Prospective randomized control study of two different types of luteal phase support in natural cycle frozen embryo transfer (FET) and its effect on pregnancy rates

The ability to transfer frozen embryos makes it possible to keep frozen the amount of embryos created in in-vitro-fertilization (IVF) treatments for later use. Advantages of frozen embryos transfer (FET) include reduced embryo loss after IVF and a higher pregnancy rate. The rate of frozen embryos transfer has increased in recent years due to a higher tendency of single embryo transfer (SET), use of preimplantation genetic testing, and prevention of ovarian hyperstimulation (1,2). Due to developments of freezing technology and techniques in recent years, embryo freezing enables the advantages mentioned above without compromising the pregnancy rate (3).

There are different methods to prepare the endometrium for FET:

1. Natural cycle (NC) - Timing of ovulation by diagnosing the luteinizing hormone (LH) Surge. The advantage of a NC is the natural preparation of the body for implantation without the need for medicinal intervention. The disadvantage is need of intense monitoring to detect ovulation and inflexibility of day of embryo transfer. As a result, NC results in higher percentages of cycle cancellation.

2. Modified natural cycle (mNC) - Inducing ovulation by administration of Human Chorionic Gonadotropin (HCG) trigger. The advantage of ovulation induction with HCG is greater flexibility and ability to plan transfer day according to the availability of the system, with fewer visits by the patient.

3. Medical - preparation of endometrium with estrogen and progesterone. This method is suitable for women who do not ovulate regularly. This allows flexibility of the treatment with fewer cycle cancellations.

Studies that compared pregnancy outcomes of natural versus adapted NC are inconclusive (4-8). In recent years, several studies showed higher clinical pregnancy and ongoing pregnancy rates in NC group compared to group treated with HCG for ovulation induction (7-8).

Recently, several studies showed advantages of FET in NCs. A study published in April 2020 by XITONG LIU in Fertility and Sterility showed a higher rate of live births

and a reduced rate of miscarriages in NC FET group compared to the drug-treated group (9). Additional studies showed higher rates of gestational hypertension (10 - 11), macrosomia and post-term pregnancies in the medicated FET groups (10).

Endogenous progesterone secreted by the corpus luteum after natural ovulation is expected to provide an adequate luteal support as in spontaneous pregnancies. Therefore, it is assumed that there is no need for luteal support when embryos are transferred in NCs, and the benefit of medical luteal support is not conclusive (12-15). Nevertheless, a meta-analysis published in Human Reproduction in 1202, examined the effect of luteal support with progesterone and showed higher pregnancy rates in the group that received luteal support compared to those who did not receive it (16). It should be noted that most of the studies were performed with induction of ovulation by HCG.

Administration of HCG for luteal support is used both in fresh and frozen cycles. A previous study showed higher ongoing pregnancy rates in a group that received HCG within the day of natural LH surge together with vaginal progesterone from the day of embryo transfer compared to luteal support with progesterone alone. The use of 1500 units of HCG on cycle day 18 and 21 resulted with a significant increase in serum progesterone levels (17).

Administration of Gonadotropin Releasing Hormone (GnRH) analog as a treatment for luteal support in fresh IVF cycles has shown higher clinical pregnancy, multiple pregnancies, and live birth rates (18). When GnRH agonist is used (as single or repeated doses) it may cause an increase in LH secretion by the pituitary gland which augments the function of the corpus luteum and may lead to direct local stimulation of the endometrium [19].

In 2015, a retrospective cohort study from Israel compared the use of progesterone versus administration of HCG and GnRH agonist in NC FET. They showed a higher clinical pregnancy rate in the group treated with GnRH agonist and HCG (20). Another randomized prospective controlled showed that the addition of a GnRH agonist to luteal support with HCG compared with HCG alone in NC FET increased clinical pregnancy and implantation rates when frozen cleavage stage embryos were transferred (21). In 2016, a randomized prospective study from Finland examined the use of GnRH agonists in addition to standard vaginal progesterone support compared to a group treated with progesterone alone in 98 women undergoing NC FET (22). Both clinical and ongoing pregnancy rates were higher in the group treated

with GnRH agonists, but due to small sample size, the difference was not statistically significant.

It is important to note that GnRH agonists are not considered teratogenic (23). In over 340 women exposed to GnRH agonists in the mid-luteal phase, the rate of birth defects and miscarriages (2.5% and 15%, respectively) was similar to the general IVF population (24-26).

There is a need for further research to examine the effectiveness of GnRH agonist treatment as luteal support in in NCs FET. Conducting a prospective randomized controlled study will allow to determine the treatment efficacy of GnRH agonist and HCG in relation to the standard luteal support with progesterone.

The aim of the study is to compare the pregnancy rate between women treated with GnRH agonist together with HCG and standard luteal support with progesterone in the return of frozen embryos in natural cycles and in vitro fertilization treatments.

Primary outcome

- Clinical pregnancy rate – visualization of intrauterine gestational sac on ultrasound divided by number of transfers

Secondary outcomes

- Overall pregnancy rate - number positive bHCG divided by total number of transfers
- Ongoing pregnancy rate – visualization of fetal cardiac activity divided by total number of transfers
- Implantation rate – number of gestational sacs divided by number of embryos transferred
- Ectopic pregnancy rate – number of ectopic pregnancy divided by number of positive bhCG
- Miscarriage rate – number of non-viable before 24 weeks divided by total number of positive bhCG
- Live birth rate – number of live births after 24 weeks divided by number of transfers.

Study methods

Women who undergo natural cycle FET at the IVF center in Shaare Zedek Medical Center will be recruited.

During the visit to the clinic, the women's demographic and clinical data will be collected. The women who are found to be suitable according to the research criteria will receive an explanation of the nature of the study and will sign a consent form for participation in the study in accordance with the procedures of the Institutional Helsinki Committee.

The women can give an agreement until the ET day.

The number of embryos that will be returned will be determined according to the age of the woman, the number of treatment cycles and the quality of the embryos in accordance with the accepted policy in the unit.

The distribution of the patients to the two study groups will be done randomly by computer in a ratio of 1:1. Sealed envelopes containing information regarding the type of treatment for luteal support will be attached to the consent forms.

144 women will be divided into two groups, and each group will receive a different luteal treatment:

Study group – patients will receive luteal support of GNRH agonist andHCG according to departmental protocol:

Cleavage stage embryo:

- ET day (embryo day 2-3) - Ovitrelle 125mcg
- Day 3 after ET - Ovitrelle 125mcg + Decapeptyl 0.1mg
- Day 6 after ET- Ovitrelle 125mcg
- Day 9 after ET - Ovitrelle 125mcg

Embryo blastocyst stage:

- ET day (embryo day 5-6) - Ovitrelle 125mcg + Decapeptyl 0.1mg
- Day 3 after ET - Ovitrelle 125mcg
- Day 6 after ET - Ovitrelle 125mcg

Control group - patients will receive luteal support of vaginal progesterone – 100 mg Endometrin twice daily until week 8 of pregnancy.

Clinical pregnancy will be defined as the demonstration of an intrauterine gestational sac. Other pregnancy outcomes will be received in a telephone conversation with the patients and computer database nine months after the treatment.

Statistical calculations

Based on the clinical pregnancy rate in the prospective study by Bjuresten, et al [13], where a clinical pregnancy rate of 32% was found among women treated with progesterone for luteal support, and given an alpha of 5% and a power of 80%, 144 women (72 women in each group) are required in order to demonstrate a clinical pregnancy rate of 55% in the study group.

Comparison of categorical variables will be carried out using the CHI-SQUARE TEST. Comparison of continuous variables will be performed using the Students t-Test or Mann–Whitney U method depending on the variable distribution (normal vs. non-normal distribution, respectively). A multivariate regression will also be conducted in order to determine which variables are significantly and independently related. Statistical significance will be defined when P values are less than 0.05

Inclusion Criteria

- Normo-ovulatory women

- Women undergoing frozen embryos transfer in a natural cycle

- Age 18-45

- BMI 18-35

Exclusion Criteria

- Women undergoing medicated frozen embryos transfer

- Women with a BMI over 35 or under 18.

- Women with hydrosalpings

- Women with defects or uterine malformations (congenital) or acquired such as myomas

- Egg donation and surrogacy

- Use of preimplantation genetic testing

Pregnant women, children and those lacking judgment will not participate in the experiment.

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