Analysis of the bleeding pattern in assisted reproduction cycles with luteal phase supplementation using vaginal micronized progesterone

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This study was designed to determine the effects of a vaginal micronized progesterone preparation on bleeding patterns and pregnancy outcomes after in-vitro fertilization and intracytoplasmic sperm injection (IVF–ICSI). The study population consisted of 149 consecutive women who had undergone IVF–ICSI using ‘long-protocol’ stimulation with buserelin–human menopausal gonadotrophin (HMG). A retrospective chart analysis of computerized medical records was undertaken. Vaginal progesterone (200 mg three times daily) was begun the day before oocyte retrieval and continued for a minimum of 16–19 days following human chorionic gonadotrophin (HCG) administration. Occurrence of bleeding following HCG injection, pregnancy rate and outcomes, and serum concentrations of oestradiol were measured. Women undergoing IVF and embryo transfer with ICSI and using vaginal progesterone for luteal support had normal luteal phase lengths (day of HCG minus day of onset of bleeding). In the absence of pregnancy, bleeding occurred after 19.2 ± 3.9 days (mean ± SD). Out of the pregnant group only three women bled within 19 days of HCG administration: two had biochemical pregnancies which spontaneously vanished and one evolved to term. The results reflect the normal bleeding pattern to be expected when vaginal progesterone is used for luteal support in IVF and embryo transfer, an approach whose efficacy has been amply proven. No shortened luteal phases were observed using vaginally administered progesterone.

Key words: assisted reproduction technology/bleeding/oestrogen/vaginal progesterone

Introduction

Luteal support is necessary in ovarian stimulation protocols, such as those commonly prescribed for in-vitro fertilization (IVF) and embryo transfer (Smith et al., 1989; Belaisch-Allart et al., 1990).

Abnormal luteal function occurs when ovulation is induced with human chorionic gonadotrophin (HCG) or when endogenous gonadotrophins are suppressed with a gonadotrophin-releasing hormone agonist (GnRHa) (Olson et al., 1983; Buvat, 1988; Smitz et al., 1992b).

Historically, luteal support was provided by exogenous HCG or i.m. injections of progesterone. Despite proven efficacy, the former approach has been progressively abandoned in view of mounting evidence that it increases the risk of frank ovarian hyperstimulation (Rizk and Smitz, 1992). Daily i.m. injections of progesterone for up to 10 weeks (if pregnancy occurs) causes pain and can potentially lead to local inflammatory reactions, possibly evolving into sterile abscesses.

Because of the practical inconvenience of i.m. injections, alternative routes for administering progesterone have been sought. In micronized form, progesterone is absorbed when given orally but is nearly completely metabolized (>90%) during its first pass through the liver (Nahoul et al., 1993). This explains the previous observation that oral micronized progesterone (300 mg/day), administered in conjunction with physiological amounts of oestradiol, failed to achieve the predecidual transformation of the endometrial stroma (Bourgain et al., 1990). In order to obtain the minimal intratissular progesterone concentration to achieve transformation of hyperstimulated endometrial tissue there would be a need for such a high oral dose that it would cause somnolence, sedation and hypnose (Arafat et al., 1988).

Transdermal administration of progesterone is also not feasible (Cooper et al., 1998), as the skin is poorly permeable to the drug and rich in the enzyme 5-α-reductase, which will inactivate the small amount of progesterone that is able to penetrate the skin. Hence, the vaginal route is the only remaining practical option for administration of progesterone for luteal support—replacing the painful i.m. injections.

In previous work, it has been shown that progesterone administered vaginally (200 mg three times daily) is capable of reproducing all the endometrial changes normally seen in the luteal phase of the menstrual cycle (Devroey et al., 1989; Smitz et al., 1992, 1993). Furthermore, vaginal progesterone supports nidation and development of pregnancy in women without ovarian function (Devroey et al., 1989).

Several authors, puzzled by the unusual efficacy of vaginal progesterone, have examined uterine tissue concentrations of progesterone and have gathered evidence that some degree of direct transport from the vagina to the uterus exists. Miles et al. (1994) observed higher endometrial concentrations of progesterone with vaginal administration, compared with the concentrations following i.m. injections (50 mg twice daily), despite lower serum progesterone concentrations following vaginal administration. Based on this evidence and our own data, it was elected to provide luteal support with vaginal progesterone for our routine IVF and IVF–intracytoplasmic sperm injection (ICSI) procedures in this centre.

From previous experience using i.m. progesterone, it is
commonly known that menstrual bleeding is postponed as long as progesterone is administered, however no real data from systematic analysis are available on this specific point in the literature.

In the present study, a retrospective analysis was undertaken of the bleeding patterns experienced by 149 consecutive IVF–ICSI cases receiving vaginal progesterone for luteal support. Outcomes obtained in pregnant and non-pregnant women were analysed separately.

Materials and methods

Study population

This study was designed to evaluate the effects of vaginal micronized progesterone on bleeding following HCG administration in IVF–ICSI procedures.

A retrospective analysis was conducted of the computerized medical records of 149 consecutive women who had undergone ICSI between January 1 and April 30, 1996. Women were eligible for review if they had completed a routine assisted reproduction–ICSI procedure [using ‘long-protocol’ stimulation with buserelin–human menopausal gonadotrophin (HMG) within the specified time period] and had received vaginal micronized progesterone for luteal phase supplementation without oestrogen or HCG supplements. No restrictions were placed on age, weight or the subsequent outcome of the assisted reproduction procedure.

Collection of information on bleeding pattern

During the luteal phase all patients were systematically instructed to have routine contact by telephone with the nursing staff to enquire about their hormonal profile, to report on eventual side-effects and to state whether any abnormal vaginal bleeding had occurred. The data were recorded in writing from the telephone conversation directly onto the patients’ individual charts kept in the laboratory and were then transferred to a computer operator to input. In the third week after oocyte retrieval, all patients were contacted by a senior scientist for women who did not become pregnant, to report on eventual side-effects and bleeding occurred after a mean of 19.2 ± 9.7 days following embryo transfer. A diagnosis of pregnancy was established using β-HCG values obtained on days 16 and 19 using the Tandem-HCG® assay (Hybritech, Liège, Belgium) with 10 IU/l as the threshold value.

Clinical evaluations

The primary evaluations were the occurrence of bleeding relative to the day of HCG injection, the onset of bleeding relative to the day vaginal progesterone supplementation ceased (expressed as the number of days before or after the end of vaginal progesterone use), and the outcome of the procedure (i.e. pregnant or non-pregnant).

Serum oestradiol concentrations were determined by commercial radioimmunoassay (Estradiol-coatia®; BioMérieux, Marcy-l’Etoile, France) in blood samples obtained on the day of HCG administration (day 0) and on days 16 and 19 (corresponding to days 12 and 15 after embryo transfer). A diagnosis of pregnancy was established using β-HCG values obtained on days 16 and 19 using the Tandem-HCG® assay (Hybritech, Liège, Belgium) with 10 IU/l as the threshold value.

Statistical methods

Statistical analyses were performed with MedCalcTM software (MedCalc Software, Mariakerke, Belgium). Variables (patient characteristics and assisted reproduction treatment outcome data, mean hormone concentrations, and bleeding patterns) were evaluated using the χ² test, Student’s test, and the Wilcoxon signed ranks test. Statistical significance was determined at P ≤ 0.05.

Results

The mean age of the 149 women was 32 years (Table I), with a median duration of infertility of 4 years (range: 1–19 years). This sample of women had experienced from one to six previous assisted reproduction attempts (median: one). Fifty-two of the 149 women (35%) became pregnant using this study protocol. No statistically significant differences were observed in these variables when comparing women who became pregnant with those who did not.

Bleeding patterns, pregnancy outcomes and serum oestradiol concentrations

For women who did not become pregnant (n = 97), bleeding occurred after a mean of 19.2 ± 3.9 days following HCG administration (median: 18 days; range: 11–41 days). Only one of the non-pregnant women experienced early menstruation; this occurred on the 11th day post-HCG. A total of 65% of the non-pregnant women experienced bleeding before vaginal progesterone treatment was discontinued. Figure 1 demonstrates the distribution of bleeding times after HCG administration in the non-pregnant women who began menstruation; this occurred on 4 days after stopping treatment. Three women with positive pregnancy tests had spotting prior to the pregnancy test: two of these had biochemical pregnancies only, and the third received double doses of vaginal progesterone from the day of spotting onward, subsequently delivering a normal singleton.

There were 40 deliveries out of 52 pregnancies in the 149 women whose charts were reviewed for this study. On these 40 deliveries, 65% were singletons, 27.5% were twins, and 7.5% were triplets. Of the remaining 12 women, three had only biochemical evidence of pregnancy, one had an ectopic pregnancy, and eight women spontaneously aborted (seven early and one late).
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pregnant (n = 52)</th>
<th>Non-pregnant (n = 97)</th>
<th>Total (n = 149)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age ± SD (years)</td>
<td>30.8 ± 4.2</td>
<td>31.2 ± 4.1</td>
<td>31.0 ± 4.1</td>
</tr>
<tr>
<td>Range</td>
<td>(22–41)</td>
<td>(22–45)</td>
<td>(22–45)</td>
</tr>
<tr>
<td>Median duration of infertility (years)</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Range</td>
<td>(1–15)</td>
<td>(1–19)</td>
<td>(1–19)</td>
</tr>
<tr>
<td>Duration of spontaneous cycles (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24 days</td>
<td>0</td>
<td>3 (3.7)</td>
<td>3 (2.3)</td>
</tr>
<tr>
<td>24–35 days</td>
<td>42 (89.4)</td>
<td>71 (86.6)</td>
<td>113 (78.6)</td>
</tr>
<tr>
<td>36 days–3 months</td>
<td>5 (10.6)</td>
<td>7 (8.5)</td>
<td>12 (9.3)</td>
</tr>
<tr>
<td>&gt;3 months</td>
<td>0</td>
<td>1 (1.2)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Median number of assisted reproduction attempts</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Number of patient files with missing data</td>
<td>6</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

As can be seen, ~90% of women investigated had a regular, normal cycle length, thus obviating any bias by polycystic ovarian disorder or 'ovarian depletion'.

Figure 1. Distribution of the onset of menses following human chorionic gonadotrophin (HCG) administration (day 0) in the 63 non-pregnant women who began menses prior to cessation of vaginal progesterone treatment.

Figure 2. Mean serum concentrations of oestradiol (pg/ml) on days 0, 16 and 19 following HCG administration to pregnant (n = 52) and non-pregnant women (n = 97). Differences between pregnant and non-pregnant women were statistically significant on days 16 and 19 (P = 0.0001; Student’s t-test). Bars represent SD.

Statistically significant differences (P = 0.0001) were seen between pregnant and non-pregnant women with regard to mean serum concentrations of oestradiol on day 19 following HCG administration (Figure 2). On the day of HCG injection (day 0), mean serum oestradiol concentrations were similar in the two groups, which indicated a similar response to ovarian stimulation in both groups. However, on both end-luteal control days mean oestradiol concentrations were significantly higher in pregnant women than in non-pregnant women (P < 0.0001).

Serum oestradiol concentrations were lower in women who bled before arrest of medication at days 16 and 19 after HCG injection (P < 0.0001; Figure 3).

Discussion

It has previously been shown that vaginal administration of progesterone (200 mg three times daily) was an effective way of priming endometrial receptivity and favouring the development of pregnancy in IVF and embryo transfer (Devroey et al., 1989; Bourgain et al., 1993). In the present
retrospective study of 149 women who used vaginal micronized progesterone as luteal phase support, the pregnancy rate per cycle (35%) and low fetal wastage observed confirmed earlier findings (Smitz et al., 1992a, 1993). In addition, using a human ex-vivo perfusion model, Bulletti et al. (1997) confirmed that a fraction of vaginally administered progesterone was transported directly to the uterus corpus. Hence, these results further support the decision to use vaginal progesterone routinely for luteal support in IVF.

The objective of this retrospective study was to evaluate the bleeding patterns in pregnant and non-pregnant IVF patients. Hence, to ensure population homogeneity, it appeared reasonable to select women undergoing the same ovarian stimulation treatment protocol. The ‘long GnRHα’ protocol with late luteal onset of GnRHα treatment was retained for the present study because it is the standard treatment for IVF in this centre. In addition, women undergoing IVF–ICSI were selected to maximize population homogeneity and because women in this subgroup were more likely to display a normal response to ovarian stimulation (Ubaldi et al., 1995; Wisanto et al., 1996; Vandervorst et al., 1997).

Since the original description by Jones (1975) that a short/inadequate luteal phase was a cause of infertility and recurrent abortions, attention has been focused on luteal phase length and bleeding pattern. The results of the current study on the bleeding profiles seen in 97 women who underwent IVF–ICSI but failed to become pregnant are most reassuring in this respect. None of the women had a shortened luteal phase with bleeding <11 days after HCG. In the group studied, one of the non-pregnant women started to bleed on the 11th day after HCG. All the other women bled later, well within the normal time frame for normal luteal phase length (Figure 1). During the observation period (until the 19th day post-HCG), bleeding occurred in three pregnant women. Two had a biochemical pregnancy and one went on to term and delivered uneventfully.

There was no evidence that the two biochemical pregnancies had a relation to any luteal supplementation used. Instead, the rate of biochemical pregnancy with this progesterone supplementation regimen has been found lower than with other forms of luteal supplementation (Smitz et al., 1992a). Occasionally however, an abnormal rescue of the corpus luteum graviditatis can be observed and an additional luteal supplement can normalize serum progesterone concentrations (Smitz et al., 1987).

Serum oestradiol concentrations were lower in women whose HCG titre was negative, compared with those who became pregnant. These results are consistent with those of Liu et al. (1991), who found that serum oestradiol concentrations rose even before HCG became detectable in peripheral blood in women who became pregnant. In the current study, women who bled before discontinuing progesterone supplementation had low concentrations of oestradiol. This confirmed that pregnancy had not occurred, making bleeding a consequence and not a cause of this non-pregnant state.

The convenience and demonstrated efficacy of vaginally administered progesterone, and the avoidance of painful, repeated i.m. injections of progesterone, should further facilitate luteal support in IVF and other forms of assisted reproduction treatment (e.g. ovarian stimulation and ovarian stimulation with IVF). The major drawback of the current regimens for luteal support with vaginal progesterone is the need for multiple (three) daily administrations (Devroey et al., 1989; Schmidt et al., 1989). New options are emerging, however, with the development of a sustained-release gel of progesterone (Crinone 8%®; Wyeth-Ayerst Pharmaceuticals, Radnor, PA, USA), which removes the need for multiple administrations (Fanchin et al., 1997).

In conclusion, this study demonstrated that early bleeding following administration of vaginal progesterone for luteal phase support occurred almost exclusively in women who had not become pregnant.

References
Vaginal progesterone in assisted reproduction technology


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