Hormonal characteristics of follicular fluid from women receiving either GnRH agonist or hCG for ovulation induction

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BACKGROUND: A recent prospective randomized study from our group compared GnRH agonist (0.5 mg buserelin) and hCG (10 000 IU) for triggering of ovulation following a flexible antagonist protocol. The agonist group showed a poor reproductive outcome despite luteal phase support with progesterone and estradiol (E2). In the present prospective observational study, the health status of follicles from the above study was monitored by analysing the hormonal content of frozen/thawed follicular fluid samples. The aim was to test whether the poor reproductive outcome could be related to a defective pre-ovulatory follicular maturation resulting in oocytes with a compromised developmental competence. METHODS: Hormone concentrations were measured in two individual follicular fluid samples from each of 32 women receiving buserelin and 37 receiving hCG, thus representing a subset of the follicles retrieved. RESULTS: Follicular fluid levels of LH in the agonist group as compared with the hCG group was 11.1 ± 0.5 versus 3.6 ± 0.3 IU/l (mean ± SEM; P < 0.001); FSH, 6.3 ± 0.6 versus 3.3 ± 0.2 IU/l (P < 0.001); hCG, not determined versus 139±8 IU/l; E2, 1.9 ± 0.2 versus 1.8 ± 0.2 μmol/l (P > 0.10); progesterone, 70 ± 4 versus 93 ± 6 μmol/l (P < 0.001); inhibin-A, 36.9 ± 3.1 versus 37.1 ± 2.5 ng/ml (P > 0.10) and inhibin-B, 35.6 ± 2.8 versus 40.1 ± 3.1 ng/ml (P > 0.10). Thus, pronounced hormonal differences exist in follicular fluid, and the collective concentration of all three gonadotropins and the follicular fluid concentration of progesterone were much higher in the group of women receiving hCG for ovulation induction. CONCLUSION: The study suggests that GnRH agonist results in proper pre-ovulatory follicular maturation, but the ovulatory signal – probably in synergy with the resulting pituitary down-regulation – is too low to support appropriate corpus luteum (CL) function.

Key words: follicular fluid/GnRH agonist/IVF/oocyte maturation/ovulation induction

Introduction

GnRH agonists have been used to create an endogenous flare-up of gonadotropins to induce ovulation in women who are without pituitary down-regulation. In contrast to hCG, the GnRH agonist-induced surge resembles the natural mid-cycle surge of gonadotropins and exposes follicles to both types of gonadotropins (Gonen et al., 1990; Imoedemhe et al., 1991). However, the GnRH agonist-induced surge is blunted as compared with the natural mid-cycle surge of gonadotropins being shorter in duration and amplitude (Hoff et al., 1983; Itskovitz et al., 1991).

The ovulatory surge induces two basic events within the follicle: oocyte maturation and the ovulatory process itself including transition of follicular cells to form a functioning corpus luteum (CL). There is evidence to suggest that these two events are activated at different levels of gonadotropins (Andersen et al., 1999). Oocyte maturation requires a much lower level of gonadotropins than ovulation itself. Furthermore, each of the two gonadotropins can substitute for one another in these processes as demonstrated in animal and primate models. Therefore, stimulation of ovulation seems to depend more on the total amount of gonadotropin rather than the actual gonadotropic hormone itself (Andersen et al., 1999). Thus, the attenuated surge induced by GnRH agonist could affect oocyte maturation differently than that of an hCG bolus.

In a recent prospective randomized study in women following a flexible antagonist protocol, patients received either hCG or GnRH agonist (buserelin) for ovulation induction (Humaidan et al., 2005). From this study, two main results emerged: (i) the frequency of fully mature oocytes (i.e. metaphase II oocytes) was significantly increased in the agonist group; (ii) the clinical
pregnancy rate was so poor in the agonist group such that the trial had to be discontinued before planned. An independent prospective randomized study has recently reached similar results (Kolibianakis et al., 2005). Both studies provided luteal phase support consisting of both progesterone and estradiol (E$_2$), in the first study until the pregnancy test was performed around 2 weeks following oocyte retrieval (OR) and in the study by Kolibianakis et al. (2005) until 7 weeks of pregnancy. It was suggested that the poor reproductive outcome in the agonist group was caused by pituitary down-regulation resulting in an insufficient stimulation and functioning of the CL – following the initial flare-up effect. This notion was supported by lower circulating levels of progesterone in the agonist group right from the day of OR until pregnancy testing (Chandrasekher et al., 1994; Humaidan et al., 2005; Kolibianakis et al., 2005). However, the poor reproductive outcome could also be related to the blunted mid-cycle gonadotropin surge in the agonist group leading to an insufficient pre-ovulatory follicular maturation and oocytes with a reduced pregnancy potential. The aim of the present study was to evaluate the latter possibility by analysing the hormonal profiles of follicular fluid from the two groups of the above study (Humaidan et al., 2005).

Materials and methods
Patients and hormonal treatment
Details on patient characteristics and the hormonal treatment given have previously been published (Humaidan et al., 2005). In brief, a total of 122 normogonadotrophic women treated with IVF or ICSI were prospectively randomized to receive either hCG or GnRH agonist for ovulation induction (discussed below). The present study included a subgroup of 69 women from which follicular fluid was analysed. This subgroup of women was similar to that of the original study. Each patient contributed with only one cycle. Inclusion criteria were as follows: (i) female aged 25–40 years; (ii) baseline FSH and LH below 12 IU/l; (iii) menstrual cycles between 25 and 34 days; (iv) BMI between 18 and 30 kg/m$^2$; (v) both ovaries present and (vi) absence of uterine abnormalities. Ovarian stimulation consisted of recombinant human FSH (r-hFSH) (Puregon; Organon, Skovlunde, Denmark) from cycle day 2 and continued until the day of ovulation induction. A fixed dose of r-hFSH was used, either 150 IU of hCG s.c. (Pregnyl; Organon) were randomized to ovulation induction with either a single bolus of LH and FSH were monitored using commercially available RIA kits (DSL-43100 & DSL-3400; Diagnostic System Laboratories). E$_2$ and progesterone were measured using commercially available RIA kits (DSL-4700 & DSL-4600; Diagnostic System Laboratories). Samples for both assays were diluted 1 : 1000 in steroid-free serum just before measurement. Inhibin-B and inhibin-A were measured using a specific enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (The Oxford Bio-innovation kit; Biotech-IgG, Copenhagen, Denmark). Before measurement, follicular fluid samples were diluted to 1 : 250 (inhibin-B) and 1 : 200 (inhibin-A) in serum obtained from a pool of five post-menopausal women (who had no inhibin-B and no inhibin-A activity), whereas serum samples were tested undiluted. The follicular fluid were pretreated with sodium dodecyl sulphate (SDS), heated and exposed to hydrogen peroxide before they were applied to the wells of the plate and incubated overnight at room temperature. Subsequently, the plates were washed and incubated with detection antibody for 3 h at room temperature. Substrate solution was applied and incubated for 1 h. The amplifier solution was added, and the plates were read with an ELISA reader at 490 nm with its reference at 620 nm [coefficient of variation (CV) <10% and <7% for inhibin-A and inhibin-B, respectively].

Statistics
The results were analysed using Student’s t-test and least square linear regression analyses using SPSS 12 (SPSS Inc., Chicago, IL, USA) where appropriate.

Results
Follicular fluid content of gonadotropins and follicular-derived hormones
In women receiving GnRH agonist for ovulation induction, concentrations of gonadotropins in follicular fluid revealed the presence of twice as much FSH and almost four times more LH as compared with the hCG group confirming the occurrence of an endogenous release of gonadotropin in response to GnRH agonist triggering (Table I). However, the total gonadotropin exposure of follicles in the group of women who received hCG for ovulation induction was almost one order of magnitude...
Hormonal concentrations in follicular fluid from women receiving either GnRH agonist or hCG for ovulation induction related to outcome of the pregnancy test.

Table I. Hormonal concentrations in follicular fluid from women receiving either GnRH agonist or hCG for ovulation induction (mean ± SEM (range))

<table>
<thead>
<tr>
<th></th>
<th>GnRH agonist group</th>
<th>hCG group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of follicular fluid samples</td>
<td>64</td>
<td>74</td>
</tr>
<tr>
<td>Number of women</td>
<td>32</td>
<td>37</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>6.3 ± 0.6* (0.4–5.7)</td>
<td>3.3 ± 0.2* (0.4–5.7)</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>11.1 ± 0.6* (0.4–5.7)</td>
<td>3.6 ± 0.3* (0.4–5.7)</td>
</tr>
<tr>
<td>hCG (IU/l)</td>
<td>ND</td>
<td>139 ± 8</td>
</tr>
<tr>
<td>Estradiol (μmol/l)</td>
<td>1.9 ± 0.2 (0.4–5.7)</td>
<td>1.8 ± 0.2 (0.2–6.3)</td>
</tr>
<tr>
<td>Progesterone (μmol/l)</td>
<td>70 ± 4* (6.4–172)</td>
<td>94 ± 5* (16–289)</td>
</tr>
<tr>
<td>Inhibin-B (ng/ml)</td>
<td>35.6 ± 2.8 (5–120)</td>
<td>40.1 ± 3.1 (5–122)</td>
</tr>
<tr>
<td>Inhibin-A (ng/ml)</td>
<td>36.9 ± 3.1 (5–116)</td>
<td>37.1 ± 2.5 (12–114)</td>
</tr>
</tbody>
</table>

ND, not determined.
*P < 0.001.

Higher than in the GnRH agonist group because levels of hCG in follicular fluid on average reached 140 IU/l (Table I). Despite the relatively large range of concentrations of E2, progesterone, inhibin-B and inhibin-A in both groups, mean levels of E2, inhibin-B and inhibin-A were similar between the two groups, whereas the concentrations of progesterone were significantly higher in the group who had a hCG-induced surge.

**Serum concentrations of inhibin-B**

On the day of ovulation induction, concentrations of inhibin-B were similar between the two groups (659 ± 83 pg/ml and 750 ± 64 pg/ml for the GnRH agonist and the hCG groups, respectively) and also on the day of OR (252 ± 28 pg/ml and 278 ± 20 pg/ml, respectively).

**Achievement of a positive pregnancy test related to follicular fluid concentrations of hormones**

Of the analysed follicular fluid samples, 10 women in the agonist group and 15 women in the hCG group achieved a positive pregnancy test, whereas 22 in either group did not. The average hormonal concentration for those who did and did not have a positive pregnancy test for each of the two groups is summarized in Table II. In the agonist group, the average follicular fluid progesterone concentration was significantly higher in women with a negative pregnancy test. Levels of inhibin-A were similar but close to significance (P = 0.068) and inhibin-B levels remained similar. In the hCG group, FSH and LH concentrations were significantly lower in women who had a positive pregnancy test, whereas that of hCG remained similar.

**Discussion**

The present study shows that ovulation induction with either GnRH agonist or hCG induces profound differences in the milieu of pre-ovulatory follicles retrieved in connection with infertility treatment. Thirty-six hours after ovulation induction, the follicular fluid content of gonadotropins differed greatly between the two groups. Because of the endogenous release of gonadotropins, FSH and LH were present in concentrations almost twice as high in the agonist group as in the hCG group. HCG was absent in follicular fluid from the agonist group, but in the hCG group, the reduced levels of FSH and LH were compensated for by high levels of hCG, which were more than one order of magnitude higher than levels of FSH and LH in the agonist group. The enhanced levels of FSH and LH in follicular fluid from the agonist group confirm an actual circulatory peak between the GnRH agonist injection and OR, but the average concentrations are unlikely to have surpassed those of hCG at any moment in time. It is well known that LH-like activity induces both ovulation and oocyte maturation without the presence of FSH. However, a large bolus of FSH, without LH activity, also induces ovulation and oocyte maturation as shown in several animal species (Schenken et al., 1984). Therefore, the present study demonstrates that the ovulatory signal, evaluated as the collective content of gonadotropins in follicular fluid, was much stronger in the hCG group as compared with the agonist group.

In the hCG group, the progesterone content in follicular fluid was significantly increased and almost 25% higher as compared with the agonist group confirming earlier findings.

Table II. Hormonal concentrations in follicular fluid from women receiving either GnRH agonist or hCG for ovulation induction related to outcome of the pregnancy test (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>GnRH agonist group</th>
<th>hCG group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of follicular fluid samples</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>11.1 ± 0.7 (11–1.1)</td>
<td>11.1 ± 0.6 (11–1.1)</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>6.3 ± 0.6 (0.4–5.7)</td>
<td>6.8 ± 0.2 (0.4–5.7)</td>
</tr>
<tr>
<td>hCG (IU/l)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Estradiol (μmol/l)</td>
<td>1.9 ± 0.2 (0.4–5.7)</td>
<td>1.9 ± 0.9 (0.4–5.7)</td>
</tr>
<tr>
<td>Progesterone (μmol/l)</td>
<td>72 ± 5* (48–58)</td>
<td>96 ± 7 (90–6)</td>
</tr>
<tr>
<td>Inhibin-B (ng/ml)</td>
<td>35.3 ± 3.3 (26–3.3)</td>
<td>36.7 ± 5.5 (26–5.5)</td>
</tr>
<tr>
<td>Inhibin-A (ng/ml)</td>
<td>40.7 ± 4.3** (35–4.3)</td>
<td>28.3 ± 2.4** (28–2.4)</td>
</tr>
</tbody>
</table>

ND, not determined.
*P < 0.01 and **P = 0.068.
(Andersen et al., 1993). The difference in follicular progesterone output between the two groups as observed in the present study was also reflected in circulatory concentrations. These data confirm that the ovulatory signal in the hCG group was stronger than in the agonist group. In contrast to these findings, the average intrafollicular concentrations of inhibin-B, inhibin-A and E2 remained similar between the two groups and seemed unrelated to whether or not a positive pregnancy test was achieved. Whereas secretion of inhibin-B, inhibin-A and E2 is related to FSH and LH stimulation, progesterone output is mainly dependent on LH stimulation (Welt et al., 2001). Thus, it may be that follicles of the agonist group selectively lack LH stimulation in order for a complete follicular luteal transition. However, the collective stimulation with FSH and LH of the agonist group may still be sufficient to secure and maybe even surpass the efficiency by which pre-ovulatory follicles mature and undertake oocyte maturation.

Taken together, the present study confirms the adequacy of GnRH agonist to elicit a signal capable of securing proper pre-ovulatory follicular maturation, but the signal – probably in synergy with the resulting pituitary down-regulation of gonadotropin secretion – is too low to support proper CL function. A defective CL function is, therefore, the likely cause of the poor reproductive outcome observed in connection with the use of GnRH agonist for ovulation induction (Fauser et al., 2002; Beckers et al., 2003; Humaidan et al., 2005; Kolibianakis et al., 2005). Thus, further studies should focus on rescuing the CL following ovulation induction with an agonist, maybe with concomitant administration of small amounts of hCG.

Women who achieved a positive pregnancy test in the agonist group showed significantly lower progesterone levels and borderline significant lower inhibin-A levels as compared with those with a negative test. This seems to support that implantation is less related to the intrafollicular hormonal milieu at oocyte collection and more to the hormone profiles of the actual luteal phase. Thus, failure of embryos to implant from the agonist group is likely to be dependant on an inadequate CL stimulation of the endometrium and points rather at an improper CL function than a defective process of oocyte maturation.

Embryos frozen in connection with the actual treatment cycle will be replaced in another cycle and provide a possibility to evaluate a possible defective implantation potential. Only limited data are available from the present study, but the embryos seem to perform equally well in the two groups: in the agonist group, three women received replacement of frozen/thawed embryos, two received a positive pregnancy test and one patient received clinical pregnancy. In the hCG-group, 10 patients received replacement, four received positive pregnancy tests and two clinical pregnancies.

The increased frequency of metaphase II oocytes in the agonist group as compared with the hCG group was monitored based on those oocytes denuded for ICSI because removal of cumulus cells allowed identification of a first polar body with certainty (Humaidan et al., 2005). However, the fertilization procedure (i.e. IVF or ICSI) was totally unrelated to the maturational status of the oocyte, which reflected conditions that the oocyte experienced within the follicle before retrieval. Consequently, we find it reasonable to assume that the figure of metaphase II oocytes is applicable to all oocytes and indeed also the subset of follicles analysed in the present study. However, conclusions from the present study are weak, because no direct correlation between individual follicular fluid hormonal data and the maturational status of the oocyte can be established. In particular, the difference in follicular fluid FSH content may warrant further investigations to evaluate a possible positive effect of FSH on human oocyte maturation.

The LH concentrations in follicular fluid from the agonist group were almost four times higher than in the circulation, whereas the reverse was the case for FSH. It seems that LH within the follicle escapes a fast clearance and shows that circulatory levels do not necessarily reflect the concentrations that the granulosa cells are exposed to. Furthermore, the relative high LH concentrations in follicular fluid may stimulate the LH receptors recently described in the oviduct and the uterus (Ascoli et al., 2002; Ziecik et al., 2005) in connection with ovulation in which the follicular fluid is expelled to the surrounding tissue. It may be speculated that activation of these LH receptors optimizes fertilization and possible implantation.

In conclusion, pronounced differences exist in follicular fluid hormonal profiles when ovulation is induced with either hCG or GnRH agonist. However, ovulation induction with GnRH agonist produces a gonadotropin signal capable of securing proper pre-ovulatory follicular maturation and release of mature oocytes, but the signal – probably in synergy with the resulting pituitary down-regulation – is too low to support proper CL function. Thus, an inappropriate CL function is the most likely explanation of the poor reproductive outcome using GnRH agonist for triggering ovulation in women following a GnRH antagonist protocol.

Acknowledgements

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References


Hormonal characteristics of follicular fluid

2129


