Title of Trial: Lutropin alfa in midfollicular phase for controlled ovarian stimulation (COS) in ovarian ageing: a randomised, comparative with parallel control group, phase II clinical trial.

Investigational Product: Lutropin alfa

Trial No.: 25186

Study Centers: This study was conducted in 1 center in Spain.

Trial Dates:

Trial Initiation Date: 10 January 2005

Trial Completion Date: 15 November 2006

Development Phase: Phase 2/4

Publication (reference): None

Study Objectives:

- To assess the effect of combining rLH with rFSH from mid luteal phase onwards versus a control group treated with rFSH alone on oocyte number and quality in infertile women aged between 35 and 40 undergoing IVF/ICSI and ET.
- To assess additional parameters of efficacy as follicular development, oocyte fertilization, embryo quality and clinical pregnancies between both study groups.
- To assess the safety of using rLH in combination with r-hFSH and that of using r- hFSH alone, including incidence of ovarian hyperstimulation syndrome (OHSS), adverse events, and local tolerance.

Methodology: Exploratory, randomised, comparative parallel group controlled trial.

Investigational treatment: rLH. Plus rFSH

Comparator: rFSH only.

Study population: Infertile women with poor ovarian reserve due to advanced reproductive age.

Therapeutic scheme: GnRH agonist long protocol followed by controlled ovarian stimulation with an initial rFSH dose of 300UI/day. From the 6^{th} day of stimulation onwards, suplementation or or not with rLH 150 UI/day according to randomization. Final follicular maturation with single administration of rHCG 250 mcg. OPU + FIV/ICSl-ET (≤ 3 embryos transferred) under luteal support with P4 from OPU onwards. The study involved a single cycl/subject.

Number of Subjects (Planned and Analyzed): A total of 131 randomized subjects (ITT population): 63 in the rFSH plus rLH group and 68 in the rFSH alone group. Out of them, a total of 128 subjects completed the study PP (63 treated with rLH plus rFSH and 65 with rFSH only).

Diagnosis and Main Criteria for Inclusion/Exclusion:

Diagnosis: Normal cycling (25-35 days) infertile women aged 35-40 years justifying IVF/ICSI.

Inclusion criteria:

- BMI between 18-30
- Baseline FSH ≤10 IU/l
- Baseline LH and E_2 within local lab range
- Scheduled for an GnRH agonist long – protocol
- The presence of both ovaries and uterine cavity able to sustain pregnancy.
- Clomiphene or gonadotropin wash out > 30 days prior to starting GnRH agonist.
- Confirmed absence of pregnancy.
- Signed informed consent

Exclusion criteria:

- Known to be HIV, HBV/HCV positive.
- Clinically sign~ficant condition preventing from gonadotropin treatment.
- More than 2 previous ART cycles
- Cancellation of two previous cycles.
- Cryopreserved embryos from previous ART
- Unexplained gynaecological bleeding.
- Polycystic ovary or unknown aetiology cyst.
- Pregnancy contraindication.
- Active substance abuse.
- Simultaneous participation in another trial or re-entry in the current one.
- Refusal or inability to comply with the protocol.

Study Treatment:

Product: Lutropin alfa, Luveris®, rLH

Dose: 150 IU/day

Route: subcutaneous self-administration

Duration of Treatment: From S6 up to hCG criteria were met. On average 5 days ranging from one to ten days.

Reference Therapies, Dose and Mode of Administration: Not applicable. The comparator was the non-administration of the investigational product.

Criteria for Evaluation:

Efficacy:

Efficacy analyses were performed on ITT (randomized) data set as the primary data, using all data available at the time of analysis.

Supportive efficacy analyses was performed on PP (protocol compliant) data set as the secondary data set.

Main efficacy endpoint was the number and maturity of oocytes retrieved.

Secondary endpoints:

- Follicles on r-hCG day or at cancellation.
- Follicles by size r-hCG/cancellation,
- Oocytes retrieved at OPU
- Fertilized (2PN) oocytes
- Fertilization rate
- Embryos and their quality
- Implantation rate per embryo transferred
- Number of biochemical pregnancies
- Number of clinical pregnancies
- Stimulation days
- Total dose of r-hFSH used,
- Serum E_2 levels on r-hCG day,

Safety

All patients who received at least one injection were analysed for:

- Incidence of OHSS,
- Adverse Events, including local tolerance.

Statistical Methods:

The statistical methods suited to nature of the variable analysed. Significance level was kept at 0.05 two-sided.

Data are presented as the mean and standard deviation when continuous and as frequencies and percent for categoric variables.

The main analysis performed on retrieved oocyctes was done by using the Student's "t". The secondary efficacy endpoints were compared by means of Student's "t" test/Mann-Whitney test or Fisher exact test/χ^2 test, for quantitative or qualitative variables, respectively.

Narratives of two severe adverse events are offered. No additional data on adverse events of different severity or injection site reactions were detected.

Results:

Subject Disposition:

- Screened: 138 subjects
- Screening failure: 7
- Randomized 131 (63 rFSH + rLH and 68 rFSH)
- Major protocol violation: 3 cases
- Evaluable 128 (63 rFSH + rLH and 65 rFSH)

Demographics and Baseline Characteristics:

No difference were found between mean baseline profiles in the participants included in each group. Mean age > 36.5 years in both groups. BMI around 22.5. Serum FSH between 5-6 IU/l. In both groups the infertility was primary in 3/4 of cases and was lasting 4.5 years. Male factor was the main cause for the infertility. About 60% had undergone 0-1 previous ART cycle.

All parameters assessing controlled ovarian stimulation evolved in similar way in both treatment groups and the mean value of each single parameter were similar in both groups at the time of starting the randomized treatment (rLH supplementation or not).

Efflcacy Results:

In the all randomised population analyses, main efficacy endpoints were similar in both study groups. Ovarian stimulation yielded similar mean results when stimulated with rLH plus rFSH and rFSH alone: 11.0 ± 5.5 vs 11.2 ± 5.7 follicles ≥10 mm on hCG day and 8.3 ± 4.7 vs 8.6 ± 5.0 oocytes retrieved at OPU, respectively.

Regarding the secondary endpoints, results were similar in both groups too but the difference in clinical pregnancy favouring the combination of rLH with rFSH. Ooocyte nuclear maturity was similar too with a mean of 6.7 ± 4.1 vs 7.0 ± 4.8 of metaphase II oocytes in rLH plus rFSH and rFSH alone group, respectively. Fertilization rate was slightly lower than expected in both groups with 48% and 49 % of the inseminated oocytes yielding 2PN zygotes. Embryo grading scale was also similar in both groups. Although two embryos were transferred on average to each patient in both groups, because of higher implantation rate (20%) in rLH plus rFSH group than in the rFSH alone group, more clinical pregnancies were clearly achieved in the group treated with rLH plus rFSH (1 7163) than in the rFSH only group (10168); i.e. 26. 6% vs 14. 7%, which was a clinically meaningful difference closet reach statistical significance (p: 0.08). The rates of singletons, twins and triplets were even distributed in both groups of treatment. Abortion rate was around 20% of pregnancies in both groups.

Safety Results:

Experimental drug, rLH, was administered 5 days on average and no other adverse reaction than a severe OHSS and a pelvic tenderness. Both required hospitalization and had subsided at discharge.

No local signs of intolerance at injection site were recorded

Conclusions:

Mid follicular phase supplementation with rLH and seems to increase embryo capacitation as expressed in terms of increased implantation and pregnancy, while being well tolerated.