Study Protocol and Statistical Analysis Plan

Analysis of the utility of freeze-all strategy in an In Vitro Fertilization program with single embryo transfer policy.

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STUDY PROTOCOL:

The study was designed as a prospective, randomized, two-arm, parallel, unblinded study, during which We will include 138 couples (69 in each group) of IVF / ICSI users. Cycles canceled or without transfer (either due to not obtaining oocytes, non-fertilization or arrest of embryonic development) will not be included in the study. The assignment of the pairs to each of the groups will be carried out by means of a random sampling procedure. The randomization list will be created specifically for this project and will be guarded by an outsider to the research team:

-Group I (experimental): fresh transfer is not performed, the best quality embryo is cryopreserved. Elective transfer in a later cycle of the cryopreserved embryo.
-Group II (control): fresh transfer of the best quality embryo.

Couples will be able to participate in the study for only one cycle. Each couple will be interviewed by a member of the team who will explain the characteristics of the study and they will be given an Information Sheet. It is necessary to obtain the written informed consent of each couple. The person outside the investigation team who is guarding the randomization list will assign the corresponding treatment group.

Before starting the study, ethical approval will be processed by the Ethics Committee through the Ethics Portal of the Biomedical Research of Andalusia.

Patients must follow ovulation stimulating treatment, in order to achieve multiple follicular development. A "long analog" GnRH agonist protocol will be used. This consists of administering from the day 22 of the cycle 0.1 mg / day of GnRH analog (Decapeptyl 0.1; Lasa, Barcelona, Spain) until the day the gonadotropin administration, in which the dose is reduced to 50% until the day of hCG. After 10-14 days of administration of the agonist, we proceed to check the pituitary braking by means of ovarian vaginal ultrasound (absence of follicles and cysts). Then, 300 IU of recombinant FSH (rFSH) is administered per day (Gonal F, Merck, Madrid, Spain) for two days, and 150 IU of FSHr from the 3rd to the 7th day. On this day, ultrasound control of the follicular development is accomplished in order to re-adjust the dose of rFSH to each patient. Once the follicular response is adequate (more than 3 ovarian follicles greater than 18 mm in diameter), we proceed to trigger ovulation using 6500 IU hCG (Ovitrelle, Merck, Darmstadt, Germany).

Oocyte retrieval will be performed by transvaginal ultrasound-guided follicular puncture 36 hours after the injection of hCG, anticipating the ovulatory process, using an ovarian puncture needle (Labotect LaborTechnik-Göttingen, Göttingen, Germany). The follicles larger than 17 mm are punctured one by one, aspirating their content into fluid collection tubes.

The content of the aspirated follicle should be transported quickly to the laboratory to immediately check for the presence of the cumulus-oocyte complex. Antibiotic prophylaxis is not routinely performed, and only in cases where the puncture is more difficult, a single dose of 1,500 mg of cefuroxime sodium will be administered intravenously (Cefuroxime Normon EGF, Madrid, Spain) or 1000 mg of erythromycin lactobionate (Pantomycin Abbot, Madrid, Spain) in patients allergic to beta-lactams. Once the follicular fluid (FF) arrive at the laboratory, the search and identification of the cumulus-oocyte complexes is performed by locating them under stereomicroscopic vision in a 100x15 mm Petri. They are placed in a new 35x10 mm Petri dish inside a few drops of MOPS culture medium Vitrolife (IVF Science Scandinavia, Gothenburg, Sweden) previously aerated and heated to 37ºC, to eliminate the remains of FF.

IVF/ICSI and embryo culture will then be performed. To perform sperm microinsemination (ICSI) it is necessary to remove the cumulus and corona radiata from the oocyte. To decumulate, the crown-oocyte cluster complex is submerged in a solution with 80 IU / mL hyaluronidase (HYASE, IVF Science Scandinavia, Gothenburg, Sweden) for 10-20 seconds aspirating the complex several times using a pasteur pipette. The microinsemination technique is performed under a microscope inverted with Eppendorf and Narishigue micromanipulators and microinjectors. In Vitro Fertilization (IVF) is performed in a five-well plate, depositing 0.5 mL of IVF medium (Vitrolife, Sweden) and 0.5 mL of Ovoil (Vitrolife, Sweden) in each well. Five clusters are left per well and 100,000 sperm/mL are added.

In both cases, the oocytes are cultured in a Thermo-Fisher incubator, within a morphokinetic platform (PrimoVision). The following days are evaluated for their classification, choosing the one of better implantation potential according to the division times measured by morphokinetics and the morphological criteria of the Spanish Association for the Study of Reproduction Biology (ASEBIR) reviewed in 2015.

The embryo transfer will be carried out in group 2 on the third day, choosing the best embryo according to the previous criteria. In Group 1 embryos will be cryopreserved by vitrification, selecting the best one for deferred transfer. The commercial vitrification media will be Medicult Vitrification, and the storage device will be Cryoleaf (McGill Cryoleaf, Medicult, Denmark). All cryopreserved embryos will be stored in liquid nitrogen at -196ºC in storage cylinders (Air Liquide, France). The day before cryotransfer, the embryo is devitrified, using the thawing kit (Medicult Warming, Denmark). At the end, the embryos are washed in G2 medium (Vitrolife), leaving them in cultivation until the following day, after evaluation of embryonic quality and cryosurvival (percentage of lysed cells). The endometrium of the woman is prepared by treatment with estradiol valerate and progesterone. The day of the cryotransfer embryonic quality is assessed again, emphasizing embryonic division. Cryotransfer is done by the same technique than fresh transfer.

Workplan:

First year:
- Our center is routinely able to carry out the vitrification, so it is not necessary to fine-tune our techniques.
- Design of the Access database with the variables to be analyzed.
- Beginning of the interviews and selection of patients
- Beginning of the IVF/ICSI cycles and cryotransfers.

Second year:
- Continue with the selection of patients until completing the expected number.
- Continuation of IVF/ICSI cycles and cryotransfer.
- Carry out an intermediate analysis of the data using "adaptive clinical dessigns".

Third year:
- Continuation of IVF/ICSI and cryotransfer cycles.
- Collection of data from IVF/ICSI and neonatal cycles and application of statistical treatment.
- Drafting of results

STATISTICAL ANALYSIS PLAN

For the purpose of testing the null hypothesis that shows equal live birth rates in the group of women who undergo elective deferred transfer of a single embryo and the group that undergoes elective transfer of a fresh embryo, a power of 80% has been established to detect statistically significant differences using a bilateral Chi-square test for two samples, taking into account that the significance level is 5%, and assuming that the proportion in the group of women undergoing a fresh transfer is 50.9%, the proportion in the group of women undergoing elective deferred transfer is 78%, and that the proportion of women out of the total is 50%, it will be necessary to include 48 women in each group, for a total of 96 experimental units in the study.

Taking into account that the expected dropout rate is three out of ten women, it will be necessary to include 69 women per group.

The assignment of the 138 women to one of the groups will be carried out by a randomized procedure, following a sequence of random numbers generated by specific software (Epidat, Epidemiological Analysis of Tabulated Data Program. Tabulated. Version 3.1)