Serum relaxin and the major endometrial secretory proteins in in-vitro fertilization and down-regulated hormone-supported and natural cycle frozen embryo transfer

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Relaxin has been postulated to be a modulator of the expression of the endometrial secretory proteins, insulin-like growth factor binding protein (IGFBP-1) and placental protein 14 (PP14). This study evaluated the expression of relaxin in relation to concentrations of these secretory proteins along with oestradiol, progesterone and human chorionic gonadotrophin in groups of pregnant and non-pregnant patients who underwent differing assisted conception treatments. Serum samples were taken from 88 patients at 8 and 12 days after embryo transfer. At 12 days after embryo transfer, relaxin concentrations in the pregnant patients who had undergone in-vitro fertilization (IVF) or natural cycle frozen embryo transfer were significantly higher than those who did not conceive in these groups (mean concentrations 8334 versus 28 and 2608 versus 62 pg/ml respectively, \( P < 0.001 \)). However concentrations in the pregnant patients who had hormone support and transfer of frozen embryos were not significantly different from the patients who did not conceive after the same treatment. Although relaxin expression was associated with corpus luteum activity, it was not related to the number of corpora lutea in IVF patients. A wide range of relaxin concentrations was seen to be compatible with a healthy pregnancy. These serum relaxin concentrations were not found to be directly related to the serum concentrations of IGFBP-1, PP14 or the other factors assessed in this study.

Key words: endometrial proteins/frozen embryo transfer/ in-vitro fertilization/ovary relaxin

Introduction

Relaxin is a polypeptide hormone secreted by the corpus luteum during the luteal or secretory phase of the menstrual cycle and during pregnancy. It is cleaved from a larger prohormone (Schwabe and Bullsbach, 1990) and has a half-life of \(< 1\) h. Two human relaxin genes have been identified, both on chromosome 9. Gene 2 (hRLX 2) is expressed in the human ovary (Hudson \textit{et al.}, 1984) and gene 1 is believed to be expressed in decidua (Hansell \textit{et al.}, 1991).

In the non-pregnant state the maximal serum concentrations of relaxin occur 10–12 days after ovulation (Stewart \textit{et al.}, 1990), when other luteal activity is decreasing. In pregnancy the serum concentration of this peptide peaks at the end of the first trimester (900 pg/ml) and then remains constant at a concentration of \(\sim 500\) pg/ml for the remainder of the pregnancy (Bell \textit{et al.}, 1987).

The influence of relaxin at the time of implantation in humans is poorly understood. It has been reported to act as a modulator of luteal steroidogenesis (Dallenbach-Hellwig \textit{et al.}, 1966) and to increase endometrial secretory activity (Tseng \textit{et al.}, 1987; Zhu \textit{et al.}, 1990). Other work has suggested that it influences decidualized endometrium (Poinsner \textit{et al.}, 1990). This may be mediated via enhanced progesterone expression (Tseng \textit{et al.}, 1992). Alternatively this may be associated with the changing expression of the major endometrial secretory proteins, insulin-like growth factor binding protein-1 (IGFBP-1) and placental protein 14 (PP14). It has been suggested that relaxin influences the production of IGFBP-1 (Thraikill \textit{et al.}, 1990) and that it may be the unidentified ovarian factor involved in the control of PP14 (Johnson \textit{et al.}, 1993; Arthur \textit{et al.}, 1995).

The aim of this study was to evaluate the expression of relaxin during early pregnancy in patients who conceived after differing assisted conception regimes, which were associated with varying corpus luteum activity. We compared these results with those from patients who failed to conceive after these treatments and assessed the relationship between relaxin and IGFBP-1, PP14, progesterone, human chorionic gonadotrophin (HCG) and oestradiol.

Materials and methods

Analyses were made from data collected from 88 patients who had undergone either in-vitro fertilization (IVF) \(( n = 53 )\), or transfer of frozen embryos in hormone-supported cycles \(( n = 23)\) or in natural menstrual cycles \(( n = 12) \). Of these patients, 52 conceived \(( n = 33, 13 \) and six respectively, from the three groups). The IVF treatment involved a long down-regulated protocol with luteal support; 2500 IU HCG was given 1 day prior to, and 2 days after, embryo transfer (Smith \textit{et al.}, 1989). The hormone support protocol prior to transfer of frozen embryos involved down-regulation and the exogenous administration of oestradiol and progesterone to simulate a normal menstrual cycle (Davies \textit{et al.}, 1991). Transfer of frozen embryos from natural cycles was performed 72 h after detecting an endogenous urinary luteinizing hormone surge. Ultrasound was used to follow the number of mature follicles and to count the number of corpora lutea. Patients involved in this study were aged \(< 37\) years at the time of their treatment and they suffered from either tubal \(( n = 60 )\) or male factor \(( n = 28 )\) infertility. All pregnancies progressed uneventfully.

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Table I. Pre-embryo transfer mean serum oestradiol and 12 day post-embryo transfer progesterone, human chorionic gonadotrophin (HCG), relaxin, insulin-like growth factor binding protein-1 (IGFBP-1) and placental protein 14 (PP14) concentrations (± SEM)

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No.</th>
<th>Oestradiol* pmol/l</th>
<th>Progesterone ng/ml</th>
<th>HCG IU/l</th>
<th>Relaxin pg/ml</th>
<th>IGFBP-1 µg/ml</th>
<th>PP14 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVF pregnant</td>
<td>33</td>
<td>5084 (617)</td>
<td>280 (39)</td>
<td>188 (46)</td>
<td>8334*</td>
<td>37*</td>
<td>82*</td>
</tr>
<tr>
<td>IVF non-pregnant</td>
<td>20</td>
<td>4390 (771)</td>
<td>25 (11)</td>
<td>-</td>
<td>28*</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Hormone-supported frozen embryo transfer pregnant</td>
<td>13</td>
<td>1079 (114)</td>
<td>72 (14)</td>
<td>125 (29)</td>
<td>20*</td>
<td>52*</td>
<td>53</td>
</tr>
<tr>
<td>Hormone-supported frozen embryo transfer non-pregnant</td>
<td>10</td>
<td>1082 (130)</td>
<td>62 (19)</td>
<td>-</td>
<td>23*</td>
<td>41</td>
<td>31</td>
</tr>
<tr>
<td>Natural frozen, embryo transfer, pregnant</td>
<td>6</td>
<td>560 (50)</td>
<td>58 (9)</td>
<td>148 (30)</td>
<td>2608*</td>
<td>20*</td>
<td>107</td>
</tr>
<tr>
<td>Natural frozen, embryo transfer, non-pregnant</td>
<td>6</td>
<td>490 (57)</td>
<td>16 (3)</td>
<td>-</td>
<td>62*</td>
<td>17</td>
<td>29</td>
</tr>
</tbody>
</table>

*2 days prior to embryo transfer.
IVF = in-vitro fertilization.

Values with different superscripts were significantly different (P < 0.001, 0.001, 0.001, 0.05 respectively).

Results

Serum samples were taken at 8 and 12 days after embryo transfer. These were analysed for relaxin, progesterone, HCG and the endometrial secretory proteins IGFBP-1 (Wang et al., 1990) and PP14 (Howell et al., 1989). The oestradiol concentration was also measured prior to embryo transfer. The newly-developed relaxin enzyme-linked immunosorbent (ELISA) assay was supplied by Genentech Inc., San Francisco, USA. It had a sensitivity of 20 pg/ml. The interassay coefficient of variation was 11.3% for a mean concentration of 321 pg/ml.

Statistical analysis

Parametric and non-parametric analyses were performed; Student’s t-test and Mann–Whitney U test respectively. When both tests confirmed a significant difference the results were expressed in the more commonly-used parametric format. Correlations were examined by a linear regression analysis.

Results

There was no significant difference in the baseline relaxin concentrations recorded in all patients 8 days after embryo transfer (20, 21 and 23 pg/ml for IVF, hormone supported and natural cycles respectively). Serum relaxin concentrations increased rapidly from day 8–day 12 after embryo transfer in the pregnant IVF and natural frozen cycle patients (Figure 1). Relaxin concentrations, 12 days after embryo transfer, in these pregnant patients were significantly higher than in the pregnant patients receiving hormone support prior to transfer of frozen embryos (P < 0.001, mean concentrations 8334, 2608 and 20 pg/ml respectively; Table I). Relaxin concentrations in the IVF and natural cycle patients who conceived were significantly higher at this time than in those who did not (P < 0.001, mean concentrations 8334 versus 28 and 2608 versus 62 pg/ml respectively; Table I). However the relaxin concentrations in the hormone-supported patients who conceived were not significantly different from those who did not (mean concentrations 20 and 23 pg/ml respectively).

The number of corpora lutea produced by the IVF patients ranged from 3 to 26. The down-regulated patients receiving hormone support had no ovarian follicular or luteal activity and patients in the natural frozen embryo transfer group each had a single corpus luteum. Although the relaxin concentrations of the pregnant IVF patients were significantly higher than the pregant natural cycle group at 12 days after embryo transfer (P < 0.001, mean concentrations 8334 and 2608 pg/ml respectively), there was no relationship between serum relaxin concentrations and the number of corpora lutea seen in these patients (Figure 2).

Those patients who did not conceive after IVF and hormone support treatment had significantly lower relaxin concentrations than the natural cycle non-conceivers 12 days after embryo transfer (P = 0.05, mean concentrations 28, 23 and 62 pg/ml respectively). The pregnant patients receiving hormone support before frozen embryo transfer had significantly higher IGFBP-
reflect a common, probably ovarian, influence on the expression of these proteins. However, we found no correlation between relaxin and PP14 in this or any of the other groups. These results, which show that relaxin is not directly related to IGFBP-1 or PP14 expression in early pregnancy, are consistent with other work that has not identified any such relationship in late pregnancy (Critchley et al., 1994).

Our results confirm previous findings that relaxin concentrations are elevated in stimulated ovarian cycles (Johnson et al., 1994); however, it also shows that these concentrations are not related to the number of corpora lutea produced by these patients. The data collected from the non-pregnant patients, showing higher concentrations of relaxin in natural cycles compared with IVF and hormone-supported cycles, suggest that the multiple corpora lutea produced after exogenous ovarian stimulation display a reduction in relaxin expression activity compared with the single natural cycle corpus luteum. This was confirmed by the similar relaxin concentrations noted in IVF patients who had experienced widely differing responses to ovarian stimulation. Johnson et al. (1994) suggested that this phenomenon was associated with reduced luteal phase gonadotrophin concentrations in these cycles.

In conclusion, a wide range of relaxin concentrations is compatible with a healthy ongoing pregnancy. Serum relaxin is likely to be directly influenced by endogenous HCG or another pregnancy factor. Although relaxin may be involved in a complex series of interactions with oestradiol, PP14, IGFBP-1 and progesterone, there does not appear to be a primary relationship between the expression of these factors.

### Discussion

The rapid increase in relaxin concentrations, between days 8 and 12 after embryo transfer, experienced by our IVF and natural cycle pregnant patients is consistent with the work of others (Norman et al., 1993). This is likely to reflect the response to endogenous HCG production or an, as yet, unrecognized pregnancy factor. The administration of exogenous HCG, as routine luteal support for our IVF group did not enhance or initiate earlier relaxin expression.

Given that relaxin is a product of an active corpus luteum (Johnson et al., 1991), it is not surprising to report that our patients who conceived after down-regulated hormone support and frozen embryo transfer showed no rise in serum relaxin concentrations. However, this does indicate that relaxin is not obligatory for a healthy ongoing pregnancy. Relaxin has been shown to be necessary to enhance endometrial IGFBP-1 expression in vitro (Bell et al., 1991). It is therefore interesting that our patients who conceived after hormone support cycles had higher than normal serum IGFBP-1 concentrations (Table I). We have previously suggested that the source of this IGFBP-1 is hepatic (Arthur et al., 1994). This implies differing control of IGFBP-1 expression within these organs and shows that relaxin is not essential for adequate serum IGFBP-1 concentrations in vivo. Our finding that relaxin did not increase in the early stages of pregnancy in the hormone-supported patients and the previous reports of reduced PP14 production in these patients at this time (Anthony et al., 1991; Arthur et al., 1995) reflect a common, probably ovarian, influence on the expression of these proteins.

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### References


Secretory proteins in IVF


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