The effect of recombinant LH on embryo quality: a randomized controlled trial in women with poor ovarian reserve

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BACKGROUND: Poor ovarian response is a common clinical problem, affecting up to 26% of IVF cycles. For these women, addition of recombinant luteinizing hormone (rLH) to ovarian hyperstimulation with recombinant FSH has a beneficial effect on ongoing pregnancy rates, but its effect on the yield of top-quality embryos is unknown.

METHODS: We conducted a randomized controlled trial in women expected to respond poorly under ovarian hyperstimulation during their first IVF cycle [all women aged 35–41 and women with FSH > 12 IU/ml and antral follicle count (AFC) ≤ 5]. Women were randomly allocated to rFSH and rLH (2:1 ratio) or rFSH alone (control group) after down-regulation with a GnRH agonist. The primary outcome was the proportion of top-quality embryos per woman on the day of transfer. Secondary outcomes were the number of stimulation days, the number of follicles ≥17 mm, the number of oocytes, the fertilization rate, the number of embryos, the number of women with ≥1 top-quality embryo, the biochemical, clinical and ongoing pregnancy rates and the miscarriage rate.

RESULTS: There were 116 women allocated to the rLH group and 128 allocated to the control group. The proportion of top-quality embryos per woman was 17% in the rLH group and 11% in the control group [mean difference 0.06; 95% confidence interval (CI) −0.01–0.14]. In the rLH and control groups respectively, 47 (41%) and 41 (32%) women had at least one top-quality embryo on the day of transfer (relative risk: 1.3; 95% CI 0.91–1.77). The ongoing pregnancy rate was 13 versus 12% (relative risk: 1.1; 95% CI 0.57–2.16) for the rLH group compared with the control group.

CONCLUSIONS: This study found no significant difference in embryo quality after the addition of rLH to rFSH for ovarian stimulation in women with poor ovarian reserve.

CLINICAL TRIALS IDENTIFIER: NTR1457

Key words: recombinant LH / ovarian stimulation / IVF outcome / embryo quality

Introduction

After the introduction of ovarian hyperstimulation in IVF, it soon became evident that ovarian response differs between women. Already in 1983, the first study that described women with poor response was published (Garcia et al., 1983). Poor response is often related to women with advanced age, in whom the low response to gonadotrophins reflects a physiologic decline in ovarian reserve of primordial follicles (Pellicer et al., 1994; Beckers et al., 2002; De Boer et al., 2002; Lawson et al., 2003). Hence, poor ovarian response is the clinical manifestation of a poor ovarian reserve. A pathologic decline in number and quality of primordial follicles may also occur in young women (Jacobs et al., 1990; El-Toukhy et al., 2002). Poor reserve is a common clinical problem, with up to 26% of IVF cycles resulting in poor response (Pellicer et al., 1987; Keay et al., 1997). In the future this percentage is likely to increase as women continue to postpone childbearing (Mathews and Hamilton, 2009).

To date, the diagnosis of poor ovarian reserve is based upon the ovarian response in an IVF treatment cycle and/or patient characteristics such as age, basal FSH, anti-Müllerian hormone (AMH) and/or...
basal antral follicle count (AFC) (Toner et al., 1991; Scott and Hofmann, 1995; Frattarelli et al., 2000, 2004; Tarlatzis et al., 2003; Hellberg et al., 2004; Mutukrishna et al., 2004; Sallam et al., 2005). In women with poor ovarian reserve, the number of mature follicles that develop during stimulation is frequently considered to be insufficient for a successful treatment, leading to cancellation of the cycle. Women with poor ovarian reserve who do proceed to ovum pick up produce low numbers of oocytes and embryos and have low pregnancy rates (Jenkins et al., 1991; Ulug et al., 2003).

The effect of the addition of recombinant luteinizing hormone (rLH) to IVF stimulation has been debated in the past decade, but increasing evidence on the beneficial effect of rLH in women with poor ovarian reserve has been published. A Cochrane review suggested that the addition of rLH to controlled ovarian hyperstimulation (COH) with rFSH increases ongoing pregnancy rates in women with poor ovarian reserve [odd ratio (OR) 1.85, 95% confidence interval (CI) 1.10–3.11] (Mochtar et al., 2007). A recent large randomized trial in women with poor ovarian reserve reported ongoing pregnancy rates of 34% for women treated with rFSH and rLH compared with 25% for women treated with rFSH (OR 1.49, 95% CI 0.93–2.38) (Bosch et al., 2011).

How exactly rLH increases ongoing pregnancy rates in this population remains unclear. An explanation could be the effect of rLH on oocyte and embryo quality. LH is known to be important in oocyte growth and maturation (Filicori et al., 1999, 2002; Sullivan et al., 1999; Filicori and Cognigni, 2003). Exogenous LH has been suggested to have an intrinsic effect on embryo competence (Tesarik and Mendoza, 2002).

To obtain better data on the relation between rLH and embryo quality, we evaluated the yield of top-quality embryos in women with poor ovarian reserve after IVF stimulation with rLH and rFSH compared with rFSH alone.

Materials and Methods

Study population

Between August 2008 and April 2010, all women 35–41 years old or women younger than 35 years old with an FSH level > 12 IU/ml accompanied with an AFC ≤ 5 and who were scheduled for their first IVF or ICSI in the Academic Medical Center or the Onze Lieve Vrouwe Gasthuis in Amsterdam, were asked to participate in a randomized controlled trial. Women were excluded from the trial if they had any endocrinopathy: Cushing’s syndrome, adrenal hyperplasia, hyperprolactinaemia, acromegaly, hypothalamic amenorrhea, hypothyroidism and diabetes mellitus type I or polycystic ovary syndrome.

After providing written informed consent, the women were randomly assigned to undergo one cycle of IVF or ICSI, with COH with rFSH and rLH (rLH group) or with rFSH alone (control group). Randomization took place during cycle scheduling (base line visit); this was usually 3 months before the treatment cycle started. During this consultation, an appointment was made for a transvaginal sonography for a basal AFC and blood sampling for AMH and basal FSH. Women who had an AFC and/or FSH sampling before randomization were asked to repeat these tests to make sure the values were from 1 to 3 months before the start of stimulation. Blood sampling (basal FSH and AMH) was only performed in women receiving treatment in the Academic Medical Centre (n = 196).

Central web-based randomization was performed prior to the start of ovarian stimulation using a computer program minimization procedure with stratification according to study centre. The study was not blinded for patients and doctors, but the embryologists and IVF technicians who evaluated the primary outcome of the study, i.e. embryo quality, were unaware of treatment allocation.

The study protocol was approved by the institutional review boards of the two participating hospitals and by the Central Committee on Research Involving Human Subjects in the Netherlands. The study was registered with EudraCT (EudraCT number 2007-007487-22) and the Dutch National Trial Register (Trial ID: NTR1457).

Treatment protocol

Women underwent COH after down-regulation with the GnRH agonist triptorelin (Decapeptyl®) in a long protocol with a midluteal start. COH was started on cycle day 5 with rFSH (GONAL-F®, MerckSerono) or rFSH with addition of rLH (Luveris®, MerckSerono) depending on the allocation.

Depending on the AFC, women started with different doses of gonadotrophins.

If the AFC was three or lower on cycle day 5, women started with a maximal stimulation of 450 IU rFSH and 225 IU rLH or 450 IU rFSH alone. If the AFC was between 4 and 14 follicles on cycle day 5, women started with 300 IU rFSH and 150 IU rLH or 300 IU rFSH alone. If the AFC was 15 or higher on cycle day 5, women started with 150 IU rFSH and 75 IU rLH or 150 IU rFSH alone.

After 7 days of stimulation, the dosage was kept the same or was adjusted according to ovarian response to a maximum of 450 IU rFSH and 225 IU rLH or 450 IU rFSH alone, always keeping the ratio of rFSH to rLH at 2:1.

Follicular maturation was induced by 6500 IU hCG hormone (Ovitrelle®, MerckSerono) when at least three follicles ≥ 17 mm had developed. Cumulus–oocyte complexes were recovered by transvaginal ultrasound-guided follicle aspiration 36 h thereafter.

If there were only one or two follicles in the first cycle and the maximum rFSH dose of 450 IU/l had not been administered, the cycle was converted to IUI. These IUI cycles were analysed separately. If these women did not conceive after IUI another IVF or ICSI cycle was started, but this time with the maximum rFSH dosage of 450 IU/l and if appropriate 225 IU/l rLH. Follicle aspiration was performed when at least one follicle of 17 mm or more was seen on transvaginal sonography and this cycle was included in the final analysis.

When women started their cycle with the maximum doses, rFSH 450 IU/l with/without 225 IU/l rLH, and there was no development of follicles, the cycle was cancelled. These women did not proceed to another IVF or ICSI cycle. These cancelled cycles were included in the final analysis.

Embryo transfer policy, 3 days after follicle aspiration was according to a standard protocol; women younger than 35 years with a top-quality embryo received one embryo; women younger than 35 years, but without a top-quality embryo received two embryos; women between 35 and 39 years received two embryos and women older than 39 years received three embryos. When less than the required number of embryos was obtained, all available embryos were transferred into the uterine cavity.

Morphological scoring

Embryos were morphologically assessed daily from fertilization (pro-nuclear morphology) until time of transfer (Puisant et al., 1987; Veeck, 1990; Steer et al., 1992). Embryos were assessed using an Olympus IX71 inverted microscope equipped with Relief Contrast optics at a magnification of x 320. On Day 3, one or more embryos were selected for transfer. Top-quality embryos were defined as embryos with a cumulative
embryo score of ≥24 on Day 3 after follicle aspiration. In the cumulative embryo score, the number of cells is amplified with the morphological score that ranges from scores 1 (excellent, 4 points) to 4 (poor, 1 point); for example: a score 2, good quality 8-cell—embryo, received $8 \times 3 = 24$ points. Morulae were considered top-quality if $<20\%$ fragments were present and at least 50% of the cells were part of the compacting process. Embryo assessment was performed blinded for the allocation of the woman.

Study end-points
The primary outcome measure was the proportion of top-quality embryos per woman on the day of transfer, i.e. 3 days after follicle aspiration. The proportion of top-quality embryos per woman was calculated by dividing the number of top-quality embryos over the total number of embryos within that cycle per woman (e.g. if one of the five embryos developed into a top embryo, the embryo rate was 0.2). Secondary outcomes were the number of stimulation days until hCG administration, the number of follicles ≥17 mm on the day of hCG administration, the number of oocytes, the fertilization rate, the number of women with ≥1 top-quality embryos, the biochemical pregnancy rate (defined as an increase in serum HCG ≥3, 14 days after follicle aspiration), the clinical pregnancy rate (defined as positive heartbeat on transvaginal sonography in week 8 of pregnancy), the miscarriage rate and the ongoing pregnancy rate (defined as a positive heartbeat at ≥12 weeks gestational age).

Power calculation
Based on a historical analysis of the data from our centre, ~20% of the total numbers of embryos are expected to develop into a top-quality embryo after COH with rFSH alone. A mean number of eight oocytes, five embryos and one top-quality embryo are available per woman with advanced age in our population (Mastenbroek et al., 2007). We expected the same proportion of top-quality embryos in women 35 years old or with an FSH level >12 IU/ml accompanied by an AFC ≤5.

To prove that the addition of rLH yields an increase of 10% in the proportion of top-quality embryos compared with a standard proportion of 20%, with a power of 80% and an alpha of 5% and a correlation coefficient ($\rho$) between embryos and women of 0.2, we required 520 embryos per treatment arm. Assuming a mean number of five embryos are available per woman this means that 104 women would have to be included per arm. To be able to account for 15% drop-out during the trial, we aimed at including a total of 242 women.

Statistical analysis
Data were analysed using the SPSS 18.0 software. All analyses were performed on an intention-to-treat basis. The effectiveness of rFSH and rLH compared with rFSH alone was expressed as mean differences with corresponding 95% CIs for continuous data such as the proportion of top-quality embryos per woman, fertilization rate, number of oocytes and number of embryos. Formal comparisons were done using independent t-tests.

Figure 1 CONSORT flow diagram assignment, treatment and analysis of women.
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Differences in dichotomous data such as pregnancy outcomes were expressed as rate ratios with 95% CIs. We used chi-square statistics to test for significance.

We expected the primary outcome to have a skewed distribution as many women will not produce a top-quality embryo. We therefore used non-parametric bootstrapping, to calculate mean and CIs for the proportion of top-quality embryos per woman.

To identify if there was an association between AFC, age or BMI and the proportion of top-quality embryos per woman corrected for treatment allocation, a linear regression with bias-corrected and accelerated bootstrapping was calculated (Efron, 1987) (Stata SE 11.1). These calculations were performed by an epidemiologist (M.v.W.).

Results

There were 270 women who met the inclusion criteria and were eligible for inclusion, 26 women declined participation. The remaining 244 women were randomized; 116 women were allocated to rLH and rFSH (rLH group) and 128 women were allocated to rFSH alone (control group). In total, six women (three in each group) were younger than 35 years and had a basal FSH > 12 IU/ml accompanied by an AFC ≤ 5. All other women were between 35 and 41 years of age.

There were 107 women in the rLH group and 117 in the control group who actually underwent the assigned intervention (Fig. 1). Completion of treatment was reached in January 2011. The baseline characteristics were similar in the two study groups (Table I).

There were 10 cycles in the rLH group and 15 cycles in the control group cancelled because of low response. Of these, three women in the rLH group and seven women in the control group had no response at all, despite maximal ovarian stimulation (450 IU rFSH with/without 225 IU rLH).

There were seven women in the rLH group and eight women in the control group who had their first IVF attempt converted to IUI. No pregnancies resulted from these IUI cycles. These women continued with the maximal stimulation in the subsequent stimulation cycle. The seven women in the rLH group all proceeded to a follicle aspiration. Of the eight women in the control group, six women went on for follicle aspiration and two women had no response.

In total 109 women in each group underwent a follicle aspiration. In the rLH group, 4 women had a follicle aspiration in which no oocytes were found and 14 women had a cycle where no oocytes were fertilized. In the control group, three women had a follicle aspiration in which no oocytes were found, 17 women had a cycle where no oocytes were fertilized and the oocytes of 2 women were vitrified because no viable spermatozoa were found on the day of follicle aspiration. Therefore, 91 women in the rLH group and 87 women in the control group had embryos available for transfer.

Outcomes

The primary outcome, the proportion of top-quality embryos per woman, was 17% in the rLH group and 11% in the control group (mean difference 0.06; 95% CI −0.01–0.14). In the rLH group, 91 women had one or more embryo(s) and of these 47 (41%) women had one or more top-quality embryo(s), while in the control group, 87 women had one of more embryo(s) and of these 41 (32%) women had one or more top-quality embryo(s) on the day of transfer (relative risk: 1.3; 95% CI 0.91–1.77).

The ongoing pregnancy rate per woman was 13 versus 12% (relative risk: 1.1; 95% CI 0.57–2.16) for the rLH group compared with the control group. All other secondary outcomes are listed in Table II. We found no association between AFC, age or BMI and the proportion of top-quality embryos per woman (AFC coefficient = 0.004, age coefficient = −0.002 and BMI coefficient = −0.006).

Discussion

We found no evidence of a statistically significant difference in embryo quality or any other outcome in women with poor ovarian reserve after addition of rLH to rFSH compared with FSH alone for COH.

Our randomized trial aimed at finding a biological explanation for the apparent beneficial effect of rLH in women with poor ovarian reserve. Although there was a higher proportion of top-quality embryos per woman and more women had at least one top-quality embryo in the rLH group, the difference was not statistically significant. One other study has investigated the number of embryos after the addition of rLH in women with poor ovarian reserve. Although there was a higher proportion of top-quality embryos per woman, the difference was not statistically significant.

A strength of our study is that rLH was given throughout the stimulation phase, in analogy to the trials that compared rFSH versus highly purified HMG, a gonadotrophin that also has LH activity and which has been demonstrated to result in a 3% higher ongoing pregnancy rate compared with rFSH alone (Coomarasamy et al., 2008; van Wely et al., 2011). Only the most recently published RCT used the same protocol.
approach, by giving 225 rFSH and 75 IU/day rLH from the beginning of the stimulation to the women assigned to rLH (Bosch et al., 2011). Other trials studying the addition of rLH to rFSH administered rLH before rFSH stimulation as a pretreatment (Durnerin et al., 2009; Kovacs et al., 2010) or as a late follicular phase treatment (Ferraretti et al., 2004; Marrs et al., 2004; De Placido et al., 2005; Fabregues et al., 2006; Barrenetxea et al., 2008; Nyboe Andersen et al., 2008; Gutman et al., 2009; Matorras et al., 2009; Pezzuto et al., 2009; Kovacs et al., 2010; Bosch et al., 2011).

Contrary to what was expected, we found 445 embryos in the rLH group and 458 embryos in the control group, instead of the anticipated 522 embryos per arm. This was due to cycle cancellations, lack of oocytes after follicle aspiration and total fertilization failure in both study groups (in total 18% in the rLH group and in total 23% in the control group). It was also due to the fact that there was no association between AFC and the proportion of top-quality embryos per woman and that the women who did have embryos had an average of four instead of the estimated five embryos per woman. This all indicates that the women in this study had an even poorer ovarian function than expected and really did represent women with a poor prognosis.

Worldwide there is no consensus on the definition of women with poor ovarian reserve or for that matter poor ovarian response. As we found it important to include all women with poor ovarian reserve, we included women of advanced age, i.e. women 35–41 years old, and women younger than 35 years old with an FSH level > 12 IU/ml accompanied by an AFC ≤ 5). In both groups, a poor response during

| Table II Outcomes in women who received IVF with rFSH + rLH or rFSH. |
|----------------------|----------------------|----------------------|----------------------|----------------------|
| Outcome              | Women assigned rFSH + rLH, (n = 116) | Women assigned rFSH (controls), (n = 128) | Mean difference (95% CI) | Relative risk (95% CI) |
| primary outcome      | Top-quality embryo rate<sup>a</sup> mean (95% CI) | 0.17 (0.06–0.28) | 0.11 (0.04–0.19) | 0.06 (−0.01–0.14) |
| stimulation phase    | No. of stimulation days mean (95% CI) | 11.6 (11.0–12.2) | 11.3 (10.6–12.0) | 0.28 (−0.66–1.23) |
|                      | Woman started stim: 450 rFSH ± 225 rLH | 38 | 35 | 1.2 (0.82–1.76) |
|                      | Woman started stim: 300 rFSH ± 150 rLH | 42 | 51 | 0.9 (0.66–1.25) |
|                      | Total amount of rFSH administrated (95% CI) | 3998.2 (2206.7–5789.7) | 3984.4 (2173.5–5795.3) | 13.8 (−459.9–487.1) |
|                      | Total amount of rLH administrated (95% CI) | 1841.8 (845–2843.6) | — | — |
|                      | Women with no response after max stim n (%) | 3 (3) | 9 (7) | 0.4 (0.10–1.33) |
| oocyte collection phase | Number of follicles ≥ 17 mm on hCG day | 6.3 (5.5–7.1) | 6.1 (5.3–6.9) | 0.22 (−0.89–1.33) |
|                      | No. of oocytes per woman mean (95% CI) | 8.6 (7.4–9.8) | 7.4 (6.4–8.5) | 1.19 (−0.39–2.77) |
|                      | Women with total fertilization failure, n (%) | 14 (12) | 17 (13) | 0.9 (0.47–1.76) |
|                      | Fertilization rate per woman mean (95% CI) | 0.40 (0.35–0.46) | 0.39 (0.33–0.45) | 0.01 (−0.07–0.09) |
|                      | No. of embryos per woman mean (95% CI) | 3.8 (3.1–4.6) | 3.6 (2.9–4.3) | 0.26 (−0.76–1.27) |
|                      | No. of top-quality embryos per woman mean (95% CI) | 0.82 (0.53–1.1) | 0.68 (0.44–0.92) | 0.14 (−0.23–0.51) |
| clinical outcomes    | Women with 1 ≥ top embryos, n (%) | 47 (41) | 41 (32) | 1.3 (0.91–1.77) |
|                      | Women with 1 ≥ ongoing pregnancies, n (%) | 15 (13) | 15 (12) | 1.1 (0.57–2.16) |
|                      | Women with 1 ≥ biochemical pregnancies | 33 (28) | 30 (23) | 1.2 (0.79–1.86) |
|                      | Women with 1 ≥ clinical pregnancies | 18 (16) | 20 (16) | 1.0 (0.55–1.78) |
|                      | Women with 1 ≥ miscarriages | 3 (3) | 5 (4) | 0.7 (0.16–2.71) |

<sup>a</sup>Total number of top-quality embryos per woman/total number of embryos per woman.
COS has been described (Croucher et al., 1998; Hsieh et al., 2001; Bancsi et al., 2002). The observed response to ovarian hyperstimulation in our study confirm the extremely poor prognosis of these women.

A Cochrane review suggested that the addition of rLH to COH has a beneficial effect on ongoing pregnancy rates in women with poor ovarian response (OR 1.85, 95% CI 1.10–3.11) (Mochtar et al., 2007). When adding the pregnancy results of our trial and those of a recently published randomized study in women with poor ovarian response (Bosch et al., 2011) to the available data in the Cochrane review, the summarized OR is 1.39 (95% CI 1.01–1.92). This substantiates the evidence that there is indeed an effect, on ongoing pregnancy rates, of the addition of rLH to FSH in women with poor ovarian response. The present trial was not powered to identify a difference in pregnancy rates between the two groups. To prove that the addition of rLH yields an increase of 10% in the pregnancy rate compared with a standard rate of 25%, with a power of 80% and an alpha of 5%, would require 700 women per treatment arm.

How would the addition of exogenous LH activity lead to higher implantation and pregnancy rates in women of increased age? Next to the biological explanation of embryo quality investigated in this trial, another possible explanation could be an intrinsic effect of LH activity on the endometrium. In an oocyte donation program, women treated with a combination of rFSH and rLH, yielded more mature oocytes and good-quality embryos and achieved higher implantation rates within the oocyte recipients compared with oocyte donor women treated with rFSH alone (Tesakir and Mendoza, 2002; Acevedo et al., 2004).

Before considering the addition of rLH to IVF stimulation for women with poor ovarian reserve, its costs should be balanced against its potential benefits with proper cost-effectiveness studies and also patient preference studies should be explored upon.

In conclusion, the present study investigated in a randomized setting if embryo quality increased with the addition of rLH to ovarian stimulation for women with poor ovarian reserve. The addition of LH favoured an increase from 11 to 17% in the proportion of top-quality embryos per woman and the proportion of women with at least one top-quality embryo increased from 32 to 41%. However the differences were not significant.

Authors’ roles
A.M.M. contributed to design of the study, recruitment of the participants, acquisition of the data and analysis of the data and drafted the manuscript. M.v.W. contributed to design of the study and analysis of the data, participated in interpretation of the data and revised the manuscript critically. E.M.K. recruited participants at the Onze Lieve Vrouwe Gasthuis, contributed to interpretation of the data and revisions of the manuscript. S.M., S.R. and F.v.V. contributed to the design of the study, interpretation of the data and to the revisions of the manuscript. M.H.M. initiated the study, contributed to design of the study, participated in interpretation of the data and revised the manuscript critically.

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