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EMBRYO TRANSFER

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Introduction

The ultimate objective of any ART program is the birth of an healthy live baby. This outcome is based on multiple parameters. These could be aptly summarized under the following headings:

1. Aetiology of infertility
2. Effective Controlled ovarian hyperstimulation protocol resulting in optimum follicular development
3. Efficient oocyte retrieval with adequate number of mature eggs
4. Proper embryological technique resulting in high quality embryos
5. A highly receptive endometrial lining
6. A very skilled atraumatic embryo transfer
7. Adequate luteal support
8. Competent antenatal management.

Most of the above mentioned parameters are more or less standardized and are operator independent. However embryo transfer technique is still very much a skill based technique. Successful outcome is dependent on the proper intrauterine a traumatic transfer of high grade embryos.

Procedure of Embryo transfer

1. Trial or Mock or Dummy or Sham Embryo transfer:

There is a wide variation in the cervical & uterine anatomy of patients. Hence a trial or practice embryo transfer done in a cycle preceding the IVF procedure is beneficial. Mansour et al (1) demonstrated a 22.8% pregnancy rate with previous trial transfer as compared to 13.1% pregnancy rate in the no trial transfer group. This transfer can be done using either a dummy catheter, or a small steel dilator. Alternately it can be done using a sterilized previously used ET catheter. It is important to map the length & direction of the uterine cavity. These points are noted for future use during the actual ET procedure. In case of difficult trial transfers due to stenosis one can subject the patients to a hysteroscopy & if need be, a dilatation of the cervix. This may be done as an office procedure, requiring minimal or no anesthesia.

2. Hysteroscopy prior to the IVF cycle

Hysteroscopy is the best method to directly visualize the uterine cavity. It can also be simultaneously used to

treat intrauterine abnormalities. This can be done in cases where there is a high suspicion of uterine or cervical pathology including those with a history of having repeated difficult intrauterine inseminations or embryo transfers. Balmaceda et al (2) have shown that up to 45% of patients undergoing IVF had a detectable uterine abnormality. These abnormalities included endometrial polyps, submucous leiomyomas, uterine malformations & cervical stenosis. Shamma et al (3) studied 28 patients with normal findings on HSG ,using office hysteroscopy. 43% of these patients had abnormal findings such as small uterine septa, small submucous fibroids, uterine hypoplasia & cervical ridges. There was a significant difference in pregnancy rates (37.5% normal v/s 8.3% abnormal findings).

The author routinely performs hysteroscopy in the cycle preceding the IVF cycle. However there is no evidence or prospective randomized study as yet to suggest that all infertile patients, especially those undergoing IVF, will greatly benefit from a hysteroscopy done in the previous cycles.

3. Timing of Embryo transfer:

Historically, routine embryo transfer has been carried out on Day 2 about 48 hours after oocyte retrieval. However, delaying the ET till Day 3 has shown to have no detrimental effect on pregnancy rates. In fact it may be of benefit in selecting embryos with a better prognosis for development. If there are 3 or more good quality embryos on day 3(6 to 8 cell blastomeres with fragmentation less than 20 %), one can grow the embryos till Day 5, when they would reach the Blastocyst stage. Although the success rate of blastocyst transfer is in the region of 50 to 60%, as many as 30 % of patients may fail to develop blastocysts, if the above mentioned selection criteria is not rigorously applied. In case the embryos are not of good grade on Day 2, it is best to transfer them back on the same day, instead of waiting for Day 3 or Day 5 transfer.

4. Grading of embryos:

It is important to select the best possible embryos for transfer. This is very important step for the success of an ART program. The only conclusive markers available to date are based on cleavage speed & morphological appearances of PN stage, 2cells stage, 4 cell stage, and 8 cell stage & blastocyst stage embryos. Thus selection of good embryos is based on the following criteria:

a. Pronuclear formation & nucleolar alignment: The markers at the 2 PN stage for the best embryo include:

   1) The abutment & proximity of two pronuclei
   2) The alignment in vertical rows of the nucleoli of each pronucleus
   3) The appearance of a cytoplasmic clear halo around the pronuclei
   4) Early appearance of the first cleavage, 2 cell stage,at around 24 to 26 hours post insemination. ICSI embryos are known to cleave earlier than IVF embryos.

b. Cleavage speed: Embryos that cleave early to the 2 cell stage, 24 to 26 hours post insemination or become 4 cells at 44- 48 hours post insemination, have a higher implantation potential.

c. Morphological Grading of Day 2, day 3& Day 5 embryos.
Grading of Day 2 embryos are based on the following criteria:

   a. cell number:1 to 8 cells
   b. fragmentation:1 to 100%
   c. Blastomere regularity
   d. Presence of multinucleated blastomeres

Granularity of blastomere cytoplasm is not considered, as it is not consistent with quality & is dependent on the type of optics used by the microscope.

Grading of Day 2 embryos

1. Grade 1 : equal size blastomeres with no fragments
2. Grade2 : unequal or irregular blastomeres with no more than 10 % fragments
3. Grade 3 :10 to 50 % fragmented or non viable blastomeres
4. Grade 4 : fragmentation or loss of more than 50 % blastomeres, but with at least one viable blastomere.

Normally ET of Grade 1 & grade 2 embryos yield similar results as compared to poor pregnancy rates with ET of grade 3 & grade 4.

Grading of Day 3 embryos

The grading system of day 2 embryos cannot be applied to day 3 embryos. Normally Day 3 embryos with less than 20% fragments are classified as good embryos. These good embryos are considered to have a good implantation potential. Bad embryos with >20 % fragments, do not yield good pregnancy rates.

Grading of blastocyst

In general any blastocyst transferred on Day 5 would result in a good pregnancy rate. The ideal blastocyst is the one that has cavitated or expanded on Day 5, has a distinct inner cell mass (ICM),a well laid down trophectoderm(TE) with sickle –shaped cells, a thin zona pellucida & a high total cell number(TCN). It is preferable to transfer blastocyst when hatching has just started .For zona free blastocyst transfer, the ability for the blastocyst to retain its cavity without collapse after pronase treatment is an added marker of good quality.Although good blastocyst can also be obtained on Day 6 , they are not given priority for ET if adequate Day 5 blastocyst are available. If there are no Day 5 Blastocysts, a transfer of Day 6 blastocyst would still result in a 30 % clinical pregnancy rate

Different grading systems have been proposed for scoring human blastocysts.

Gardners(4) system of grading Blastocysts

BC are given a score from 1 to 6 based on the degree of expansion & hatching status

Grade 1 : Early BC with cavity (blastocoel) less than half the embryo volume

Grade 2 : BC with more than or equal to half the embryo volume

Grade 3 : A full BC with blastocoel completely filling the embryo

Grade 4 : an expanded BC with cavity volume larger than early embryo with a thinning zona

Grade 5 : A hatching BC with trophectoderm starting to herniated through the zona

Grade 6 : A hatched BC with complete escape of BC from zona

Blastocyst of Grade 3 or more are assessed for Inner Cell Mass (ICM) development:

Grade A : Tightly packed, many cells

Grade B : Loosely packed, several cells

Grade C : very few cells

Blastocyst of Grade 3 or more are assessed for trophectoderm:

Grade A : Many cells forming a cohesive epithelium

Grade B : few cells forming a loose epithelium

Grade C : very few large cells

Using this grading system it was shown that clinical pregnancy rates in excess of 60 % could be attained by the transfer of at least one high scoring blastocyst equal to or greater than 3AA.

5. Number of Embryos to be transferred

Most ART units transfer the 3 best good grade embryos. In patients with repeated implantation failures, older patients or in patients with poor grade embryos, more number of embryos (4 to 5) may be transferred. Although there is an increase in pregnancy rates with transfer of three or more embryos, there is also a corresponding increase in multiple births & associated complications. Presently, there is an increasing trend to transfer two or even single "Top" embryo. This can be done in patients with good prognosis (young age, tubal infertility only, past history of pregnancy or delivery)As mentioned previously, if there are many good quality embryos on Day 3 , one can go for blastocyst culture, select a single 3AA or higher grade of Blastocyst(5) for transfer & freeze the remaining blastocysts. This would probably be the way to go, in the near future.

6. Ultrasound guidance

The use of transabdominal ultrasound guidance during ET has many advantages over blind clinical touch ET. This has been supported by many prospective randomized trials. . There was a high pregnancy rate of 50 % in the ultrasound guided ET as compared with the clinical touch group of 33.9 % studies. (corleu et al 2000)(6) .The patient is asked to not pass urine for about an hour and a half, prior to the ET. Generally a semi full bladder is preferred to a very full bladder. Besides causing major discomfort & a possible incontinence on the ET table, a full bladder can flatten the utero cervical axis. This may result in the inability to pass the embryo transfer catheter through the endo cervical canal. An overfull bladder may also cause difficulty in the visualization of the uterine cavity. In such conditions, the patient is asked to half empty her bladder. Alternately, she could be catheterized on table. There is a risk of causing bladder infection, as one cannot use an antiseptic prior to catheterization. The patient is allowed to empty her bladder 15 minutes after embryo transfer and remains at bed rest for no more than an hour.

Advantages of USG guided ET:

a) Direct visualization of the whole ET process

b) Proper placement of the embryos 1 to 1.5 cm below the fundus.

c) Confirmation of the retention of the embryos at the spot of deposition of Embryos. The small air bubble (5 microlitre volume) which is expelled with the embryos is called the transfer bubble. Retention of this bubble at the site of transfer is considered to be favorable.

d) Correction or straightening of cervicouterine axis, by the full bladder helps transfer, especially in an acutely anteverted uterus.

e) USG helps with the insertion of the soft catheter. When the placement is difficult, the problem can be visualized and modification of angle between the cervix & uterus can be accomplished by the manipulation of the speculum or usage of an artery forceps to redirect the tip of the catheter.

f) USG can prevent the catheter from digging into the endometrium or tubal ostia, which could stimulate junctional zone contractions. These contractions can expel the embryos out of the uterine cavity, either out of the cervix or into the fallopian tube causing ectopic pregnancies.

g) Recently Woolcott & Stanger (7) have recommended the placement of embryos in the lower uterine cavity at a depth of 5 to 6 cm from the external cervical os., there being a belief that this increases the pregnancy rates. USG can help in this objective.

h) USG identifies the curling of the catheter with the tip pointing to the cervix. This is found in difficult transfers, especially when there is resistance to the catheter & the clinician tries to force the catheter through, without a stylet. Blind catheter transfer has shown to result in inadvertent location of the catheter tip outside the endometrial cavity in 25 % of cases. (woolcott et al 1997)(7)

i) It also helps the clinician to avoid hitting the fundus with the catheter, and enables him to confirm that the catheter tip has passed the internal os by at least 1 cm prior to injection of embryos. This is particularly significant in patients with a long endocervical canal.

j) The patients ovarian status can be reevaluated to make sure that the risk of OHSS is not great & that the ET can be accomplished safely.

k) High power USG machines can visualize the intensity, frequency & direction of endomyometrial contractions.

l) Colour Doppler USG prior to ET can be used to score the endometrium (Applebaums score) & thus predict successful implantation.

Thus ultrasound helps in the traumatic & bloodless placement of the embryos 1-2 cm from the uterine fundus, into the lumen of the endometrial cavity without inducing uterine contractions.

Disadvantages of USG guided ET

a. Discomfort of maintaining a semi full bladder

b. Difficult visualization in cases of overfull bladder. This is commonly seen in patients who have originally a retoverted uterus

c. Overfull bladder can cause uterocervical compression, resulting in resistance to the catheter placement, at the internal os.

d. During the procedure the catheter tip may sometimes not be clearly visualized. This is seen in patients who are obese, have previous surgical scars on the abdomen, or who have uterine fibroids or in retroverted uterus. The operator may try to locate the catheter tip for a longer than normal period. This can cause inadvertent trauma to the endometrium, resulting in blood on catheter tip and a traumatic transfer. In such cases, it is best to go by the findings of previous mock transfer & transfer the embryos blindly by the touch technique.

e. Some studies have shown no improvement in pregnancy rates with ultrasound guided ET (Al –Shawaf et al)(8). Kan et al (9) failed to find any major difference in USG guided ET except in older women & those with difficult transfers

7. Embryo transfer Catheter type:

There are various catheter designs available:

a) Soft (wallace) or stiff catheter(frydman)

b) Single(frydman) or double-coaxial outer sheath with an inner catheter(Wallace, Cook, labotect)

c) End or side opening

d) Quality of material

e) Finish: rounding of catheter tip edge v/s a sharp edge

f) Proper washing, sterilization & embryo toxicity testing of the catheter.

The common catheters used in clinical practice are:

a) Edward Wallace coaxial catheter

b) Labotect coaxial catheter

c) Cooks trans soft coaxial catheter

d) Cooks SIVF catheter

e) Frydman catheter

f) Tomcat catheter

The benefits of one catheter over the other are controversial. Wisanto et al (10) in a retrospective study of 400 patients found better pregnancy rate with Frydman(32%) as compared to Wallace (19%) or TDT(19%). On the other hand Al –Shawaf (8) did not find any difference between Frydman(31%) & Wallace (31%).

Soft catheters such as Wallace allow the catheter to gently follow the curvatures of the uterocervical canal thus minimizing trauma to the endometrium. However their extreme flexibility makes them a liability in difficult transfers. On the other hand catheters with rigid outer sheath make catheter placement much easier. However their rigid nature may cause bleeding, mucous plugging, retention of embryos & uterine contractions- factors which may hamper pregnancy rates.

On the whole, variations of soft catheter like the Edgar Wallace catheter is used in many ART programs. In cases where the mock transfers have been easy, one can use the soft catheters for transfer. In patients with difficult mock transfers or previous difficult Embryo transfer one can use the catheter system with more rigid outer sheaths, or a TDT system with a malleable mandarin can be used. Alternately one can use a Wallace catheter with a malleable stylet. This catheter can be negotiated beyond the internal os. The stylet is then removed with the outer sheath left in place. The inner embryo transfer catheter loaded with embryos is then threaded under USG control till the tip is placed in the uterine cavity.

The author is used to the Labotect catheter, which has a rigid outer sheath. The outer sheath is having distal markings at 1 cm intervals from the tip .The inner catheter has also got proximal markings at 1 cm interval. The embryos are loaded in the inner catheter. This catheter is then withdrawn into the outer sheath. The outer

sheath is opaque. This prevents any damage to the loaded embryos from the light in the embryo transfer room. The outer sheath is passed gently through the cervical canal till it just lodges into the canal just below the internal os. There is no effort made to pass beyond the internal os. There is thus no bleeding & trauma that is associated with such catheters. The inner catheter is gently threaded into the uterine cavity. This protects the inner catheter from mucous plugs. At the same time if there is difficulty in passing the inner catheter, the inner catheter is withdrawn into the outer catheter, the outer sheath is negotiated beyond the internal os & then the inner catheter is re-advanced.

The ultimate decision of catheter make is based on the skill, experience & catheter familiarity of the clinician performing the ET.

8) Loading of Catheter

The 1 ml tuberculin syringe which is used for loading the embryos should be nontoxic & sterile. If possible, one should use a two piece syringe, which does not contain the rubber piece which is at the tip of the plunger. The rubber is the source of toxicity. The syringe is flushed with 1 ml culture media, prior to use. This helps in washing of the toxic particles in the syringe. The syringe is now filled with 0.8 ml of media & the air bubbles are dispelled. The filled syringe is attached to the hub of the inner embryo transfer catheter. The entire medium is dispelled out rinsing the lining of the inner catheter. This result in the entire inner catheter filled with media, without the existence of any dead space. The embryos are loaded in a continuous column of total 30 microlitres of transfer media, taking care to keep the embryos preferentially toward the tip of the embryo column closest to the catheter tip. An 5 microlitre air column is taken at the tip to visualize the catheter tip on USG during transfer as well as visualize the transfer bubble in the uterine cavity after transfer. If a larger volume of more than 30 microlitres is taken during transfer, the embryos may get expelled out of the cervix.

During the transfer of embryos into the cavity, a steady pressure should be exerted on the syringe piston, for ejecting the embryos. A sudden thrust may result in damage to the embryos or lodging of embryos into the fallopian tube.

After the expulsion of embryos one can wait for 30 to 60 seconds before withdrawing the catheter. A quick withdrawal after transfer is thought to trigger uterine contractions.. Martinez et al (11) compared immediate withdrawal with withdrawal after 30 seconds wait. There was no difference in outcome.

While withdrawing the catheter after transfer, a steady pressure is maintained on the piston. This prevents the sudden suction of embryos back into the catheter. It is also advocated to rotate the catheter through 90 degrees in its axis before withdrawing it gently from the uterus.

9) Transfer medium

It has been postulated that higher concentration of proteins in the culture medium would be better for embryo transfer. However a study analyzing pregnancy outcomes with different concentration of proteins in transfer media did not demonstrate a difference in pregnancy rates. Replacing proteins with newer macromolecules such as hyaluronan have been found to improve pregnancy rates. The newer embryo transfer media such as embryo glue (vtirolife) & universal transfer medium (medicult) have incorporated the hyaluronan molecule in their composition, with excellent outcomes.

10) The removal of Cervical Mucus prior to Transfer

Cervical mucus can cause the plugging of the catheter tip during transfer. This can lead to retention of embryos in the catheter& embryo damage in case the embryos have undergone assisted hatching prior to transfer. It can also cause improper embryo placement as well as contamination of the endometrial cavity & embryos. (Egbase et al). (12)

There are various ways of removing the cervical mucus:

a) Gentle aspiration of the mucus with a tuberculin syringe. During aspiration, care should be taken not to suck or abrade the cervical mucosa, which may start bleeding.

b) One can also use a sterile Q tip (similar to an ear bud) dipped in culture media to clean the mucus from the cervix.

c) One can vigorously lavage the cervical canal with culture media, taking care not to push the media into the uterine cavity. This can be done making use of a tuberculin syringe filled with culture media. The endocervical canal can be cleaned up by using an IUI catheter or a previously used but resterilised ET catheter mounted on an tuberculin syringe, filled with 1 to 2 ml of media. (sallam 2000)(13)

11) Difficult Embryo Transfer

The various events that can be classified as difficult ET are:

1. Difficulty to negotiate the catheter into the uterine cavity
2. Use of a tenaculum or volsellum during transfer
3. Inability to remove the cervical mucous plug during transfer.
4. Need for cervical dilatation due to inability to pass the catheter
5. Need for transmyometrial uterine transfer due to impassable cervix
6. Touching the fundus with the catheter
7. Bleeding during transfer or blood on the tip of catheter following transfer.
8. Retention of embryos in the catheter following ET, thus necessitating a repeat ET.

Difficult ET was associated with very poor pregnancy rates (4%) as compared to easy transfers (20%).

Difficult embryo transfer may provoke sub endometrial-myometrial contractions.. If there are more than 4 uterine contractions per minute, on ultrasound, there is a lowering of pregnancy rate. (fanchin et al).(14)

Normally the following sequence of events is followed by the author in case of difficult transfer:

a) All ETs are done under ultrasound guidance as a rule. Manipulation of cervix with speculum & or guiding of the catheter tip with long artery forceps or ring forceps is done to negotiate the internal os, wherever necessary.

b) In case of previous history of difficult embryo transfer or difficult mock transfer, one can use an outer catheter sheath with a malleable stylet or mandril. Once this catheter is negotiated under USG guidance into the ideal spot, the inner ET catheter is loaded with the embryos, the mandril is removed, & the inner catheter is threaded in, & ET is performed.

c) In case the catheter cannot pass through the canal, one can hold the anterior lip with a tenaculum or ellis & gently tract on the cervix. This helps in straightening out of the uterocervical canal. One should not clamp the tenaculum, as the pressure will create immense pain & muscular contractions. According to lesny et al, (15)1999, tenaculum can release prostaglandins, cause uterine contractions & decrease pregnancy rates

d) A constant watch is kept on the time, once the embryos are loaded into the ET catheter. In case the embryos are not replaced into the uterine cavity 2 minutes from the time of loading, they are returned to the laboratory & replaced in the Petri dish to prevent cooling effect & ph damage to the embryos. (brinsden et al) (16)

e) If everything else fails, one may have to resort to cervical dilatation. This may be done without anesthesia or under sedation or mild general anesthesia. In case the patient is not starving, one can wait for a few

hours & then do the dilatation & ET. The dilatation is done with 1mm or 2 mm diameter steel dilators. Alternately one can do it with rigid catheters like labotect or TDT or soft catheters like Wallace with malleable mandarin (stylet). Groutz et al (17) achieved a single pregnancy amongst 41 patients who underwent cervical dilatation prior to ET.

F) In extreme cases, where the cervix is impassable, one can go for transmyometrial embryo transfer. (kato etal)(18)The ET is done using a Towako set(Cook Australia)The patient is given general anesthesia & the prepuncture preparations are similar to an oocyte retrieval procedure). An 18 gauge needle with stylet is passed under transvaginal sonographic guidance. The needle is advanced through the myometrium till it just reaches the endometrium. The stylet is removed & The ET catheter loaded with embryos is passed through the needle, so that the tip projects into the endometrial cavity.. After release of the embryos, the catheter is withdrawn & checked.

G) In case there is difficulty in passing the cervix & the tubes are normal, one can do a laparoscopic transfer of the embryos into the fallopian tube (Tubal embryo transfer or TET)

H) In worst case scenarios where one has achieved dilatation, but caused a lot of bleeding, one can retransfer the embryos into long term culture media & transfer the embryos the next day.

The presence of blood on the catheter or bleeding from the cervix is poor prognostic sign for a successful pregnancy outcome. (Englert et al)(19)Blood on the catheter in itself implies a difficult transfer. Embryos can get trapped in the blood & hamper implantation. Release of prostaglandins may cause contractions. Gaudas et al (20) demonstrated a 7 fold decrease in pregnancy rates, if there was blood on the catheter.

Similarly, touching the fundus with an ET catheter can decrease the pregnancy rates.. Waterstone et al (21) demonstrated a 24 % pregnancy rate when the fundus was intentionally touched by the ET catheter, then withdrawn by 0.5 cm prior to expulsion of the embryos. On the other hand a routine injection of the embryos 5 cm from the external os without touching the fundus achieved a 46 % pregnancy rate. A preliminary dummy transfer, ultrasound controlled ET & use of a soft catheter, can reduce the chances of clinicians touching the fundus.

12) Retained embryos

In the early luteal phase the uterine contractions tend to be cervico fundal. This can sometimes result in ectopic pregnancy. Occasionally there may be alteration in the normal pattern of contractions, resulting in expulsion of embryos outside the cervix. (poindexter et al)(22).

Embryos may be retained in the catheter tip due to many reasons:

a) Plugging of catheter tip with inadequately removed cervical mucus.

b) Inadequate transfer volume

c) Failure to place the embryos at the top of the embryo fluid column.

d) Reverse suction on the catheter tip while withdrawing the catheter after transfer.

13) Rest after Embryo transfer;

Generally the embryo transfer is carried out in volumes less than 30 microlitres. This small volume, coupled with sticky secretions of the endometrial lining & the contractile peristaltic forces of the apposing endo myometrial surfaces result in the proper retention of embryos. A standing patient coupled with gravity is not adequate enough to expel the embryos out of the uterus. Many studies have shown that immediate getting up of the

patient, does not in any way affect the result. Hence a patient can easily walk home following 15 to30 minutes of rest, following ET. (sharif et al)(23)

14) Methods to reduce uterine contractions:

Some people advocate that ET be done under General anaesthesia. Others advocate that indomethacin be administered orally, one hour before ET.

A relatively atraumatic ET, under USG guidance, without touching the fundus, along with usage of Soft ET catheter, and transfer volumes of less than 30 ul will minimise Uterine contraction.

However till date progesterone (oral/vaginal/intramuscular) has been the only drug that has been shown to relax the uterus. This may be one of the reasons for greater success in Blastocyst culture, where the uterus is quiescent after 7 days exposure to progesterones , prior to transfer.

Pre ET instructions to patient:

1. Not to come starving for ET, except when Et is being planned under General anaesthesia.
2. Not to pass urine 1 hour prior to ET
3. Not to insert the progesterone pessary, 2 hours prior to ET
4. Not to shave, prior to ET. The patient can shave herself, at the beginning of gonadotrophin stimulation.
5. Not to wear perfumes, talcum powder etc on the day of ET procedure..

Procedure of Embryo transfer

1. Confirmations of the name & identity of patient who is made ready for transfer
2. Tally the embryo dishes with the patients' identity. This is a very important step to avoid mismatched transfer & medico legal litigation
3. Grading & selection of embryos for ET.
4. The selected embryos are washed & stored in 0.8 ml transfer media either placed in an well of 4 well nunc dish or centre well of a falcon 3037 petridish. Many people cover this with oil. However the author likes to prepare the dish 5 minutes prior to transfer & does not like to cover the dish with oil. This dish is placed in the incubator.
5. Patient is placed in lithotomy position, with semi full bladder & an abdominal USG is performed to see whether bladder is adequately full to demonstrate proper visualization of uterus as well as to confirm correction of anteversion. Jelly is not used with USG probe as it is embryo toxic. Saline is used instead.
6. The person doing the embryo loading as well as the person performing the embryo transfer procedure wears sterile plastic gloves. These gloves are rinsed with normal saline & then dried with a sterile towel.
7. Cervix is visualized with either a cuscos speculum or two Sims speculums. Cervix is cleaned & cervical mucus is removed. The cervix is rinsed with buffered culture media.
8. ET catheter is opened, using sterile no touch technique. Care is taken not to touch the tip of the catheter. The airtite syringe is opened. It is rinsed, filled with medium, & then attached to the ET catheter. The ET catheter is now flushed with the medium.
9. The assistant now opens the incubator & places the petridish with embryos under stereomicroscope; the microscope is generally set at 1.5 X magnification.

10. The embryologist now loads the embryos in less than 30 ul of media, taking care to keep the embryos in the last 10 ul of media.At the tip one tends to take 5 ul of air. This not only protects the column, but also helps in locating the catheter tip during ET. Following expulsion of embryos, it acts as a good marker for the location of embryos.
11. The catheter s passed under USG control, 1.5 cm below the fundus or 5 cm from the external os.
12. Midcavity deposit of the embryos is made by depressing the piston of the syringe, slowly. After deposition there should be gentle pressure maintained on the syringe piston. One can withdraw the ET catheter immediately or 30 seconds after the expulsion. An real time USG is done to confirm the proper placement of embryos.
13. While withdrawing one can rotate the catheters tip in its axis, prior to removing the catheter. During withdrawal a slight pressure may be applied on the syringe piston, so that there is no sucking in of the embryos.
14. Following ET the embryologist checks the catheter for blood, mucus & retained embryos. If embryos are retained, they are rewashed, a new ET dish is prepared & a repeat ET is done with a new catheter.
15. The patient is rested on the ET table for 20 minutes. Following this, she is made to recline on a chair for half an hour, After that, the patient is discharged with proper instructions.
16. Proper documentation is done.

Post ET Instructions

1. To take antibiotics for two more days. Many specialists do not give any antibiotics following ET.
2. To be on adequate luteal support:

   Tab Micronised progesterone 600 mg vaginally per day or

   Inj progesterone 50/100 mg IM per day or

   Oral micronised progesterone 600 mg per day or

   Progesterone gel for vaginal application or

   Inj HCG 2000 to 3000 IU IM every 3 to 4 days
3. To report on any problems such as pain in abdomen, nausea, giddiness or bleeding
4. To come for an USG scan 5 to 7 days after ET, in patients at high risk of OHSS.
5. To undergo a B HCG test 14 days after ET. To repeat the test after 2 to 3 days.
6. Not to stop luteal support unless told to do so, by the Doctor.

Conclusion

The main goal of Embryo transfer is the skillful, gentle atraumatic transfer of embryos near the fundus, withoutcausing uterine contractions, pain, bleeding or damage to the endometrium or the embryos. If this goal can be attained, it will result in a very successful ART program.

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EMBRYO CRYOPRESERVATION

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Introduction

Ever since the first reported birth of a baby from a frozen embryo transfer (FET) approximately 250,000 babies from FET have been born. Cryopreservation of embryos and human oocytes, zygotes, cleavage stage embryos and blastocysts has become an integral part of any in vitro fertilisation –embryo transfer (IVF-ET) programme. Efficient embryo freezing has several advantages as it helps reducing cost and increases cumulative pregnancy rates. It also helps in case embryo transfer is cancelled in fresh cycles due to Ovarian Hyperstimulation Syndrome (OHSS) or a difficult ET. Also as multiple pregnancies are a major concern these days, the recent trend has been to transfer less number of embryos with some units resorting to selective single embryo transfer. This results in excess embryos being available for freezing in many patients and necessitates an excellent freezing program in routine IVF practice.

The technology used should be embryo friendly and oocyte friendly and should ensure optimal survival of the embryos and oocytes that are stored and subsequently thawed for transfer. This implies choosing low-temperature technology that is likely to minimize damage and enhance survival rates of the living material that is stored for infertility treatment. Traditionally this has been accomplished using slow freezing with low concentrations of cryoprotectants but this has relatively poor outcome in terms of post thaw survival, implantation and pregnancy rates. Vitrification has recently emerged as an alternative procedure in the preservation of oocytes, embryos and blastocyst and it may confer some time-saving and survival advantages over this traditional procedure.

A) Vitrification VS Conventional slow freezing :

Vitrification was first investigated and described in the 19th century. Luyet,[1] the founder of cryobiology, first recognized the potential of achieving an ice-free, structurally arrested state for cryopreservation[2,3].

Vitrification [6, 7] can be defined as a physical process by which a highly concentrated solution of cryoprotectant solidifies from the liquid phase, by an extreme elevation in the viscosity while cooling at a low temperature. The solid which is called glass retains the normal molecular and ionic distribution of liquid state and can be considered to be an extremely viscous super cooled liquid[4]. The process avoids intracellular and extracellular ice formation[5]. It thus avoids the possible damage which can be caused by intracellular ice formation and the osmotic effects related to extracellular ice formation.

Vitrification of water inside cells can be achieved in two ways: by increasing the speed of temperature conduction, and by increasing the concentration of cryoprotectants. In addition, by using a small volume [<1 ul] of high-concentration cryoprotectants, very rapid cooling rates from 15000 to 30000°C/min can be achieved [e.g. T from – 196 to 25°C=221°C/ 0.5S=26 520°C/ min].

During Vitrification the entire solution remains unchanged and the water does not solidify and therefore no ice crystals are formed. Most living organisms are composed of large amount of water. Since water is not viscous it can be vitrified by little increase in the viscosity level when cooled at a rate of millions of ºC per second. Under this condition, water molecules do not have time to arrange themselves into a crystalline lattice structure. Water can also easily vitrify if mixed with cryoprotectants.

The Studies on vitrification of embryos:

The studies have mainly been conducted on the blastocyst stage by Lane *et al*.[8], 1999;Reed *et al*[9], 2002; Liebermann and Tucker[10],2004; Kuwayama *et al*, 2005 [11].with fewer studies on human cleavage-stage embryo by Rama Raju *et al*[12] , 2005; Desai *et al*[13] ,2007; Kuwayama[14], 2007. Pyruvate uptake by embryos was significantly higher in vitrification than that after slow freezing. (Lane and Gardner, 2001[15]). This may, be the probable reason for the reduced developmental ability of embryos after slow freezing. Clinical pregnancy rate of 49.3%.was demonstrated by B. Balaban, B. Urman *et al* [16] 2008.Desai et al. (2007) showed that embryo metabolism and its progression to the blastocyst stage, with the cryoloop vitrification is superior to slow freezing i.e cryo survival rate of more than 85%.The cryo survival rate with vitrification reaching ~95% was reported by Rama Raju et al.2005. Zhao XM et al showed that the survival rate of 2-PN stage embryos vitrified by OPS method with EDFS30 vitrification solution was higher than that of those cryopreserved by conventional freezing. In a study by B. Balaban, B. Urman et al [16] 2008, the cryosurvival rate of 95% was reported in vitrification as compared to 88% in conventional freezing. They also demonstrated embryos with 100% blastomere survival rate and blastocyst formation rate of 78% and 60% respectively in vitrification as compared to 60% and 50% respectively in conventional freezing. But comparison of results between above two methods is really difficult since different cryoprotectants and carriers have been used (Loutradi et al, 2007[17]).

Table 1:A comparison of vitrification with slow-cooling procedures.

	Vitrification	Slow cooling
1. Control of solute penetration	yes	no
2. Control of dehydration rate	yes	no
3. Maintenance of physiological temperature during equilibration procedure	yes	no
4. Duration out of incubator	10-15 min	3 h
5. Prolonged temperature shock	no	yes
6. Interference with oocyte or embryo	low	high
7. Fracture of zona pellucida	no	possible
8. Capture by growing ice crystals	no	possible
9. Equipment and running costs	inexpensive	expensie

Kuleshova. Vitrification of oocytes and embryos. Fertil Steril 2002

Advantages of vitrification:

1. Due to the speed of the vitrification process, it minimizes the period of embryo exposure outside the incubator.
2. It requires minimal setup time.
3. There is no need of the extensive and programmable freezing equipment.
4. Post biopsy embryo survival following vitrification was better than slow freezing.
5. It is better for the blastocyst stage freezing due to its better survival rates.
6. I t has better cumulative pregnancy rates, as more number of embryos are available for multiple embryo transfer.

Disadvantages of vitrification:

It results in more zona thickening compared to slow freezing. Hence assisted laser hatching of zona should be done to improve implantation rates.

B) Open Vs Closed Systems :

Open method:

In this method, a high freezing rate is achieved by avoiding any delay that may be caused by the carrier walls. This method was conventionally the gold standard for vitrification until there were concerns about liquid nitrogen cross contamination. e.g. EM grid, cryotops etc.

Closed method:

In a closed system, the specimen is not allowed to come directly in contact with the nitrogen by a carrier that delivers the maximum heat transfer rate to the embryos. The closed systems are the CryoTip and Cryo-leaf and the high security straws (HSS).

C) Different Cryoprotectants : Dmso Vs Ethylene Glycol

Higher concentration of cryoprotectants can damage the embryo by osmotic or chemical shock. Hence, this can be tackled by reducing concentrations of cryoprotectant. But this concentration should be reduced without loosing their effectiveness. This can be done in various ways:

a. Increasing the cooling and warming rates this can be achieved by the following methods:

1) Sudden plunging of the carrier system from room temperature (25 Deg C) to Liquid nitrogen (minus 196 Deg C)
2) Having no barrier between the embryo and the liquid nitrogen (open system: cooling rate of 24,000 deg C / minute) or minimal barrier between embryo and liquid nitrogen (closed system: cooling rate of 12,000 deg C / minute)
3) Increasing the thawing or warming rates

b. Minimising the volume of vitrification solution as much as is possible

Improvements have been made by using less toxic & more permeable chemicals, by using combination of cryoprotectants to reduce toxicity, using a stepwise approach to equilibration & increasing cooling and warming rates.

D) The rate of cooling slow Vs fast :

Factors that determine whether a vitrification solution will remain uncrystallized are the total solute concentration, the capacity of the cryoprotectant to form glass, and the rate of cooling/ warming. Increasing the cooling/ warming rate can also decrease the amount of solute required to form a stable glass.

The newer technique of vitrification is by use of VitMaster. The cooling rate in VitMaster is increased from 25,000ºC/min (traditional technique) to 130,0000ºC/min. VitMaster avoids chilling injury and lipid phase transition allowing sensitive oocytes at all stages of development to be successfully Cryopreserved. Moreover, the cryoprotectant concentration used can be decrease with a consequent decreased in the associated toxicity and oocytes and embryos can be sealed within straws, eliminating potential liquid nitrogen contamination.

E) Single versus multiple cryoprotectants :

In 1990s, embryologists often used single exposure to a highly concentrated one cryoprotectant. In 1991, Li and Trounson et al[18] found that the use of DMSO, 1, 2-propanediol and glycerol in combination with Macromolecules yielded better blastocyst survival rate.

Conclusions:

Vitrification has demonstrated significantly better embryo survival rates and implantation rates than conventional freezing. Embryo cryosurvival rate of 92%,Clinical pregnancy rate of 49.3%,Blastocyst formation in 60% was demonstrated by B. Balaban, B. Urman *et al* [16] 2008. Desai *et al*.[13] (2007) showed that embryo metabolism and its progression to the blastocyst stage, with the cryoloop vitrification is superior to slow freezing and cryo survival rate of more than 85%. The cryo survival rate with vitrification reaching

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