Cover page

Title: Cumulative Pregnancy Rate with Lower and Higher Gonadotropin Dose During IVF Among Poor Responders

Trial registration: NCT05003228

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Design: Investigator initiated prospective, randomized, multi-center, non-blinded trial to compared higher and lower gonadotropin in poor responder patients undegoing IVF-ICSI **treatment identified based on Posedion III-IV criteria**

Inclusion Criteria:

- Expected poor responder based on:

a) AMH: 0.3-1.2 ng/ml or AFC <5,

b) AMH>1.2 ng/ml or AFC≥5 but the retrieval of ≤ 4 oocytes during previous IVF treatment.

- motile sperm with normal morphology obtained from the ejaculate of testicular biopsy

- no more than 3 previous failed IVF cycles (if the patient had 2 or more previous cycles cancelled for poor response she cannot be included)

- BMI: 18-35 kg/m2

- regular 24-35 day cycles

- intact uterine cavity

- indication for in vitro fertilisation treatment (tubal factor, male factor, low ovarian reserve, endometriosis, unexplained infertility)

- age 18-421 yrs

Exclusion Criteria:

- presence of hydrosalpinx

- positive HIV, hepatitis B, C screening tests

- planned preimplantation genetic testing of the embryos

- planned elective cryopreservation

- lack of consent

- not meeting the inclusion criteria

Treatment protocols and treatment monitoring/ steps:

Lower-dose group:

- 150 IU follitropin alpha + 75 IU highly purified human menopausal gonadotropin (hpHMG)
- 10 mcg follitropin delta + 75 IU hpHMG

Higher-dose group:

- 225 IU follitropin alpha + 150 IU hpHMG
- 15 mcg follitropin delta + 150 IU hpHMG

Treatment protocol:

- The stimulation will get started on day 2 or 3 of the spontaneous menstrual cycle, or on the 5th day after oral contraceptive pill use or after luteal estradiol pretreatment.
- During the stimulation ultrasound +/- serum hormone measurements will be used to monitor response. The first ultrasound is scheduled for day 5 or 6 of stimulation. At the time of the ultrasound blood test for serum estradiol is planned too. At the time of the last scan serum estradiol and progesterone levels will be measured.
- According to the study protocol the patient will continue with the assigned medication dose throughout her treatment. Dose increase is not allowed. If there is evidence for hyper-response [estradiol level > 4000 pmol/l on day 6 or more than 15 follicles over 10 mm any time during the stimulation] the dose can be reduced.
- As soon as two or more follicles reach >17 mm in diameter human chorionic gonadotropin (hCG) or gonadotropin-releasing hormone agonits [GnRH a] trigger injection will be used and 35-36 hours later transvaginal ultrasound guided follicle aspiration will be performed.
- The luteal phase will be supported by vaginal progesterone starting on the day after the retrieval.
- IVF or ICSI fertilization will be performed based on the semen parameters or previous fertilization records.
- The day after the retrieval the success of fertilization will be checked and the embryo(s) will be transferred 2-5 days after the retrieval. Embryo(s) will be transferred transcervically using soft transfer catheters under ultrasound guidance using the afterload technique.
- Surplus good quality embryos will be cryopreserved using vitrification. Elective cryopreservation (no transfer in the fresh cycle) will be performed if: 1) risk of ovarian hyperstimulation syndrome, 2) serum progesterone level over 1.5 ng/ml [5.5 nmol/l]

prior to the oocyte collection, 3) any complications between the retrieval and planned fresh transfer (bleeding, infection, illness).

- 12-14 days after the transfer serum human chorionic gonadotropin (HCG) measurement will determine whether implantation has occurred. If the test is positive in 2-3 weeks a vaginal ultrasound will be done to determine the size, location and number of gestational sacs. Viable pregnancies will be referred to a formal prenatal care around week 8-9 of pregnancy. Delivery and perinatal outcome data will be collected by phone call after the delivery.

- If the fresh IVF cycle is not successful but embryos have been cryopreserved then the patient will undergo a frozen embryo transfer treatment cycle either in her own cycle, or in a minimally stimulated or completely artificial cycle.

The care of the patients will not differ from the care of non-study patients in terms of potential medication dose, the number of clinic visits, retrieval and embryology procedures as well as prenatal care.

Randomization: Randomization will be performed according to a computer generated list. (www.randomizer.org) The planned sample size is 700 patients (350 in both the low- and high-dose groups).

Sample size calculation: In order to determine the sample size, the investigators calculated with a 20% pregnancy rate in a patient population that fits the inclusion-exclusion criteria. The investigators expect higher oocyte yield in the higher dose group that should result in more available embryos and therefore more frozen embryo transfers. The investigators calculate that this could increase the cumulative pregnancy rate by 50%. The investigators also believe that about 20% of the patients will drop out for various reasons. Therefore, 350 participants are needed in both arms of the study.

Statistical analysis: After the enrollment of the first 350 patients, a planned interim analysis will be performed to decide whether the planned sample size is sufficient to achieve our goal. Logistic regression analysis using a generalized, mixed linear model will be used to assess the impact of various Gn doses on pregnancy rates. Chi-square test will be used to test significance and OR will be calculated. Results are reported as intention-to-treat (ITT).

Data will be collected for the following parameters:

- Age
- Cycle day 3 follicle stimulating hormone (FSH), estradiol level
- anti-Müllerian hormone (AMH)
- antral follicle count (AFC)
- Indication for IVF: male, tubal, unexplained, endometriosis, diminished ovarian reserve
- Smoking (yes-no)
- Body mass index
- Cycle number (fresh + frozen together)
- Estradiol level on day 6
- Estradiol + progesterone level at the last scan
- Number of follicles >10 mm at the end of stimulation
- Stimulation duration
- Endometrial thickness
- Gn dose (daily, total)
- Trigger mechanism (HCG vs. GnRh agonist)
- Sperm parameters
- Oocyte number
- Mature (MII) oocyte number
- Proportion of mature oocytes (MII/ oocyte number)
- IVF vs. ICSI fertilization
- Number of fertilized (2 pronuclei (PN)) oocytes
- Fertilization rate per oocyte
- Fertilization rate per mature oocyte
- The number of good quality embryos (morphology better than score "6B2" on day 3, or score >2BB on day 5) and proportion of good morphology embryos among all embryos
- Number of transferred embryos

- Number of cryopreserved embryos
- Pregnancy rate (positive hCG)
- Clinical pregnancy rate (sac with viable embryo in it)
- Cumulative pregnancy rate (fresh + frozen embryo transfers)
- Pregnancy/ neonatal outcome