-7 weeks of pregnancy or a serum β-HCG level of ≥1400 mIU in the absence of a scan [14].

Ongoing pregnancies were gestation that reached 20 weeks of gestation.

Results

Pregnancy rate

The percentages of biochemical pregnancy per transfer were 45.7, 30.6 and 12.5 in Group A (intramuscular P), in Group B (vaginal gel P) and in Group C (controls), respectively. The differences were statistically significant.

At the same time, the clinical and ongoing pregnancy rates were higher in the group treated with intramuscular progesterone (Group A: 34.3 and 28.9) than in the group treated with the vaginal gel progesterone (Group B: 19.1 and 11.0) or not treated (Group C: 6.8 and 3.0). Also in this case, the differences were statistically significant.

Moreover, what is most important is that in the group treated with intramuscular progesterone, the living birth rate was also significantly higher than in the group supported with the intravaginal gel progesterone (22.1 versus 8.0) (see Tab. 1).

Serum Progesterone and 17 ß-Estradiol levels

On day +1, before the beginning of treatment, there were not any statistical significantly differences in the serum levels of progesterone and 17 ß-Estradiol.

Moreover, there were not any statistical differences in the plasmatic 17 ß-Estradiol among the groups during the whole period of treatment. On days +1 and +2, after the beginning of the protocols, intramuscular P induced a statistically significant increase in the serum P with a maximum value of 47.5±13 ng/ml (mean±SD). Vaginal progesterone induced also a significant increase of serum P with a maximum value of 25.2±11 ng/ml (mean±SD) but it was significantly lower if compared to Group A. Group C (not supplemented) showed serum P

Table 1. — *Pregnancy Rate per Transfer*

Parameters	Group A (IM P)	Group B (Vaginal P)	Group C (Conrtrol)
No. of Transfers	52	52	52
Biochemical Pregnancy (%)	45.7	30.6	12.5
Clinical Pregnancy (%)	34.3	19.1	6.8
Ongoing Pregnancy (%)	28.9	11.0	3.0
Living Births (%)	22.1	8.0	2.8

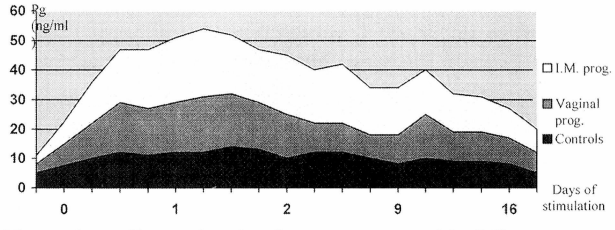

Figure 1. — Serum levels of progesterone with different routes of administration.

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** Crinone 8, Wyeth Lederle, U.S.A.

levels significantly lower than the other two groups (18±19 ng/ml; mean±SD).

On days +9 and +16, all the supplemented patients showed mean serum P levels significantly higher than controls (mean±SD; 42.5 ± 13 in Group A and 20.2±10 in Group B). Also in these cases the mean values were significantly higher in Group A than in Group B (see Figure 1).

Discussion

After ovarian stimulation for IVF, the endogenous secretion of progesterone from the corpus luteum acts to transform the endometrium at least during the early luteal phase. The necessity of luteal phase supplementation after a long protocol is caused by the dramatic decrease in serum progesterone concentration during the mid- or late luteal phase. This drop is probably due to the LH secretion suppression induced by the presence of GnRH-a and can induce the appearance of menstruation before the secretion of HCG by an implanting embyro, leading to an extremely early miscarriage [15]. Progesterone support avoids this premature necrosis of the endometrium and permits the pregnancy to continue.

Post reports have documented the difficulty of obtaining synchronously developed glands and stroma. Sauer *et al.* [16] attribute this discrepancy to the inadequate delivery of progesterone.

On the basis of these observations, exogenous progesterone given orally, intramuscularly or vaginally is commonly used to support the luteal phase after an in vitro fertilization procedure [13].

It has already been demonstrated [18], that these different ways of administering progesterone induce different concentrations of serum P. After oral administration of micromized progesterone (300 mg/day), no adequate endometrial response was noted. A very recent study demonstrated that if oral progesterone is administered at an adequate posology (200 mg three times daily), progesterone levels do not differ if compared to those obtained with intramuscular administration but it has negative effects on embryo implantation [18]. This negative result can be related to the high concentration of circulating progesterone metabolites, including deoxycorticosterone, estrone and E_2. These metabolities circulating at high levels, may bind to progesterone receptors and interfere with normal progesterone action by interfering with transcription cofactor or DNA binding [18]. Alternatively, the 5 α and 5 ß reduced pregnanolones are known to have high affinity for γ-aminobutyric acid receptors [19]. Such receptors are present in the reproductive tract [20], and their activation may adversely affect pregnancy outcome. After the I.M. injection of progesterone in oil (100 mg/day), maturation of the endometrium was heterogeneous. On the other hand, Simon *et al.* [21] have shown that the pharmacokinetics of progesterone differ for I.M. and oral administration. Moreover, the nutritional status of the patient has a large impact on serum progesterone concentration and bioavailability.

It has been demonstrated that high doses of progesterone (150 mg/day) delivered intramuscularly abolish glandular-stromal disparity in ovarian failure patients preparing for ET [22].

Oral administration involves the metabolic inactivation of progesterone during the first liver pass and is frequently associated with drowsiness [23].

It has been observed that after vaginal administration of progesterone, uterine tissue concentration exceeds by more than 10-fold the levels achieved by systemic administration, despite plasma levels in the latter case that are more than seven times higher, suggesting a direct transit into the uterus or «first uterine pass effect» [24, 25].

The administration of progesterone by the vaginal route results in lower levels and great variability in absorption of steroids compared to intramuscular injections [26].

Despite a previous study showing that intravaginal P yields a higher PR than the intramuscular P [13], our data showed that the ongoing pregnancy rate was higher in the group supplemented with intramuscular than with vaginal progesterone.

Also Maraschio and colleagues in 1996 [27], comparing three different protocols for luteal phase support (Group I treated with intramuscular P, Group II with vaginal P and Group III unsupported), demonstrated that the pregnancy rate was significantly higher in Group I treated with i.m. P versus the other two groups, and there were no statistically significant differences between group II, supported with vaginal P and group III, not supported.

Moreover, it has been suggested that the vaginal preparation might have an adverse effect on the embryo implantation through the direct stimulation of P-dependent IGFBP-1 [28].

This effect could be responsible for the lower pregnancy rate in the intravaginal than in the intramuscular P group.

Therefore, we emphasize the use of the injectable natural progesterone for luteal phase support after in *vitro* fertilization with embryo transfer and reserve the vaginal gel progesterone only as a second choice for patients showing high discomfort with the injectable administration.

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