Gonadotrophin receptor expression on human granulosa cells of low and normal responders to FSH

P.Thiruppathi1, S.Shatavi1, J.A.Dias2, E.Radwanska1 and J.L.Luborsky1,3

1Department of Obstetrics and Gynecology, Rush Medical College, Chicago, IL and 2Wadsworth Center, New York State Department of Health, Albany, NY, USA

3To whom correspondence should be addressed at: Rush Medical College, 1653 W Congress Parkway, Chicago, IL 66012, USA. E-mail: jluborsk@rush.edu

The aim of this study was to determine if follicle stimulating hormone receptor and luteinizing hormone receptor (FSH-R and LH-R) expression is altered on granulosa cells (GC) of women with low oestradiol responses to FSH. Cells were obtained from mature follicles (>17 mm) following controlled ovarian stimulation. For comparison, chinese hamster ovary (CHO) cells transfected with FSH-R or LH-R were also assessed. FSH-R and LH-R expression were detected by flow cytometry. Receptors were labelled with FSH-R antibodies, or with excess FSH or human chorionic gonadotrophin (HCG) and anti-FSH or HCG antibodies, and compared to multiple controls. Receptor expression on GCs was more heterogeneous than on CHO cells. Gonadotrophin receptor levels on GCs were not correlated with the number of FSH ampoules administered or peak oestradiol response. Low and normal response groups were defined using a ratio of peak oestradiol/number of FSH ampoules. FSH receptor expression was not different on GCs from low and normal responders. However, LH-R expression was higher on GCs of low responders compared to those of normal responders (P = 0.04) suggesting a trend to more advanced luteinization. Access of hormone to follicles was not reduced in low responders. Thus, differences in gonadotrophin receptor expression, hormone binding, and access of hormones to follicles do not appear to account for low oestradiol responses to FSH.

Key words: FSH/LH/low responders/receptors

Introduction

Approximately 20% of women with infertility have an inadequate oestradiol response to gonadotrophin stimulation administered during infertility treatment (Vargyas et al., 1984; Forti et al., 1998). The basis for low responses to gonadotrophin during ovarian stimulation remains an enigma. Although increasing the dose of FSH produces a moderate increase in serum oestradiol and the number of oocytes retrieved in some low responders, the oestradiol response is generally limited (Benadiva et al., 1988; Land et al., 1996; Scott, 1996; Karande et al., 1997). Furthermore, higher doses of FSH do not improve pregnancy outcome even when oestradiol production is increased (Benadiva et al., 1988; Jenkins et al., 1991; Land et al., 1996; Scott, 1996; Karande et al., 1997).

The expression of a hormone receptor and its ability to respond to the hormone is a central determinant of hormone action. Altered receptor expression and function may account for some forms of reduced ovarian function. For example, reduced fertilization is associated with lower LH receptor expression on the surface membrane of granulosa cells (Hill et al., 1987; Enien et al., 1998; Whitman et al., 1988, 1989). In addition, specific mutations in FSH or LH receptors in humans are associated with altered receptor expression or trafficking and varying degrees of reduced reproductive function (Clayton, 1996; Conway, 1996; Gromoll et al., 1996; Arnhold et al., 1997, 1999; Beau et al., 1998; Huhtaniemi and Aittomaki, 1998; Latronico et al., 1998; Simoni et al., 1998; Layman 1999; Levallet et al., 1999). However, there is no systematic information on the expression of FSH or LH receptors on granulosa cells from individuals with low ovarian responses to FSH stimulation.

The aim of this study was to determine if altered cell surface expression of gonadotrophin receptors is associated with low responses to FSH stimulation during ovarian stimulation. The approach was to compare the expression of gonadotrophin receptors as detected by flow cytometry on human granulosa cells from women with low and normal responses to gonadotrophin stimulation.

Materials and methods

Study group

Follicular fluid was collected during oocyte retrieval from 32 women undergoing IVF. The average age of the study group was 34.5 ± 4.6
(mean ± SD) with a range of 27–42 years. The average duration of infertility was 3.3 ± 2.0 (range 1–8 years). The average number of prior intrauterine insemination cycles was 2.6 ± 2.0 (range 0–7). The average number of prior IVF cycles was 1 ± 1.1 (range, 0–4). The primary infertility diagnosis included male factor (n = 12), pelvic factor (n = 5), uterine factor (n = 2), tubal factor (n = 4), ovulatory dysfunction (n = 5) and unexplained (n = 4). All procedures were reviewed and approved by the Institutional Review Board.

Ovarian stimulation included desensitization of the pituitary with GnRH started during the midluteal phase of the preceding cycle. Daily injectable fertility medication included human menopausal gonadotrophin (HMG), urofollitropin, or urofollitropin HP until ovulation. The hormone preparations used did not differ between the two response groups. The average peak oestradiol achieved in response to HMG was 134 ± 605 pg/ml and the average number ampoules of HMG administered was 52 ± 28. Ovulation was induced with 10,000 IU of human chorionic gonadotrophin (HCG) (Profasi®, Serono, Norwell, MA, USA). Oocytes and granulosa cells were obtained 34 h after HCG administration under transvaginal ultrasound guidance from follicles >17 mm in diameter.

**Detection of FSH and LH receptors**

Cell surface receptors were detected on human granulosa cells and on Chinese hamster ovary (CHO) cells transfected with intact human FSH (CHO-FSH-R) or LH (CHO-LH-R) (Ares Advanced Technologies, Randolph, MA, USA) by flow cytometry (De Neubourg et al., 1996, 1998). Transfected CHO cells were used to screen primary and secondary antibodies, determine appropriate antisera dilutions, cell concentrations and incubation times for binding saturation. Furthermore, CHO-FSH-R and CHO-LH-R were used for comparison to human granulosa cells since they are clonally derived and would be less heterogeneous.

CHO-FSH-R and CHO-LH-R were cultured in selection media to 70% confluence as described previously (Kelton et al., 1992). The parental CHO cell line without gonadotrophin receptors (CHO-N) was used as a control. Cells were harvested in Hanks Balanced Salt Solution with low Ca²⁺ and Mg²⁺ (GIBCO, Grand Island, NY, USA) containing 2 mmol/l EDTA (Sigma, St Louis, MO, USA). Human granulosa cells were isolated from follicular fluid of 3–4 follicles and pooled for each patient. Cells were incubated in 10 ml of 2 mmol/l EDTA for 5 min to reduce clumping. Cells were washed and suspended in phosphate buffered saline containing 3% BSA (Sigma) and 0.02% sodium azide (Sigma) (PBS-BA). Azide was included to block receptor internalization (West and Cooke, 1991; Cooke et al., 1992). The average cell yield was 3–10×10⁶ per patient.

A rabbit antiserum to peptide domain R265–S296 of the extracellular domain of the human FSH receptor (Liu et al., 1994), a monoclonal antibody to the human FSH receptor binding domain (Lindau-Shepard et al., 2000), human FSH (NIH) and anti-human FSH (NIH), and HCG (NIH) and anti-HCG (NIH) were used to detect immunoreactive receptor or hormone bound to receptor. Control incubations included substitution of primary antiserum with normal rabbit serum or mouse IgG2b as appropriate (Biosource International, Camarillo, CA, USA). Cells (1×10⁵) were incubated with rabbit anti-FSH receptor antiserum (1:50, 0.2 ml) or monoclonal anti-FSH receptor (5 μg, 0.3 ml). Other wells were pre-incubated with excess FSH or HCG (1 μg/0.1 ml; 60 min, 24°C), washed and the corresponding rabbit antiserum added (FISH antiserum at 1:500; 0.2 ml; HCG antiserum at 1:1000; 0.2 ml for 30 min). All antisera and hormones were diluted in PBS-BA. After washing, mouse monoclonal anti-rabbit-immunoglobulin-fluorescein isothiocyanate (FITC) conjugate, (Sigma) was added (0.1 ml at 1:50) to tubes containing rabbit primary serum, and goat anti-mouse immunoglobulin-FITC conjugate (Sigma) was added (0.1 ml at 1:50) to tubes containing monoclonal primary antibody (30 min, 24°C). The samples were washed and white blood cells were labelled with 5 μl of monoclonal anti-human CD45-phycocerythrin (PE) (Sigma) (30 min, 24°C) (De Neubourg et al., 1996). In the final wash, BSA was omitted from wash buffer and cell pellets were suspended in 0.25 ml of PBS containing 0.02% azide. Red blood cells were lysed and the granulosa cells fixed by addition of 50 μl of Optilyse B (Becton Dickinson) according to the manufacturer’s protocol.

**Flow cytometric analysis**

Flow cytometric analysis was performed on a FACScan (Becton Dickinson) using standard settings: FITC fluorescence (FL1), detector 600 V, compensation 1.1%; PE fluorescence (FL2), detector 496 V, compensation 22.8%. 10,000 cells (events) were counted. Specific binding to receptors was analysed using the CellQuest (Becton Dickinson) program. Lymphocytes labelled with anti-CD45-PE were ‘gated out’ in the dot plot of FL1 versus FL2 before analysis of receptor labelling, and histograms created for the remaining CD45 negative cells. The histograms were evaluated statistically with CellQuest for possible shifts and analysed in relation to the appropriate controls. The geometric mean peak fluorescence was used since histograms were not symmetrical. To calculate specific geometric mean peak fluorescence, control histograms were subtracted from experimental histograms for each receptor label. To calculate the proportion of labelled cells, markers were used to define histograms corresponding to unlabelled and labelled cells in the CD45 negative cells. Unlabelled cells were those that corresponded to fluorescence histograms of control incubations.

**Measurement of FSH and HCG in follicular fluid**

The follicular fluid was centrifuged to remove cells and debris and frozen (–20°C) until assayed. FSH was measured with an immunosay kit (Medix; Genzyme, CA, USA) with inter- and intra-assay coefficients of variation of 3.1 and 3.3% respectively. HCG was measured with a sandwich immunoassay (Medix; Genzyme) for intact HCG. The inter- and intra-assay coefficients of variation were 7.9 and 6.2% respectively.

**Statistical analysis**

Statistical differences between experimental and control incubations, or between low and normal responders, were assessed with the unpaired Student’s t-test assuming unequal variance. The Kolmogorov–Smirnov test was also used for analysis of histogram similarity (Young, 1977). Linear regression and Pearson’s correlation analysis was used to compare receptor levels and the dose of gonadotrophin, and follicular fluid levels of FSH or HCG. Power analysis based on comparison of specific peak fluorescence of low responders to normal responders showed 80% power to detect a difference in peak fluorescence for α = 0.05.

**Results**

**FSH and LH receptor on CHO cells**

The typical fluorescence histogram of gonadotrophin receptors detected on the surface of CHO cells transfected with gonadotrophin receptor showed a single, relatively symmetrical peak (Figure 1). Immunoreactive FSH receptor detected with either rabbit anti-FSH receptor antiserum or monoclonal anti-FSH receptor antibody showed significantly different from control incubations (Figure 1A,B) (P < 0.001). Controls included normal rabbit serum, antigen (peptide 265–296) adsorbed
antiserum and CHO-N without receptor or a non-relevant mouse immunoglobulin of the same class. There was no difference among the controls since the control histograms were indistinguishable when overlaid on the same plot. The average proportion of cells labelled with rabbit anti-FSH receptor was 83.4 ± 12% or monoclonal anti-FSH receptor was 95.4 ± 3.0%.

Detection of receptor with receptor antibodies does not address the ability of receptor to bind hormone. Therefore, hormone binding was also investigated. FSH bound to receptors of FSH/LH receptors on granulosa cells of low responders antiserum and CHO-N without receptor or a non-relevant address the ability of receptor to bind hormone. Therefore, hormone binding was also investigated. FSH bound to receptorsmouse immunoglobulin of the same class. There was no difference among the controls since the control histograms on CHO-FSH-R was signi significantly different from controls (Figure 1C) (P < 0.001). The peak fluorescence observed with FSH + FSHβ antiserum was lower than that seen with antibodies to FSH receptor (106 versus 181 respectively). The average proportion of cells labelled with bound FSH was 60.9 ± 14.7%. HCG bound to LH receptor on CHO-LH-R cells was significantly different from controls (Figure 1D) (P < 0.001). Controls consisted of omission of hormone, substitution of normal rabbit serum for anti-hormone antiserum, and use of parental CHO cells without receptor. There was no difference among the control histograms. The average proportion of CHO-LH-R cells labelled with bound HCG was 83.7 ± 16.7%.

**FSH and LH receptors on human granulosa cells**

In the study group, the peak serum oestradiol concentration reached in response to FSH ranged from 564–2714 pg/ml (average 1341 ± 605 pg/ml). The number of ampoules of FSH ranged from 16–120 (average 52 ± 28). The number of oocytes retrieved ranged from 2–34 (average 19.5 ± 8.3), and the number of mature oocytes ranged from 0–17 (average 9 ± 4). Thus the study group was composed of individuals with low to normal oestradiol responses to FSH and did not contain non-responders.

Cells retrieved at oocyte retrieval contained 16.2 ± 9.7% (range 1.6–28.6%) CD45 labelled lymphocytes. The peak fluorescence for labelled receptors on granulosa cells (CD45 negative cells) compared to controls was significantly higher for rabbit FSH receptor antiserum (P = 0.006), monoclonal FSH receptor antibody (P = 0.045) and anti-HCG antiserum (P = 0.0007). For anti-FSH antiserum only 11/21 samples showed significant FSH binding (P = 0.03) compared to control rabbit serum. However, FSH binding was correlated with FSH receptor detected with rabbit anti-FSH receptor antiserum or monoclonal anti-FSH receptor antibody (P = 0.004 and P = 0.001 respectively). The lower FSH binding could be due either to less efficient detection of low receptor concentrations, or that not all receptors on the plasma membrane bind hormone. The average specific peak fluorescence for FSH receptor was similar for rabbit anti-FSH receptor (180 ± 90) and monoclonal anti-FSH receptor (124 ± 15), and FSH + anti-FSHβ (136 ± 88). The proportion of labelled

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**Figure 1.** FSH receptor (FSH-R) expression on CHO cells transfected with hFSH-R as detected by flow cytometry. Fluorescence histograms of (A) immunoreactive FSH receptor detected with a rabbit anti-FSH-R antiserum (FSHR A/S) compared to two controls, normal rabbit serum (NRS) and FSH-R antiserum adsorbed with peptide antigen (FSHR A/S + Peptide) at the same dilution. (B) Immunoreactive FSH receptor detected with a monoclonal antibody (FSHR/MAB) compared to control immunoglobulin (IgG2b). (C) Bound FSH detected with anti-FSH antiserum compared to control cells incubated similarly but without FSH. (D) Bound HCG detected with anti-HCG antiserum compared to normal rabbit serum. Autofluorescence (AF) is shown for reference.

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cells for FSH receptor was similar for rabbit anti-FSH receptor (72.2 ± 26.3% labelled) and monoclonal anti-FSH receptor (66.7 ± 22.6% labelled), FSH + anti-FSHβ (57.9 ± 30.0% labelled). The average specific peak fluorescence for LH receptors was 108 ± 45 and the proportion of labelled cells was 81.2 ± 25.0%. When the relationship between study group responses and FSH receptor expression on granulosa cell surfaces was assessed as a continuum, there was no correlation between the FSH receptor and the number of ampoules of FSH (P = 0.8) or the peak oestradiol response (P = 0.6).

Comparison of gonadotrophin receptor expression on granulosa cells of low and normal responders

A low oestradiol response was defined initially as a peak oestradiol value below 1000 pg/ml at HCG administration, since it is often the primary clinical criterion. By this definition, some individuals with low total oocytes, low rates of fertilization and those requiring high doses of FSH were classified as normal. Also, the number of ampoules of FSH administered did not differ significantly between low and normal responders (P = 0.76). Furthermore, a relationship between the dose of FSH and serum oestradiol in normal responders was not apparent (correlation = −0.23, P = 0.34).

The number of FSH ampoules or the peak oestradiol concentrations alone are incomplete indicators of response. In order to integrate the FSH dose and the oestradiol response, the ratio of the peak oestradiol/number of ampoules was explored as another definition. An empirical threshold value of 42 was used to differentiate between low and normal responders. The cut-off value represented the upper limit of the 95% confidence interval of the mean ratio of peak oestradiol/ampoules (range, 7–89). When the ratio of peak oestradiol/number of ampoules of FSH was used to separate the study group, there were 21/32 low and 11/32 normal responders (Table I). A significant correlation between the number of FSH ampoules administered and peak serum oestradiol was apparent for normal responders (correlation = 0.74, P = 0.009), but not low responders (correlation = 0.24, P = 0.29). Furthermore, individuals with a low number of oocytes retrieved were included in the low responder group. This method of defining low responses was more consistent with resistance to FSH and was used to define response groups.

Examples of fluorescence histograms for FSH or LH receptor on granulosa cells are shown in Figure 2. Individual histograms were less uniform than for transfected CHO cells and there was a wide range of peak values and proportion of labelled cells between individuals. There was no significant difference in the average peak fluorescence value for FSH receptor between low and normal responders, regardless of the receptor label (P > 0.5) (Figure 3 and Table II). In addition, a similar proportion of cells from normal (60–70%) and low responders (57–71%) expressed FSH receptor regardless of the detection reagents (P > 0.6) (Table II). The average peak fluorescence value for LH receptor was higher and had a wider range in the low response group (215 ± 167; range, 50–619) compared to the normal response group (144 ± 101; range, 57–381) (Figure 3 and Table II). As seen in Figure 3, the box plot analysis identified an extreme outlier in the normal responder group. The difference in LH receptor level was not significant (P = 0.2) unless the extreme outlier was eliminated (115 ± 52; range 57–212) (P = 0.04). The proportion of granulosa cells expressing LH receptor was similar in low responders (77 ± 26%; range 33–99%) and normal responders (86 ± 24%; range, 28–99%) (P = 0.27). The proportion of cells labelled became significant when the extreme outlier in the normal responder difference in the group was omitted (96.1 ± 5.9; range 83–99%) (P = 0.014).

FSH and LH in follicular fluid

Another component of the response to FSH is the ability of FSH and HCG to reach the follicle. The average FSH level in follicular fluid was highly correlated with the number of ampoules of FSH administered (correlation coefficient 0.71, P < 0.001) (Figure 4). The level of FSH in follicular fluid of low responders (30.9 ± 23.8 mIU/ml) was significantly higher than FSH in follicular fluid of normal responders (11.9 ± 9.1 mIU/ml) (P = 0.002), consistent with the higher doses of FSH administered to low responders (Table I). The average concentration of HCG in follicular fluid did not differ between low and normal responders (P = 0.84) (Table I),

<table>
<thead>
<tr>
<th>Table I. Comparison of normal and low responders</th>
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<td>Normal n = 11</td>
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<tr>
<td>---------------</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Peak oestradiol (pg/ml)</td>
</tr>
<tr>
<td>Ampoules</td>
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<tr>
<td>FF FSH (mIU/ml)</td>
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<tr>
<td>FF HCG (mIU/ml)</td>
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<tr>
<td>Oocytes (total)</td>
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<tr>
<td>Oocytes (mature)</td>
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</table>

Comparison of patient information for normal and low responders. Responses were defined by the ratio of peak oestradiol/number of ampoules (range, 7–89). Low responses were those with a ratio below 42, and included women with low oocyte retrieval and no mature eggs. The peak serum oestradiol concentration, the number of ampoules of FSH administered and follicular fluid (FF) FSH concentrations were significantly different between normal and low responders.
consistent with the equivalent dose of HCG administered to all individuals.

**Discussion**

This study shows that reduced oestradiol responses to gonadotrophin do not appear to be associated with reduced expression of gonadotrophin receptors on human granulosa cells. FSH receptor expression was similar and LH receptor expression was higher on granulosa cells of low responders, compared to normal responders. Gonadotrophin receptor expression has been demonstrated on human granulosa cells (Polan et al., 1984, 1986; Hill et al., 1987; Whitman et al., 1988, 1989; Lane and Chen, 1991) and on cells transfected with gonadotrophin receptors (Babu et al., 2000; Liu et al., 2000; Mann et al., 2000; Nechamen and Dias, 2000). However, this is the first report comparing gonadotrophin receptor expression on granulosa cells from women with different oestradiol responses to FSH.

Although LH receptor expression was higher, LH receptor appeared to be expressed on fewer cells in low responders compared to normal responders. The altered expression and distribution of LH receptor could reflect altered processing and cell surface trafficking of LH receptor. Whitman and co-workers showed reduced internalization of HCG by granulosa cells from follicles with poor fertilization and embryo cleavage (Whitman et al., 1988, 1989). Another interpretation is that cells of low responders are more luteinized. Accelerated luteinization has been suggested in association with ovarian aging (Klein et al., 1996, 1998; Ubaldi et al., 1996; Anasti et al., 1998). The wide range of LH receptor levels suggests that there may be a subgroup of low responders with dysregulated LH receptor expression. This subgroup would be better differentiated in a larger study.

Since there is a gradient of FSH and LH receptor expression on granulosa cells in maturing follicles, uniformity of cell labelling was not expected (Visintin and Luborsky, 1989; Camp et al., 1991; Yamoto et al., 1992; Minegishi et al., 1997; Oktay et al., 1997; Tukao et al., 1997). The shape of the fluorescence histogram for CHO cells transfected with receptor is consistent with a relatively uniform cell surface expression of gonadotrophin receptor on CHO cells. In contrast, the width and asymmetry of the fluorescent histogram corresponding to receptors on granulosa cells is consistent with a large variation in the expression of gonadotrophin receptor among individual granulosa cells. The variation is probably not due to variations between follicles that were pooled since a similar result was obtained with individual follicles in preliminary experiments.

The peak values of receptor detected on transfected CHO cells and human granulosa cells were similar. However, the

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**Figure 2.** Gonadotrophin receptor expression on human granulosa cells as detected by flow cytometry. Cells were obtained during oocyte retrieval for IVF. Normal and low responses were defined by the ratio of peak oestradiol/number of ampoules. White blood cells labelled with anti-human CD45 were gated out during histogram analysis. M1 = marker corresponding to unlabelled cells and M2 = marker corresponding to labelled cells. Only one FSH receptor label is shown (anti-FSH receptor antiserum), since similar results were obtained among the different FSH receptor labels. LH receptor (LH-R) was detected with excess HCG (1 µg) and anti-HCG antiserum. An example of granulosa cells of normal responders showing (A) FSH-R and (B) LH-R. An example of granulosa cells of low responders showing (C) FSH-R and (D) LH-R. The differences between histograms are consistent with variations among cells from one individual to another, rather than between response groups.
Figure 3. Comparison of gonadotrophin receptor expression on granulosa cells of normal and low responders. Box and whiskers plots show the median (horizontal dark line), 25 to 75th percentile values (box), range of values (whiskers), outliers (○) and extreme outliers (*). Response groups were defined as the ratio of peak oestradiol/number of FSH ampoules. (A) Relative levels of FSH receptor (FSH-R) and LH receptor (LH-R) expression. FSH-R did not differ between normal and low responders ($P > 0.5$). LH-R expression was significantly different between the two groups when the extreme outlier was removed from the normal response group ($P = 0.04$). (B) Proportion of cells expressing FSH receptor (FSH-R) and LH receptor (LH-R). The number of cells expressing FSH-R was similar between normal and low responders ($P > 0.6$). The slightly lower number of cells expressing LH-R from low responders was not significant ($P > 0.27$) unless the extreme outlier identified in the normal responder group was omitted ($P = 0.014$).

Table II. Gonadotrophin receptor expression on granulosa cells of normal and low responders (mean ± SD).

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Label</th>
<th>Peak fluorescence</th>
<th>Percentage cells labelled</th>
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<tr>
<td></td>
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<td>Normal Low $P$ value</td>
<td>Normal Low $P$ value</td>
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<tr>
<td>FSH</td>
<td>Rabbit antiserum</td>
<td>235 ± 131</td>
<td>74.5 ± 30.9</td>
</tr>
<tr>
<td>FSH</td>
<td>Monoclonal antibody</td>
<td>177 ± 88</td>
<td>70.1 ± 18.4</td>
</tr>
<tr>
<td>FSH</td>
<td>FSH + anti FSHβ</td>
<td>207 ± 204</td>
<td>60.2 ± 26.4</td>
</tr>
<tr>
<td>LH</td>
<td>HCG + anti HCG</td>
<td>115 ± 521</td>
<td>96.1 ± 5.9</td>
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</table>

Comparison of gonadotrophin receptor expression on granulosa cells of normal and low responders was defined by the ratio of peak oestradiol/number of ampoules. The average geometric mean peak fluorescence and the percentage of labelled cells are shown. There was no difference in FSH receptor expression between low and normal responders. For LH receptor both the difference in geometric mean peak fluorescence and the proportion of cells labelled became significantly different when the extreme outlier, which was identified in statistical analysis in the normal group, was omitted (see text and Figure 3).
et al. expressed on the cell surface (Grogan et al., 1990). Although the values of the peak fluorescence for labelled receptors are similar between granulosa cells and CHO cells, the number of receptors/cell was not expected to be the same, particularly in this case where a strong promoter was used to express FSH receptor in CHO cells (Kelton et al., 1992). Since the cell size and density of receptor is likely to differ between transfected CHO cells and luteinizing human granulosa cells, direct comparison of receptor levels is probably not valid. Nonetheless, it is valid to compare gonadotrophin receptors labelled with the same antibody on granulosa cells from women with low and normal oestradiol responses to FSH.

Although FSH receptor expression was similar on granulosa cells of low and normal responders, there may be alterations in functional capacity of the granulosa cells, or in the ability of receptors to translate hormone binding into intracellular signals. The in-vitro response of human granulosa cells to FSH has been reported to differ between low and normal responders. For example, cultured granulosa cells from low responders secreted less basal oestradiol, but responded to FSH with a greater increase in aromatase activity and oestradiol than higher responders (Hurst et al., 1992). Also, basal inhibin B secretion is lower from cultured human granulosa cells of women with low ovarian reserve (based on day 3 FSH) than from cells of women with normal ovarian reserve (Seifer et al., 1996). However, since the expression of LH receptor is dependent on appropriate FSH receptor activity, the presence of LH receptor suggests that FSH receptors are functionally intact in low responders.

Altered blood flow has also been suggested as a basis for low oestradiol responses (Pellicer et al., 1994, 1998). Reduced blood flow has also been observed in unexplained infertility by Doppler ultrasonography (Battaglia et al., 1998). VEGF is a marker of angiogenesis (Ferrara et al., 1998) and low VEGF levels in follicular fluid have been associated with poor conception rates (Doldi et al., 1997; Friedman et al., 1998). In the current study, blood flow was not measured directly. However, follicular fluid concentrations of FSH were correlated with the dose of FSH administered. Thus, low oestradiol responses were not explained by a reduced ability of FSH to reach mature follicles. Similarly, HCG concentrations were similar in follicular fluid of normal and low responders consistent with the equivalent dose of FSH administered.

An association between resistance to FSH and LH and various mutations in the FSH receptor and LH receptor has been described (Conway, 1996; Layman, 1999). For example, a homozygous microdeletion of helix 7 of the LH receptor in a woman with ovarian resistance was associated with low cell surface expression of LH receptor. The available surface LH receptor bound HCG but was unable to activate the Gs subunit of adenylate cyclase (Latronico et al., 1998). In addition, allelic variations in FSH receptor at position 680 were reported to predict the number of FSH ampoules needed for successful stimulation of follicles (Perez Mayorga et al., 2000). The similarity of gonadotrophin receptor expression observed in this study does not rule out receptor polymorphism or genetic mutations in receptor signalling as a basis for variations in response to gonadotrophin.

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