Luteal support with micronized progesterone following in-vitro fertilization using a down-regulation protocol with gonadotrophin-releasing hormone agonist: a comparative study between vaginal and oral administration

S. Friedler 1, A. Raziel, M. Schachter, D. Strassburger, I. Bukovsky and R. Ron-El

IVF and Infertility Unit, Department of Obstetrics and Gynecology, Assaf Harofeh Medical Center, Zerifin 70300, Israel

1 To whom correspondence should be addressed

Introduction

The need for luteal support following ovulation induction for in-vitro fertilization (IVF)–embryo transfer in cycles using gonadotrophin-releasing hormone agonist (GnRHα) has become a consensus.

Methods of luteal phase support include corpus luteum stimulation to secrete endogenous oestrogen and progesterone by serial injections of human chorionic gonadotrophin (HCG) (Smith et al., 1989; Ballaisch-Allart et al., 1990) or with exogenous replacement of progesterone (Van Steirteghem et al., 1988; Smitz et al., 1988). Although a meta-analysis of randomized trials of luteal support (Soliman et al., 1994) has shown a preference for HCG, it cannot be recommended in patients with high response because it may aggravate the risk of ovarian hyperstimulation syndrome (OHSS).

Micronized progesterone induces endometrial maturation and leads to pregnancies following IVF–embryo transfer or during cycles of oocyte donation. Micronized progesterone is absorbed after oral or vaginal administration but various protocols for its administration have been proposed in the literature (Salat-Baroux et al., 1988; Devroey et al., 1991; Smitz et al., 1992a; Pouly et al., 1996) and the optimal effective route or dosage for luteal phase support following ovulation induction using GnRHα down-regulation for IVF has not yet been established.

The aim of our study was to compare the efficacy of micronized progesterone administered as luteal support, either orally or vaginally at different dosages, and to characterize the luteal phase hormonal profile during such treatments.

Materials and methods

Study population

The study population included 64 couples with male factor infertility requiring intracytoplasmic sperm injection (ICSI), using fresh ejaculated spermatozoa, treated at Assaf Harofeh Medical Center’s IVF unit. All patients included in this study were high responders, with serum oestradiol concentration of ≥2500 pg/ml on the day of HCG administration and all had >1 embryo available for intrauterine transfer following IVF/ICSI.

Ovulation induction and oocyte retrieval

Ovulation induction and oocyte retrieval were performed according to a routine protocol using GnRHα (DTRP α, Decapeptyl 3.75 mg i.m.; Ferring, Malmo, Sweden) suppression, with human menopausal gonadotrophin (HMG) (Pergonal®; Teva, Petah Tikva, Israel) for ovarian stimulation. Oocytes were retrieved 36 h after administration of 5000 IU of HCG (Chorigon®; Teva), by vaginal ultrasound guided follicular puncture and those at metaphase II were selected for ICSI.

ICSI procedure, embryo transfer and pregnancy evaluation

ICSI was performed according to previously described methodology (Van Steirteghem et al., 1993) using, preferably, motile spermatozoa. Fertilization was assessed on the following day, 16–18 h post sperm injection. If two distinct pronuclei were observed, then fertilization was judged to have occurred. Embryonic cleavage and morphological quality was assessed ∼24 h later and embryo transfer was performed. Our policy is to limit the number of embryos transferred to three, except in older women (age >38 years) or in cases with recurrent failures of implantation.
Luteal support

The patients included in this study were prospectively randomized by order of embryo transfer into two groups of 32 patients each, for luteal support with micronized progesterone [Urogestan®; Basins Iscovesco (CTS), Paris, France] administered either orally (200 mg×4/day) or vaginally (100 mg×2/day), starting the day following embryo transfer (+1), until serum βHCG measurement 14 days following embryo transfer. The minimal dosage administered vaginally represented the dosage used successfully (Salat-Baroux et al., 1988) for luteal support in cycles following transfer of cryopreserved–thawed embryos. Only clinical pregnancies including sonographic demonstration of a gestational sac were counted.

Hormonal profile of the luteal phase

The day of the ovulatory stimulus was considered to be day 0 of the luteal phase. Oestradiol and progesterone concentrations in the serum were measured by microparticle enzyme immunoassay (MEIA), performed using the Axsym system (Dainabot Co., Tokyo, Japan), an automated immunoassay analyser specifically designed to accommodate MEIA (Smith et al., 1993). This method utilizes a microparticle coupled with each ligand (oestradiol, progesterone) as a competitor against the ligand in the sample. The accuracy of serum progesterone measurement was validated as reported elsewhere (Momoeda et al., 1998). Blood samples were obtained on the morning of days 0, +5, +8, +11 and +15. Luteal phase endocrine profiles were analysed in conception and non-conception cycles better to demonstrate the effect of exogenous administration of progesterone in the presence and absence of endogenous early concentrations of HCG.

Statistical analysis

Statistical evaluation was performed using Student’s t-test, χ² test and Fisher’s exact test, where appropriate. Difference was considered significant at P < 0.05. Results are expressed as mean ± SD.

Results

The two groups were comparable in age (31.9 ± 6.1 versus 30.6 ± 5.2), number of oocytes retrieved per cycle (17 ± 8.2 versus 18 ± 7.0), and number of embryos transferred per cycle (3.1 ± 1.2 versus 2.7 ± 0.9), orally versus vaginally respectively.

Luteal phase endocrine profiles including mean concentrations of oestradiol, progesterone and oestradiol/progesterone ratio in the serum, according to conception and non-conception cycles in the two treatment groups, are depicted in Figure 1a–c. Regarding luteal serum oestradiol concentrations no significant differences were noted between the groups. Serum oestradiol concentrations decreased constantly until day +11 in all groups, with no significant difference, although following oral treatment a tendency for lower oestradiol concentrations was noted both in conception and non-conception cycles. After day +11, oestradiol concentrations increased in conception cycles, in both treatment groups, probably reflecting the rise in HCG concentrations affecting corpus luteum function. Luteal serum progesterone concentrations declined gradually with no significant difference between the groups until day 8 post HCG administration. Later on, in conception cycles, progesterone concentrations increased, probably reflecting the rise in endogenous HCG concentrations stimulating the corpora lutea, with no significant difference between the treatment groups. Comparing non-conception cycles at day +11 and day +15, significantly higher (P = 0.032) serum progesterone concentrations were found using 800 mg micronized progesterone and oral compared to vaginal treatment of 200 mg micronized progesterone. Oestradiol/progesterone ratios reflected the serum oestradiol concentrations, being higher from day +8 onwards after vaginal treatment but with no significant difference between conception and non-conception cycles.

The clinical outcome in the two treatment groups is described in Table I. A significantly higher (P < 0.01) rate of implantation was observed following luteal support administered vaginally compared with oral treatment, although the differences in the pregnancy, miscarriage and ongoing pregnancy rates were not significant statistically.

No side-effects resulting from vaginal administration of micronized progesterone, such as local intolerance or pruritus, were reported in this study. Following oral treatment two patients complained of somnolence.

Discussion

Ovarian stimulation cycles preceded by GnRHα down-regulation necessitate luteal phase supplementation (Smits et al., 1988; Smith et al., 1989; Bellaisch-Allart et al., 1990; Buvat et al., 1990; Herman et al., 1990). This prevents luteal phase insufficiency caused by prolonged suppression of gonadotrophin excretion leading to insufficient stimulation of the corpora lutea and impairment in production of late luteal oestradiol and progesterone (Calogero et al., 1987; Smits et al., 1987, 1988). Recently, it was suggested that luteal phase deficiency may result not from insufficient gonadotrophin stimulation but from failure of a malfunctioning corpus luteum to react to stimulation and produce progesterone (Momoeda et al., 1998). However, this study was conducted in natural cycles, whereas after ovulation induction, when pituitary down-regulation precedes ovarian stimulation, insufficient gonadotrophin stimulation seems a more plausible aetiology for the luteal phase insufficiency. Significant improvement of all endometrial features showing impairment of progesterone bioavailability (i.e. endometrial histological maturation assessed by histology, ultrastructure by electron microscopy and oestradiol and progesterone receptor distribution by immunocytochemistry) has been reported after luteal phase supplementation of progesterone (Bourgain et al., 1994). Luteal phase supplementation by progesterone increased the pregnancy rate significantly following IVF according to a meta-analysis (Soliman et al., 1994). Vaginal administration leads to lower serum concentrations of progesterone but has fewer side-effects compared to its administration either orally or progesterone in oil by i.m. injection (Miles et al., 1994). Principal metabolites found in blood following oral administration of micronized progesterone are 17-alpha-hydroxyprogesterone, 11-desoxycorticosterone and 20-dihydroprogesterone. Vaginal administration causes synchronous secretory maturation in the endometrium and elicits only minor changes in plasma concentrations of psychotropic metabolites, such as 5-alpha and 5-beta-pregnanolone, which are known to act directly on GABA receptors of the central nervous system.

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Figure 1. Luteal phase endocrine profile in conception and non-conception cycles using luteal support with micronized progesterone administered orally or vaginally. (a) Luteal phase serum oestradiol. (b) Luteal phase serum progesterone. (c) Luteal phase serum oestradiol(E2)/progesterone(P) ratio.

Table I. Clinical outcome of intracytoplasmic sperm injection, according to luteal support administered vaginally or orally

<table>
<thead>
<tr>
<th></th>
<th>Orally</th>
<th>Vaginally</th>
<th>(P^a) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implantation rate (%)</td>
<td>10/93 (10.7)</td>
<td>28/91 (30.7)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pregnancy rate/embryo transfer</td>
<td>10/30 (33.0)</td>
<td>16/34 (47.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Miscarriages (%)</td>
<td>4/10 (40.0)</td>
<td>2/16 (12.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Ongoing pregnancy rate/embryo transfer</td>
<td>6/30 (20.0)</td>
<td>14/34 (41.1)</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^a\) By Fisher’s exact test.
NS = not significant.

(CNS) (sedative/hypnotic, anxiolytic or anti-epileptic effects). The notion of the first uterine pass effect, referring to a mechanism of direct vagina to uterus transport of progesterone, was suggested by De Ziegler (De Ziegler et al., 1994, 1995) and has gained further support in the literature since then (Miles et al., 1994; Balasch et al., 1996; Buletti et al., 1997; Fanchin et al., 1997; Cicinelli et al., 1998). The exact mechanism of transport is not yet clarified; however in an ex-vivo uterine perfusion model a time dependent, wave-like progression of \(^{14}\)C-progesterone from cervix to fundus was observed reminiscent of passive diffusion (Buletti et al., 1997). A countercurrent distribution mechanism could be involved.
with diffusion from vaginal/uterine vein to artery, as progesterone concentrations in the uterine artery were found to be higher than in the radial artery following vaginal administration (Cicinelli et al., 1998).

The optimal effective dosage for luteal support has not been established yet. In women lacking ovarian function and receiving osteodiol, even a dose of progesterone as low as 45 mg/48 h, as given by a sustained release vaginal gel, caused complete secretory changes in endometrial glands (day 20) and stroma (day 24) as indicated by endometrial biopsies. This was despite low (1–5 ng/ml) plasma progesterone concentrations (Fanchin et al., 1997). In the context of clinical luteal phase support following IVF, use of between 200 and 600 mg progesterone/day has been reported. Endometrial preparation for cryopreserved–thawed embryo transfer was successfully performed using a protocol combining oestrogen (transdermal and oral preparations) with micronized progesterone given vaginally at a daily dose of 100 mg (Salat-Baroux et al., 1988). The efficacy of 400 mg/day micronized progesterone administered orally or vaginally has been compared with HCG injected every third day following embryo transfer at a dose of 1500 IU (Buvat et al., 1990). When serum peak oestradiol concentration was >2700 pg/ml, implantation rate was 5–6 times higher with vaginal administration, compared to oral treatment (26 versus 5%). Pregnancy rate and implantation rate with vaginal treatment were both similar to those following HCG injections.

Further studies comparing natural progesterone administered i.m. or vaginally reported a significantly lower early spontaneous abortion rate, resulting in higher ongoing pregnancy rate/embryo transfer following vaginal administration. However, the daily dosage of natural progesterone used in these studies was 600 mg/day micronized progesterone given vaginally compared with 50 mg/day progesterone in oil given i.m. Vaginal administration resulted in a better ongoing pregnancy rate (30.5 versus 19.1%) as well as a lower rate of early spontaneous abortions (2.9 versus 9.1%) (Devroey et al., 1991; Smitz et al., 1992b).

Another study compared luteal support using 90 mg progesterone gel administered vaginally with 300 mg/day micronized progesterone taken orally and found no difference regarding pregnancy rate/embryo transfer, rate of spontaneous abortions or number of babies born per embryos replaced (Pouly et al., 1996).

Therefore we examined the efficiency of this low daily dosage of micronized progesterone in the context of luteal support following ovulation induction using GnRHa/HMG.

Our results indicate that supplementation of luteal phase with a low dose micronized progesterone absorbed vaginally, 100 mg twice daily, leads to significantly higher embryo implantation rates compared to oral ingestion. In these cycles the endometrium was receptive in spite of lower plasma progesterone concentrations, representing probably higher local tissue progesterone concentrations achieved by vaginal administration, and reflecting the strong uterine first-pass effect (Cornet et al., 1991; De Ziegler et al., 1994, 1995; Miles et al., 1994; Balasch et al., 1996; Buletti et al., 1997; Fanchin et al., 1997; Cicinelli et al., 1998).

The luteal endocrine profile in our patients is similar to those reported elsewhere (Smitz et al., 1987; Herman et al., 1996), showing a decline in oestradiol and progesterone production until day +8 post-HCG. Later on, probably due to endogenous secretion of HCG in conception cycles, the corpora lutea are stimulated to produce further oestrogen and progesterone indicated by the rise in their serum concentrations. Our data show clearly that, in spite of lower serum progesterone concentrations following vaginal administration, the conception rate is not impaired. Indeed the superior clinical outcome obtained using a lower dose of micronized progesterone administered vaginally indicates better bioavailability of progesterone absorbed vaginally.

Specific techniques should be used to determine plasma concentrations of progesterone after oral administration, since progesterone metabolites which are produced in significant amounts may interfere with the radioimmunoassay (Nahoul et al., 1987, 1993), thus causing difficulty in comparing the oral and the vaginal administration of micronized progesterone. The method of plasma progesterone measurement used in this study is based on MEIA, a technique that minimizes cross-reaction with the metabolites of orally ingested progesterone, allowing reliable comparison of plasma progesterone concentrations between the two treatment groups.

The tendency to lower late luteal phase osteodiol concentrations following oral administration could reflect a different pattern/rate of steroid metabolism in this group. As conceptions have occurred over a wide range of luteal phase osteodiol/progesterone serum ratio, this parameter may not have a clear predictive value regarding establishment of conception. This finding concurs with previous reports showing that endometrial morphology is not altered by extreme shifts in luteal phase osteodiol concentrations and osteodiol/progesterone ratios (De Ziegler et al., 1992, 1993).

As low dose micronized progesterone absorbed vaginally is an easy, well tolerated and simple method of luteal support, it could be recommended as the method of choice for luteal support, especially for high responder patients at risk for OHSS.

References


Cicinelli, E., Cignarelli, M., Sabatelli, S. et al. (1998) Plasma concentrations of progesterone are higher in the uterine artery than in the radial artery


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