Effect of oral administration of dydrogesterone versus vaginal administration of natural micronized progesterone on the secretory transformation of endometrium and luteal endocrine profile in patients with premature ovarian failure: a proof of concept


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BACKGROUND: We aimed to explore the endometrial histology and endocrine profiles on day 21 of an artificial cycle in patients with premature ovarian failure (POF) treated with oral dydrogesterone (DG) or vaginal micronized progesterone. METHODS: The study was designed as a prospective pilot study at an academic reproductive medicine unit. Six POF patients were included in the study. After estrogen endometrial priming, patients were randomized to receive DG or progesterone in two subsequent cycles. The main outcome measure was the endometrial histology and the endocrine profiles on day 21 of the cycle. RESULTS: Development of endometrial glands corresponded to an early secretory phase in five out of six cases supplemented with DG (out-phase). In contrast, five out of six cases treated with micronized progesterone showed an endometrium corresponding to a mid-luteal phase (in-phase) \( (P = 0.021 \text{ versus } \text{DG}) \). There was a significant difference in the mean progesterone value [8.6 versus 0.3 ng\( \text{ml}^{-1} \)] \( (P = 0.013) \), the mean LH value [12.9 versus 22.5 IU\( \text{l}^{-1} \) \( (P = 0.049) \)] and the mean FSH value [13.0 versus 23.9 IU\( \text{l}^{-1} \) \( (P = 0.047) \)] between the progesterone and DG group, respectively, on day 21 of the cycle. CONCLUSIONS: After estrogen endometrial priming in POF patients, exogenous vaginal micronized progesterone is more effective than oral DG in creating an ‘in-phase’ secretory endometrium and induces significantly higher progesterone and lower LH and FSH serum concentrations on day 21 of the cycle.

Key words: dydrogesterone/luteal phase/micronized progesterone/oral progesterone/premature ovarian failure

Introduction

Hormonal support of the luteal phase in patients undergoing oocyte retrieval and embryo transfer is routinely used (Albano et al., 1999). Various formulations of progesterone are available, including oral, vaginal and i.m. administration routes. A recent meta-analysis by Nosarka et al. (2005) showed clearly that vaginal and i.m. micronized progesterone had comparable implantation and clinical pregnancy rates.

Progesterone administered orally is subjected to a first-pass prehepatic and hepatic metabolism. This metabolic activity results in progesterone degradation to its 5α- and 5β-reduced metabolites (Penzias, 2002).

Dydrogesterone (DG), a retroprogesterone with good oral bioavailability, is a biologically active metabolite of progesterone, which has an anti-estrogenic effect on the endometrium causing a secretory transformation (Whitehead et al., 1980; Chakravarty et al., 2005). Recently, Chakravarty et al. (2005) in a prospective, randomized study compared the efficacy, safety and tolerability of vaginal micronized progesterone with oral DG as luteal-phase support after IVF. Both DG and micronized progesterone were associated with similar rates of successful pregnancies (24.1% versus 22.8%, respectively, \( P = 0.81 \)).

Prior to initiation of a large randomized controlled trial to compare these two treatment schemes for IVF cycles, we decided to conduct a pilot study in patients with premature ovarian failure (POF) (Beck-Peccoz and Persani, 2006), who were on the waiting list of our oocyte donation programme. The subjects were to receive oral DG or vaginal progesterone as the progestins in subsequent cycles in protocols of
cyclic steroid replacement after endometrial priming with estradiol (E2).

In reproductive research, the menopausal patients treated for oocyte donation are the best study paradigm for endometrial receptivity (De Ziegler and Fanchin, 2000). There, in the absence of an endogenous source of progesterone, the bioavailability and efficiency of different administration routes of progesterone can be compared easily.

The objective of this study was to compare the endometrial histology on day 21 of the artificial cycle in patients with POF treated with oral DG versus vaginal progesterone as progestins in protocols of cyclic steroid replacement. Additionally, day 21 luteal-phase hormonal profiles were measured in the two groups.

Materials and methods

Patients and protocols

Six women with a diagnosis of POF were included in the study, after informed consent had been obtained. All patients had a normal uterine cavity, assessed by a diagnostic hysteroscopy. No steroid replacement therapy had been administered in the 8 weeks preceding the study.

This was a prospective pilot study carried out between January and July 2006. The study consisted of two consecutive cycles of steroid replacement. Participants were randomized using a computer-generated randomization list, in two groups to receive for the luteal-phase two different types of progesterone with two different administration modes. Both groups received estradiol valerate (Zumenon®, Solvay Brussels, Belgium) 2 × 2 mg day⁻¹ orally, once in the morning and once in the evening for 7 days. From days 8 to 15, the dose was increased to 3 × 2 mg daily for 6 days. DG and progesterone were used as progestins. Three patients started using DG in the first cycle and switched to progesterone in the subsequent cycle, whereas the remaining three women started with progesterone and used DG in the subsequent cycle. Oral DG (Duphaston® Solvay Pharma, Brussels, Belgium) was administered at a dose of 10 mg Duphaston twice daily (morning and evening) from day 15 of the artificial cycle. Vaginal administration of progesterone (Utrogestan® Besins, Drogenbos, Belgium) was started also from day 15 of the artificial cycle with three times two tablets of 100 mg (morning, afternoon and evening). The oral Zumenon was decreased to 2 × 2 mg day⁻¹ (morning and evening) from day 15 onwards. The treatment was taken until day 26 of the stimulated cycle. There was a hormone-free interval of eight weeks between the two treatment schemes. The patients participating in this trial did not have an embryo transfer during the trial.

Hormone measurement

Blood samples were obtained at days 1 and 21 of the artificial cycle. Serum LH, FSH, E2 and progesterone were measured with the automated Elecsys Immunoanalyzer (Roche Diagnostics, Mannheim, Germany). Intra-assay and inter-assay coefficients of variation were <3% and <4% for LH, <3% and <6% for FSH, <5% and <10% for E2 and <3% and <5% for P.

Endometrial biopsies and tissue preparation

Endometrial biopsies were obtained by a four-quadrant scraping technique using a Novak curette at noon on cycle day 21 in all study patients. Day 21 of the artificial cycle was considered as day 7 of the luteal phase.

Figure 1. Representative endometrial biopsy on day 21 of an artificial cycle after micronized progesterone. Patients with premature ovarian failure received estrogen from days 1 to 21 and vaginal progesterone from days 15 to 21. (Coiled glands with active secretion and minimal residual vacuoles. Stromal oedema.) Absence of mitotic activity. The maturation corresponds to day 6 of the luteal phase (haematoxylin and eosin staining, ×200).

Results

The patients participating in this trial were aged between 29 and 40 (mean 37.2 ± 4.2). The mean value of the body mass index was 22.6 (± 3.4) kg m⁻².

Endometrial specimens were evaluated histologically (Figures 1 and 2). Figure 3 describes the results of the endometrial biopsies for each patient under the different modes of luteal progesterone supplementation.

Development of endometrial glands corresponded to an early secretory phase in five out of six cases supplemented with oral DG (out-phase). In contrast, five out of six cases treated with vaginal micronized progesterone showed an endometrium corresponding to a mid-luteal phase (in-phase) (P = 0.021 versus DG).
The intra-observer variation was 0% for the 12 biopsies for the senior pathologist and 1 out of 12 (8.3%) for the second pathologist. The inter-observer variation was 1 out of 12 (8.3%), whereby the difference in dating was only 1 day, which did not make any difference for the outcome.

Luteal-phase endocrine profiles on day 21 of the artificial cycles including mean concentration of progesterone, E2, LH and FSH are presented in Table I. There was a statistically significant difference in the mean progesterone value [8.6 versus 0.3 μg l⁻¹ (P = 0.013)], the mean LH value [12.9 versus 22.5 IU l⁻¹ (P = 0.049)] and the mean FSH value [13.0 versus 23.9 IU l⁻¹ (P = 0.047)] between vaginal micronized progesterone and oral DG groups, respectively.

### Discussion

The present study shows that vaginal administration of micronized progesterone in women with POF results in significantly more in-phase endometria, when compared with oral DG (P = 0.021). Additionally, as expected, serum progesterone values on day 21 of the cycle were significantly different in the two treatment modes (P = 0.013). Moreover, on day 21 of the artificial cycle, there was a significant difference in serum LH and FSH levels between the two groups (P = 0.049 and 0.047, respectively).

The dose of DG was selected because it has been reported to induce optimal secretory transformation of the endometrium (King and Whitehead, 1986) and it was the same dose as used in the publication of Chakravarty et al. (2005).

From our group, Bourgain et al. (1990) and Devroey et al. (1989) reported the absence of any secretory transformation of the endometrium in patients treated with oral micronized progesterone compared with patients treated with i.m. injections or vaginal micronized progesterone, suggesting a reduced bioavailability of this hormone, if taken orally. The three most important metabolites of progesterone taken orally are pregnanediol-3 α-glucuronide, 17-hydroxyprogesterone and 20-α-dihydroprogesterone (Whitehead et al., 1980). 20-α-Dihydroprogesterone is a biologically active metabolite of progesterone (Whitehead et al., 1980).

DG, a retroprogesterone, is a stereoisomer of progesterone, wherein the methyl group attached to the 10th carbon is located in the α-position (as opposed to the β-position in micronized progesterone). The hydrogen attached to the carbon in the ninth position is in the β-position (as opposed to the α-position in micronized progesterone). There is also an extra double bond between carbons 6 and 7. These changes in configuration make DG metabolically stable and orally effective (Chakravarty et al., 2005).

It has been documented that oral DG induces a normal endometrial secretory pattern in infertile (Balasch et al., 1982) and post-menopausal (King and Whitehead, 1986) patients. In contrast, the current study documents an incomplete secretory pattern.

### Table I

<table>
<thead>
<tr>
<th></th>
<th>Oral dydrogesterone (n = 6)</th>
<th>Vaginal micronized progesterone (n = 6)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone (μg l⁻¹)</td>
<td>0.3 (± 0.05)</td>
<td>8.6 (± 2.20)</td>
<td>0.013²</td>
</tr>
<tr>
<td>Estradiol (ng l⁻¹)</td>
<td>201.7 (± 26.70)</td>
<td>190.0 (± 21.46)</td>
<td>NS</td>
</tr>
<tr>
<td>FSH (IU l⁻¹)</td>
<td>23.9 (± 6.14)</td>
<td>13.0 (± 3.35)</td>
<td>0.047²</td>
</tr>
<tr>
<td>LH (IU l⁻¹)</td>
<td>22.5 (± 4.16)</td>
<td>12.9 (± 3.10)</td>
<td>0.049²</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SD.

²Statistically significant.
transformation of the endometrium with orally administered DG on day 21 of the artificial cycle, supporting the reduced efficacy of this formulation. The relatively retarded endometrial development in artificial cycles treated with oral DG was also reported in the previous studies (Pellicer et al., 1989; Li et al., 1994).

During the luteal phase, the increased levels of progesterone and E2 play an important role in the maintenance of the low FSH and LH levels. This suggests that it is the combined action of E2 and progesterone that mediates the negative feedback effect on gonadotrophin secretion during the luteal phase of the cycle (Messinis, in press). It seems that the co-administration of progesterone and E2 in the luteal phase could express a stronger negative feedback effect on LH and FSH levels than E2 alone. Whether progesterone alone could express a similar action has not been investigated, although under experimental conditions the negative feedback effect of progesterone is apparent in the presence of estrogen (Soules et al., 1984).

The effect of progesterone on the endometrium was assessed on day 21 of the artificial cycle, since in human it is currently accepted that the window of implantation, the time during which the endometrium is most conducive to trophoblast-endometrial interactions, is restricted to days 20–24 of an idealized 28 day cycle (Coutifaris et al., 2004).

It is doubtful that the very low progesterone levels, measured on day 21 of the artificial cycle treated with oral DG, could induce the observed onset of secretory transformation of the endometrium. Moreover, the early secretory transformation of the endometrium caused by oral DG seems to be insufficient.

It has been assumed that deficiencies in progesterone could result in delayed endometrial maturation (Dallenbach-Hellweg, 1984). The histological appearance, together with the endocrine findings, suggest that the endometrium supplemented with oral DG fails to present optimal conditions for the implantation of human embryos according to our current method of evaluation of the endometrium. Whether the results may be improved by increasing the dose of DG is uncertain and requires further investigation. However, final conclusions cannot be drawn, since no embryo transfer was performed in this study. These findings cannot be referred to a stimulated IVF cycle. The oral DG might be sufficient for luteal supplementation in IVF cycles; however, more large randomized controlled trails are needed before a conclusion can be made.

This experimental study is a test for assessing the ability of progestins to prime endometrial receptivity, which also describes the endocrine profile at an important moment in a cycle (day 21).

In conclusion, after sufficient estrogen endometrial priming in POF patients, exogenous vaginal micronized progesterone is more effective than oral DG in creating an in-phase secretory endometrium and induces significantly higher progesterone and lower LH and FSH serum concentrations on day 21 of the artificial cycle.

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References

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