Clinical guidelines

The clinical guidelines are an aid to doctors treating infertility. They should be viewed as the best recommendations available at the time of writing for principles relating to the examination and treatment of infertile couples. The examinations and treatments discussed should be seen as suggestions from among several alternatives, and not as the only options that can be used. Each clinical situation, including the condition of the patient and other information, must be assessed individually by the doctor, who should then act based on his or her experience, knowledge, and evaluation. Medical science is constantly developing. New examination and treatment options may therefore arise. Some of these may be internationally recognised, but not yet assessed in relation to the existing guidelines.

 The NFS guidelines have been prepared in complete independence from pharmaceutical and instrument companies. No direct support has been received from such companies. Please also refer to guideline number 1: Introduction to clinical guidelines.

15. Frozen embryo replacement (FER)

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Definition and scope

Frozen Embryo Replacement (FER) refers to transfer of thawed, fertilised eggs from a previous IVF/ICSI treatment cycle, during a time in the woman's cycle where the endometrium is receptive. FER is used where treatment using fresh embryos has not led to pregnancy, or where the patient has been pregnant, and now wants to have another child. Finally, there will be situations where all fertilised eggs are frozen following egg retrieval, for later use (where there is a risk of OHSS, or in order to preserve the couple's fertility potential prior to an expected cytotoxic treatment such as chemotherapy or radiotherapy).

Recommendations

FET can be carried out through different cycle regimens including: spontaneous ovulatory cycles (natural cycle); cycles in which ovulation is induced by drugs (ovulation induction cycle); and cycles in which the endometrium is artificially prepared by exogenous oestrogen and progesterone (artificial cycle). All regimens seem to be similar in their efficacy. For ovulatory patients, except where necessary for logistical reasons, the natural cycle should be used, as this is the simplest method and leads to the same probability of pregnancy.	A
During FER in a natural cycle, either hCG administration or urine LH testing may be used to determine ovulation timing, as the two methods lead to the same probability of pregnancy.	A
Progesterone supplementation during the luteal phase is recommended for ovulatory and anovulatory women undergoing FER.	A
Anovulatory patients (WHO group I, II, III) and patients with irregular menstruation should be offered FER treatment during a hormone replacement cycle or ovulation induction.	A,B
Hormone replacement FER treatment can be carried out within a window of at least two weeks, as an oestrogenised endometrium will be susceptible to progesterone influence for a prolonged period.	C
Where implantation takes place in a hormone replacement FER cycle, oestradiol and progesterone treatment should be maintained until around 8-10 weeks of gestation.	B
Suppression using GnRH agonist prior to a hormone replacement cycle is not indicated, as the pregnancy rate is not increased and the treatment is more involved and expensive.	A
Frozen embryo transfer is not recommended in a cycle where the endometrium is measured to be less than 5 mm thick, as the probability of implantation will be low. With respect to the receptivity of the endometrium, it seems to be appropriate to start progesterone administration of cryopreserved/thawed cells as soon as the endometrium is developed sufficiently (> or =8 mm, trilaminar pattern), and to perform the embryo transfer not before day 3-4 of progesterone treatment, i.e. embryo development on day 2-3.	B,C
There is evidence of moderate quality that the implantation, clinical and ongoing pregnancy rates of ART cycles may be improved by performing FER compared with fresh embryo transfer because of the asynchrony between the embryo and endometrium in COH cycles.	A
Embryos should only be transferred where at least 50% of the blastomeres are intact following thawing, as there will be a low probability of pregnancy if less than 50% of the embryo has survived. It is also recommended that embryos be cultivated for 24 hours prior to transfer, as transferring embryos which have continued to divide following thawing leads to a higher pregnancy rate.	C
The maximum of transferred embryos should be two.	A
Assisted hatching in connection with FER treatment cannot be recommended as there is no evidence that the procedure increases the birth rate.	A

Introduction/background

Cryopreservation of surplus embryos following egg retrieval during IVF/ICSI treatment makes it possible to store these for a period of time, such that a single aspiration may result in several transfers, and thereby be fully utilised. This approach offers several advantages. Firstly, the pregnancy rate per aspiration is increased, and the patient is spared having to undergo a new treatment process and subsequent egg retrieval. Secondly, the number of embryos transferred on each occasion can be limited, without impacting on the patient's overall probability of achieving pregnancy and also reducing the risk of multiple pregnancy. The risk of developing OHSS among susceptible patients will thereby also be reduced. An effective cryopreservation program is dependent on the quality of the embryos frozen, the age of the woman, the freezing and thawing procedures, the receptivity of the endometrium at the time of transfer, and a sufficient luteal phase.

Literature review
Searches were carried out in:
1. PubMed
2. The British guidelines: “Fertility. Assessment and treatment for people with fertility problems”, Clinical guidelines, February 2004, National Institute for Clinical Excellence.
3. The Cochrane library
4. The American society of reproductive medicine guidelines: “Progesterone supplementation during the luteal phase and in early pregnancy in the treatment of infertility”, an educational bulletin, 2008

There are three ways to ensure that development of the endometrium and the embryo thawing process are synchronised:

1. by monitoring endometrium development in the patient's natural cycle
2. by stimulating endometrium growth in an oestradiol/progesterone replacement cycle
3. by using ovulation induction for endometrial preparation

Cycle regimens
In Cochrane Database of Systemic Review (2008) seven randomised controlled trials including 1120 women were determined whether there is a difference in outcome between natural cycle FET, artificial cycle FET and ovulation induction FET. The authors´ conclusion was that at the present time there is insufficient evidence to support the use of one intervention in preference to another (1) (evidence level 1a).
Another study in which 4470 frozen ET cycles between 2006 and 2010 were compared in three different Scandinavian clinics showed that there were no differences in clinical pregnancy and delivery rates between natural cycle followed by progesterone (NC + P, 26% of cycles), natural cycle with hCG (NC + hCG, 10% of the cycles) or substituted cycle with oestrogen and progesterone (E + P, 64% of the cycles). A higher positive pregnancy test rate was obtained in E+P (34.3%) and NC + hCG (35.5%) cycles as compared with the NC + P cycles (26.7%). However, the clinical pregnancy rate (27.7%, 29.1%, and 24.3%, respectively), and the life-birth rate (20.1%, 23.5%, 20.7%, respectively.) were similar in all groups (2) (evidence level 2a).

There are contradictory results comparing frozen blastocyst-stage transfer cycles in natural cycle or in exogenous hormone stimulation. A retrospective study for 648 cycles (611 patients) who underwent blastocyst FER using either the natural cycle (n=310), the natural cycle with ovulation induction employing human chorionic gonadotropin (n=134), or a hormonally manipulated artificial cycle with oestrogen and progesterone supplementation (n=204) showed that employment of natural cycles with (PR 41.8%) or without (PR 41.9%) hCG treatment was associated with better clinical pregnancy rates than was the use of hormonally manipulated cycles (PR 30.4%)(P=0.006) (3), (evidence level 2a). A contradictory result became from a retrospective cohort study of 1391

frozen-thawed blastocyst-stage embryo transfer cycles where GnRh agonist followed by estrogen and progesterone was compared with natural protocol. The life birth rate for synthetic protocol when two embryos were transferred was 32.3% vs 20.4% in natural cycle (RR 1.58; 95% CI, 1.22-2.06) (4) (evidence level 2a).

Hormone replacement FER cycles are used for anovulatory patients, patients with irregular menstruation or also for the treatment of ovulatory patients for planning purposes (e.g. in clinics which are closed on the weekend). The transfer time may be planned days or weeks in advance, as the endometrium will be receptive for several weeks during continued oestrogen treatment, once it has reached the desired thickness (5,6) (evidence level 3). Oestrogen treatment should be initiated immediately when menstruation begins, and the patient should undergo an ultrasound scan, not only to ensure adequate endometrium thickness, but also to ensure there are no signs of follicle growth in the ovaries.
A number of randomised clinical studies have been carried out to compare hormone replacement FER cycles with or without GnRH treatment. In Cochrane Database Systemic Review 22 RCT were included to evaluate the most effective endometrial preparation for women undergoing transfer with frozen embryos or embryos from donor oocytes. Five studies analysed the use of GnRh agonist versus control. No significant benefit was demonstrated when using GhRh agonists (7) (evidence level 1a).

The third, not so common method to ensure the synchronization of endometrium and embryo is to use ovulation induction. Two hundred and seventy patients (n=270) between 28 and 40 years of age undergoing IVF due to bilateral tubal blocks were included in a study in which three different protocols preparing endometrium was compared. All of the patients had a previous unsuccessful single IVF attempt or a postponed embryo transfer due to the threat of ovarian hyperstimulation syndrome or poor endometrial development. One hundred patients had endometrial preparation by gonadotrophin-releasing hormone agonist down-regulation and with hormone replacement therapy, 55 had natural cycle FET, and the remaining 115 patients had letrozole-induced ovulation induction for endometrial preparation. The clinical and biochemical pregnancy rate or live birth rate was higher in the letrozole group than in the other groups. So, the use of ovulation induction is also an alternative for endometrial preparation for anovulatory patients when the use of hormone replacement for different reasons is difficult to use (8) (evidence level 2a).

Endometrial thickness and receptivity
It is not completely clear what effect the thickness of the endometrium has on implantation.
An Israeli overview based on 27 cohort and observational studies found insufficient data for a correlation between endometrium thickness and the probability of implantation during IVF cycles. The median endometrium thickness was the same (8.6-12.0 mm) for cycles where implantation failed or was successful. However, a study based on 1605 cycles in 13 studies found that no pregnancies occurred where the endometrium thickness was < 5 mm (9) (evidence level 2b-3).
In a meta-analysis of three trials for 633 cycles in women aged 27-33 years showed that FET resulted in a significantly higher ongoing pregnancy rates compared with the fresh embryo transfer, possibly because of the asynchrony between the embryo and endometrium in COH cycles. It may be advantageous to cryopreserve all viable embryos and use them in a subsequent FER in normal and high-responder patients if the method of cryopreservation is well-functioning (10) (evidence level 1a).

Luteal support
The necessary or optimal duration of supplemental P therapy has not been established firmly. Evidence derived from the classic luteectomy studies indicates that P supplementation is most important during the first 5 weeks after conception (7 weeks' gestation) and almost certainly unnecessary beyond 7 weeks after conception (9 weeks' gestation) (11,12) (evidence level 2b).
Progesterone replacement may be given in the form of vaginal tablets containing micronised progesterone or vaginal gel. Both are equally effective (7) (evidence level 1a). It seems to be appropriate to start progesterone administration of cryopreserved/thawed cells as soon as the

endometrium is developed sufficiently (> or =8 mm, trilaminar pattern), and to perform the embryo transfer not before day 3-4 of progesterone treatment, i.e. embryo development on day 2-3 (13) (evidence level 3).
There are varying practices among clinics using progesterone supplementation if an embryo is transferred during natural cycle. Generally, there is a belief that endogenous production of progesterone is sufficient to support implantation in a natural cycle. Bjuresten et al showed in prospective randomized study (n=435) that progesterone supplementation (400 mg micronized vaginal progesterone twice a day) starting from the evening of the embryo transfer increased significantly (P=0.0272) the live birth rate (LBR 30%) compared with no progesterone (LBR 20%) supplementation at all. There were no differences in early miscarriage rate, clinical pregnancy rate, or spontaneous abortion rate between the groups. Vaginally administered progesterone increases serum concentration to a peak level after approximately 8 hours and thereafter there is a gradual fall during the next 8 hours. Therefore, progesterone was given twice a day (14, 15) (evidence level 1b).
In Cochrane analysis (2011) of luteal support for assisted cycles sixty-nine studies with total of 16 327 were included. The review showed a significant affect in favour on progesterone for luteal phase support versus placebo or no treatment. The addition of other substances such as oestrogen or hCG did not seem to improve outcomes. There was no evidence favouring a specific route or duration of administration of progesterone (16) (evidence level 1a).
In a retrospective study (n=346) of FER in hormone replacement cycle showed that doubling the dose of vaginal progesterone from 90 mg (Crinone) once a day to twice a day significantly decreased the early pregnancy loss rate (67.4% -> 43.7%). This resulted in a significantly higher delivery rate (20.5% versus 8.7%, respectively) (17) (evidence level 2a).

Number of embryos per transfer
Embryos will often be unusable following thawing. In practice, only around 80% of all thawed embryos will be transferred (18). It has been shown earlier that the probability of implantation is significantly correlated to embryo quality before and after thawing, including whether the thawed embryos have divided prior to transfer (18,19) (evidence level 3).
A retrospective Danish study (20) analysed the effect of +/- division for thawed embryos following 24 hours cultivation prior to transfer in 701 cycles involving frozen embryos. 459 transfers involving embryos which had divided (defined as at least one divided embryo) resulted in an implantation rate of 10% – significantly more than for the group where embryos which had not divided were transferred (n=153, ~ 4%, P= 0.0003). 130 pregnancies were achieved (28% per transfer) in the first group, compared to only 17 pregnancies (11% per transfer) in the group without division (P= 0.0001). However, the average number of embryos transferred was significantly higher in the group with the embryos which had divided (2.46 +/- 0.03 versus 1.82 +/- 0.07) (evidence level 3). Another retrospective study involving 891 FER transfers in the period 1998-2003 found no pregnancies where blastomere survival was less than 50%, and that the probability of pregnancy was greater, the more intact the embryo was. The survival of 25-50% of the blastomeres led to implantation and pregnancy rates of 3.2% and 3.2% respectively, while > 75% survival lead to a significant increase to 17.3% and 16.6%, respectively (P = 0.007) (21) (evidence level 3).
A retrospective Finnish study involving 1647 FER transfers between 1998-2003 found an overall birth rate of 22.6% per FER – significantly higher for DET (25.7%, versus 19.2% for SET), but the multiple pregnancy rate was 21.9% among the 872 women who underwent DET. With the introduction of eSET, the difference in birth rates compared to DET disappeared (25.7% versus 28. 6%) (22) (evidence level 3). The above retrospective study of 891 FER cycles (21) found a pregnancy rate of 19.6% following DET. As already mentioned, the embryo quality, age of the women, and previous treatment outcomes are the major factors determining the number of implantations where more than one embryo is transferred under FER treatment, as is the case for the transfer of fresh embryos.
In a meta-analysis of individual patient data (n=1367) from randomised trials where the effectiveness of elective single embryo transfer versus double embryo transfer was compared showed that although the strategy of single embryo transfer yields to a lower life birth rate than a double embryo transfer in a fresh IVF cycle (27% v 42%, OD 0.50, 95% CI 0.39 – 0.63), this

difference is almost completely overcome by an additional frozen single embryo transfer cycle (cumulative LBR 38% v 42%) with a minimal cumulative risk of multiple birth (1% v 32%, OD 0.85, 95% CI 0.62 – 1.15) (23) (evidence level 1a).
The number of frozen embryos transferred should therefore be based on the same guidelines as apply to the transfer of fresh embryos. These factors should also be considered in the decision regarding how many embryos to freeze in the same batch.

Does assisted hatching (AH) increase implantation in relation to FER treatment?
In a Cochrane review (2012) of assisted hatching, 31 RCTs was analysed involving a total of 1992 clinical pregnancies in 5728 women. There was no significant difference in the odds of live birth in the AH group compared with the control group (9 RCTs; OR 1.03, 95% CI 0.85 – 1.26), with no evidence of significant heterogeneity (P= 0.38) or inconsistency (I(2) = 6%). Analysis of all of the studies showed that the clinical pregnancy rate in women who underwent AH was slightly improved, but the level only just reached statistical significance (OR 1.13, 95% CI 1.01 – 1.27). However, it is important to note that the heterogeneity for this combined analysis for clinical pregnancy rate was statistically significant (P= 0.001) and the I(2) was 49%. However, sub-analyses of women who had had previous failed attempt at IVF found improved clinical pregnancy rates in the women undergoing AH compared with the women in the control group (9 RCTs, n=1365; OR 1.42, 95% CI 1.11-1.81) with I(2) = 20%. Miscarriage rates per women were similar in both groups (14 RCT; OR 1.03, 95% CI 0.69-1.54, P=0.90). Multiple pregnancy rates per women were significantly increased in women who were randomised to AH compared with women in the control group (14 RCT, 3447 women; OR 1.38, 95% CI 1.11-1.70, P=0.004) (24) (evidence level 1a).

Is there a greater risk of malformations following transfer of frozen embryos?
This issue lies outside the scope of these guidelines, but based on the knowledge available from several large follow-up studies, embryo freezing does not adversely affect perinatal outcome. Birth weight of singletons born after cryopreservation of embryos is higher compared with singletons born after fresh embryo transfer (25-32) (evidence level 1a).

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