Mid-cycle serum levels of endogenous LH are not associated with the likelihood of pregnancy in artificial frozen-thawed embryo transfer cycles without pituitary suppression

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BACKGROUND: The aim of the present study was to evaluate the association between clinical pregnancy and serum luteinizing hormone (LH) levels, assessed after 14 days of endometrial preparation with estradiol (E2) in the absence of pituitary suppression during a frozen-thawed embryo transfer (FRET) cycle. METHODS: A total of 513 patients undergoing their first FRET cycle (01/99 to 11/05) participated in this prospective study. Endometrium preparation for FRET was started on cycle day 1 and continued for a fixed period of 14 days with trans-dermal E2 patches. On day 14, serum LH, progesterone and E2 levels were assessed. On day 15, progesterone supplementation was initiated and patients underwent embryo transfer on day 17 or day 18. The association between clinical pregnancy and LH levels was evaluated in groups of patients defined according to Tukey’s Hinges percentile analysis of LH levels on day 14. In addition, robust logistic regression was performed with the dependent variable clinical pregnancy and independent variables LH, progesterone, embryos score, cycle rank and gravidity. RESULTS: Age, BMI, parity, cycle rank, embryo number, embryo score, endometrial diameter, E2 and progesterone were not significantly different in cycles with low (0.1–8.1 IU/l; n = 132), intermediate (8.2–19.4 IU/l; n = 238) and high (20.0–78.0 IU/l; n = 143) levels of LH, respectively. Clinical pregnancy rates were not significantly different in cycles with low [12.1%, 95% confidence interval (CI) 7.6–18.8], intermediate (13.4%, 9.7—18.4) and high levels of LH (16.1%, 11.0–23.0). Robust logistic regression analysis indicated that embryo score [Odds ratios (OR) 1.04, 95% CI 1.02–1.06, P < 0.01] was statistically significantly associated with the likelihood of clinical pregnancy achievement, but not day 14 levels of LH or progesterone, gravidity or cycle rank. CONCLUSIONS: The likelihood of clinical pregnancy is not associated with serum LH levels on day 14 of an artificial FRET cycle. Hormonal monitoring of LH levels does not yield useful information with regard to cycle management and patient prognosis, and should therefore not be conducted.

Keywords: frozen-thawed embryo transfer; luteinizing hormone; progesterone; clinical pregnancy

Introduction
The role of LH during ovarian stimulation for IVF still remains controversial (Kolibianakis et al., 2006). Besides the well known indirect effects of LH, which are mediated through ovarian steroid production, LH might directly affect endometrial receptivity and implantation, since endometrium is one of its target organs (Ziecik et al., 1992; Rao, 2001).

Supplementation with estrogen (E2) from the early follicular phase without pituitary suppression in an artificial endometrium preparation cycle for frozen-thawed embryo transfer (FRET) can lead to a rise in LH level, similar to that observed before ovulation. As E2 administration suppresses follicular recruitment, this LH rise is usually not accompanied by a rise in progesterone (de Ziegler et al., 1991; Simon et al., 1998). By direct action on the endometrium, however, such a rise of LH might interfere with endometrial receptivity (El-Toukhy et al., 2004) during a FRET cycle, in which no pituitary suppression is used.

The aim of the present study was to evaluate the association between clinical pregnancy and serum LH levels, assessed after 14 days of endometrial preparation with E2, in the absence of pituitary suppression, during a FRET cycle.

Materials and Methods
The present study was a single-centre, prospective, clinical cohort study, which was approved by the local ethics committee. Since January 1999, all patients with supernumerary frozen 2-PN embryos were scheduled for FRET in an artificial cycle (Bals-Pratsch et al., 1999). Patients could enter the study only once.
Endometrium preparation and freezing-thawing protocol

Briefly, at menses, preparation of the endometrium was started and continued for 14 days with transdermal E2 patches (Estraderm TTS 100®) as previously described (Bals-Pratsch et al., 1999). On day 14 of the artificial cycle, endometrial thickness and serum levels of LH, progesterone and E2 were assessed. Embryo transfer was performed irrespectively of hormone values and endometrial thickness in all patients with available embryos for transfer. Transfer of embryos took place 24, or occasionally 48 h (according to the preferences of the laboratory and the patients) after thawing of 2-PN embryos on day 3 or 4 of treatment with vaginal progesterone (Crinone 8%). Embryos at the pronuclear stage were cryopreserved and thawed as previously described (Al-Hasani et al., 1999).

Hormone measurements

Serum E2, LH and progesterone levels were assayed with the commercially available electrochemiluminescence immunoassay ‘ECLIA’ (Roche Diagnostics Inc., Germany) on the Roche Elecsys 2010 automated immunoassay analyser by the local laboratory. Intra-assay and inter-assay coefficients of variation were routinely monitored at regular intervals throughout the time period of this study, and were <2.0 and <7% for E2, <2.5 and <5% for LH and <2.5 and <5% for progesterone, respectively.

Outcome measures

Primary outcome was clinical pregnancy rate per FRET, which was defined as the presence of fetal heart beat on ultrasonography 4–5 weeks after embryo transfer. The cumulative embryo quality score (Steer et al., 1992) of the FRET was assessed as previously described (Ludwig et al., 2000).

Data analysis and statistics

The association between the achievement of clinical pregnancy and LH levels was examined by robust logistic regression. Independent variables that could enter the regression model were identified by univariate analysis (Mann–Whitney U-test.) Furthermore, the likelihood of pregnancy achievement was compared by chi-squared test for trend between three groups of patients objectively defined by Tukey–Hinges percentile analysis of LH levels on day 14 of the FRET cycle (<25th, 25–75th, and ≥75th percentile). Normally distributed (Kolmogorov–Smirnov test with Lilliefors correction) metric variables were compared across groups by one-way analysis of variance, while non-normally distributed metric variables were compared by Kruskal–Wallis analysis of variance. Correlations of LH with endometrial diameter, E2 and progesterone were tested by Pearson correlation.

Power analysis

No data were available in FRET cycles to estimate accurately the necessary sample size for the present study and the power analysis was based on the assumption that pregnancy rates would be higher (20%) in the middle LH group and lower (10%) in the low and high LH groups, respectively. Power analysis indicated that a sample size of 506 patients achieves 80% power to detect an effect size W (0.138) using a 2 d.f. chi-squared test with a significance level (alpha) of 0.05.

Results

A total of 513 patients had a FRET cycle performed after thawing of 2-PN embryos between January 1999 and November 2005. ICSI had been performed in 89.9% of the fresh cycles that led to a FRET cycle. Less than 2% of all embryo transfers were performed 48 h after thawing. All embryos available for transfer originated from the same fresh cycle.

Hormone values and endometrial thickness on day 14

Mean LH values were 15.8 ± 11.0 mIU/ml. The distribution of LH values on day 14 in the 513 artificial endometrium preparation cycles performed is shown in Fig. 1. Mean E2 values were 340.6 ± 175.6 pg/ml, with E2 values ≥450.0 pg/ml found in 109 (21.2%) of the 513 cycles. Mean P-values were 0.64 ± 0.4. In 4 (0.7%) of the 513 cycles, progesterone was

Figure 1: Distribution of serum LH levels on day 14 (n = 513)
>2.0 ng/ml (2.4, 2.7, 2.8 and 2.8 ng/ml, respectively). Mean endometrial thickness was 10.2 ± 2.0 mm. Endometrial thickness ≥6 mm was present in 512 (99.8%) of 513 cycles.

Table 1 shows that LH levels were significantly negatively correlated to E2 values, however, no correlation to endometrial thickness, progesterone, body weight and BMI was found.

### Patient and cycle characteristics

Demographic and FRET cycle characteristics in groups of patients defined according to percentile analysis of LH levels on day 14 are shown in Table 2. No significant differences between the groups compared were observed regarding patient (age, BMI, gravidity and cycle rank) and cycle characteristics (number of embryos transferred, embryo score, E2 on day 14 and progesterone on day 14).

### Pregnancy likelihood according to LH value

The overall clinical pregnancy rate was 13.8% [95% confidence interval (CI) 11.1–17.1]. Table 3 shows pregnancy outcome in groups of patients defined according to percentile analysis of LH levels on day 14. No significant differences were present between the groups compared. A robust logistic regression was performed with independent variables day 14 LH, day 14 progesterone, embryo score, cycle rank and gravidity. Table 4 shows that embryo score (OR 1.04, 95% CI 1.02–1.06, \( P < 0.01 \)) was significantly associated with the likelihood of clinical pregnancy achievement, which was not the case with levels of LH or progesterone on cycle day 14, gravidity or cycle rank.

No significant differences were present between the LH percentile groups with respect to ongoing pregnancy at 10–12 weeks of gestation [9.8% (95% CI 6.2–15.2), 10.5% (8.1–13.5) and 14.4% (9.6–21.0) ongoing pregnancy rate in patients with low, intermediate and high levels of LH, respectively; chi-square for trend \( P = 0.36 \)]. However, follow up beyond clinical pregnancy was incomplete with data missing in five patients.

### Discussion

The present study addressed the question whether endogenous LH levels are associated with the likelihood of clinical pregnancy, in patients undergoing a FRET cycle in which endometrium is prepared with \textit{trans}-dermal E2 patches in the absence of pituitary down-regulation. The data presented herein show that clinical pregnancy likelihood is similar in FRET cycles in the presence of low, intermediate or high LH levels on day 14 of endometrium preparation.

The results of the present prospective study originate from a large series of patients undergoing their first FRET cycle, irrespective of serum hormone levels and endometrial thickness. Mean hormone values and mean endometrial diameter were similar in the present study as compared to mean hormone values and endometrial diameters as reported in previous publications on artificial FRET cycles using folliculare phase \textit{trans}-dermal E2 (de Ziegler \textit{et al.}, 1991; Queenan \textit{et al.}, 1999; Bals-Pratsch \textit{et al.}, 1999).

As previously reported, progesterone elevations above follicular phase reference values after \textit{trans}-dermal E2 application appear to be rare events with an incidence of 0.7% progesterone elevation ≥2.0 ng/ml in the present study (0% incidence in the studies of de Ziegler \textit{et al.}, 1991; Queenan \textit{et al.}, 1997; Bals-Pratsch \textit{et al.}, 1999). However, no upper threshold value for progesterone above which a FRET cycle should be cancelled has been determined up to now. It has to be noted that the present study was only powered to study the association of LH levels with pregnancy likelihood, and thus no solid conclusions can be drawn regarding the association between progesterone elevation and pregnancy rates. Similarly, no consensus exists as to what constitutes the minimal endometrial diameter below which a cycle should be cancelled. Also, it has to be acknowledged that only patients reaching embryo transfer were analysed in this study, while hormone and endometrial thickness measurements had also been performed during the study period in patients who did not undergo embryo transfer.

### Table 2: Demographic and FRET cycle characteristics according to LH value percentiles on day 14 of an artificial endometrium preparation cycle for frozen-embryo transfer (\( n = 513 \))

<table>
<thead>
<tr>
<th>Groups of cycles according to LH level percentiles</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–25th (( n = 132 ))</td>
<td>25–75th (( n = 238 ))</td>
</tr>
<tr>
<td>Female age (years)</td>
<td>32.4 ± 4.9</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>24.2 ± 4.3</td>
</tr>
<tr>
<td>Gravidity (( n ))</td>
<td>0.5 ± 0.8</td>
</tr>
<tr>
<td>Cycle rank (( n ))</td>
<td>2.9 ± 1.5</td>
</tr>
<tr>
<td>Transferred embryos</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td>Embryo score</td>
<td>22.3 ± 10.8</td>
</tr>
<tr>
<td>Endometrium (mm)</td>
<td>10.1 ± 2.0</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>552.4 ± 203.7</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>0.6 ± 0.4</td>
</tr>
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</table>
As yet, no published study has addressed the question asked in the present study. The only comparable information comes from a study published so far as an abstract, the results of which are in line with those presented here, showing no association between endogenous LH levels and the probability of clinical pregnancy (Cheung et al., 2005).

This information might be useful in clinical practice since it indicates that a wide range of LH values is compatible with establishment of clinical pregnancy, and thus there is no value in measuring LH levels during a FRET cycle. It might also be assumed that endogenous LH levels probably have no direct effect on the endometrium and the process of implantation. Furthermore, the present data suggest that LH levels in an artificial FRET cycle are negatively correlated to E2 levels, probably through a negative feedback mechanism operating between serum E2 and the pituitary gland. Therefore, the LH level under such circumstances is possibly mostly an epiphenomenon of the serum level of E2 achieved in an individual patient by trans-dermal application.

It is tempting to speculate that patients in the present cohort might not have benefited from pituitary suppression by a GnRH agonist. This is in line with a number of experimental studies indicating no benefit of pituitary suppression in artificial FRETs (Simon et al., 1998; Dal Prato et al., 2002; Yee et al., 2005; Gelbaya et al., 2006). The significant difference in pregnancy rates between pituitary suppressed and unsuppressed patients in the study of El-Touky et al. (2004) might be due to the absence of hormonal monitoring to ascertain ovarian quiescence, after preparation of the endometrium with oral E2, in patients not receiving GnRH-agonists. Moreover, it might be associated with the ~3 weeks duration of E2 administration in the non-down-regulated group that might have predisposed patients to a higher rate of ovulation compared with a regimen utilizing a fixed (14 days) regimen of trans-dermal E2 supplementation prior to initiation of progesterone.

It is also important to note that the findings from the present study are only valid in FRET cycles with endometrial preparation by E2 administration, but not in FRET cycles with active ovaries and naturally occurring secretory changes of the endometrium following ovulation. Similarly, the results of the present trial cannot be extrapolated to cycles with fresh embryo transfer following ovarian stimulation for IVF.

No indication can be obtained from the current study that LH supplementation is necessary to compensate for low endogenous LH in a certain proportion of patients undergoing FRET cycles with E2 supplementation and no pituitary down-regulation.

In conclusion, we suggest that in artificial endometrium preparation cycles without pituitary down-regulation, assessment of LH levels after 14 days of E2 treatment does not appear to yield useful information with regard to cycle management and patient prognosis and should therefore not be conducted.

Table 3: Pregnancy outcome in groups of patients (n = 513) categorized according to percentile analysis of LH levels measured on day 14 of an artificial endometrium preparation cycle for FRET

<table>
<thead>
<tr>
<th>Groups of FT-ET cycles according to hormonal levels</th>
<th>LH levels (mIU/ml)</th>
<th>cPR/ET</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–25th Percentile (Tukey)</td>
<td>Mean ± SD</td>
<td>Min</td>
</tr>
<tr>
<td></td>
<td>5.1 ± 2.3</td>
<td>0.1</td>
</tr>
<tr>
<td>25–75th Percentile</td>
<td>13.6 ± 3.5</td>
<td>8.2</td>
</tr>
<tr>
<td>75–100th Percentile</td>
<td>29.3 ± 10.7</td>
<td>20.0</td>
</tr>
</tbody>
</table>

cPR/ET, clinical pregnancy rate per embryo transfer; 95% CI, 95% confidence interval of PR/ET.

Table 4: Robust logistic regression on the likelihood of clinical pregnancy achievement in FRET cycles (Model P < 0.001; Hosmer and Lemeshow test P = 0.84)

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>P-value</th>
<th>OR</th>
<th>95% CI</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Embryo score</td>
<td>0.01</td>
<td>1.04</td>
<td>1.02–1.05</td>
</tr>
<tr>
<td>Cycle rank</td>
<td>0.06</td>
<td>0.86</td>
<td>0.73–1.01</td>
</tr>
<tr>
<td>LH</td>
<td>0.15</td>
<td>1.02</td>
<td>0.99–1.04</td>
</tr>
<tr>
<td>Gravity</td>
<td>0.16</td>
<td>0.77</td>
<td>0.54–1.11</td>
</tr>
<tr>
<td>Progesterone</td>
<td>0.87</td>
<td>0.95</td>
<td>0.50–1.80</td>
</tr>
</tbody>
</table>

References


Queenan JT, Jr, Veeck LL, Toner JP, Oehninger S, Muasher SJ. Cryopreservation of all prezygotes in patients at risk of severe hyperstimulation does not eliminate the syndrome, but the chances of pregnancy are excellent with subsequent frozen-thaw transfers. *Hum Reprod* 1997;12:1573–1576.


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