Reviews

Clinical practice of embryo transfer*

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Abstract

In this review, several embryo transfer methods are considered, together with factors involved in achieving an effective transfer. The approach most used is transcervical intrauterine transfer. This is described in detail, together with the many variables influencing success, e.g. technical ability and training of personnel, catheter choice, value of a previous 'dummy transfer' and the need to minimize trauma during transfer and so prevent damage to the uterine lining, bleeding and uterine contractions. These factors can each negatively impact on pregnancy rates. Emphasis is put on quality, developmental stage and number of embryos to be transferred to limit multiple pregnancies and their unwanted side-effects. Culture to blastocyst stages and single embryo transfer when optimal quality embryos are available are discussed as means of avoiding multiple pregnancies. Reference is made to embryo cryopreservation and fertility following frozen embryo transfer. Other techniques, such as ultrasound-controlled transcervical intrauterine transfer, and ultrasound-controlled transmyometrial transfer, are reviewed. More invasive procedures, generically grouped as surgical embryo transfer, including gamete intra-Fallopian transfer (GIFT), zygote intra-Fallopian transfer (ZIFT), pronuclear stage transfer and embryo intra-Fallopian transfer (EIFT), are also described. These techniques had a place in IVF when the need to apply assisted reproductive techniques exceeded the capacity of most laboratories, but not today thanks to refined laboratory technology and improved understanding of implantation. Alternative assisted reproductive technologies, such as direct intra-follicular insemination (DIFI), Fallopian spermatic perfusion (FSP), peritoneal oocyte stage and sperm transfer and intra-vaginal culture (IVC), are mentioned briefly.

Keywords: *embryo transfer, implantation rate, IVF, pregnancy, transfer catheters*

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Introduction

Embryo transfer is one of the most critical steps among all those involved in assisted fertilization procedures. Errors at this stage negate all previous work. On the whole, embryo transfer has not received due attention, and the significance of this apparently simple step in success or failure of assisted reproduction is frequently neglected. When considered with other improvements in the clinic and laboratory, it is clear that this area has evolved poorly. It is not encouraging to observe that highly valuable embryos, achieved after so much effort, care and cost, are transferred almost blindly to the virtual cavity known as the endometrial space. Many times, this procedure is performed without a concomitant clear knowledge of the processes happening before, during and after transfer.

Transcervical approaches to embryo transfer

One of the most critical factors affecting success is the technical ability of the person performing the embryo transfer. Estimates show that up to 30% of all cycle failures can be considered as caused by defects in the transfer technique.

Variations among transfer personnel

Operator variation is shown when results in an assisted fertilization programme are ranked on the basis of the physician performing the transfer. Differences arise among the various operators, highlighting the importance of such subtle issues as the gentleness of inserting a catheter, or the injection technique. One study comparing embryo transfer by different individuals, recording success rates for each of them, revealed several key factors (Hearns-Stokes *et al*., 2000).

Clinical pregnancy rates were found to vary significantly among providers for 393 clinical pregnancies resulting from 854 embryo transfers, in which the number or quality of embryos transferred did not differ significantly. Fluctuations from 17.0% (47 transfers) to 54.3% (57 transfers) ($P < 0.05$) were observed, suggesting that individual skills in embryo transfer technique greatly influence pregnancy outcome in assisted reproductive technology.

Improvement in success rates may occur with greater acquired experience, as shown in a recent analysis (Papageorgiou *et al*., 2000). Results in terms of pregnancy rate were analysed for five different individuals undergoing training in transfer methods. At the onset of the trial, results from four of them were lower than the mean standard of the programme. The results improved following experience in performing 35 transfers, when these individuals reached the same proficiency as the other practitioners in the programme. Generally, but not always, transfers are performed by clinicians, although nurses are trained in the procedure in some clinics. Comparisons of the performance of clinicians and nurses (Cheung *et al.,* 2000) revealed that, if trained properly, both groups achieved similar pregnancy and implantation rates. Whatever the academic qualifications of the person performing this step, it is clear that good technical ability and properly supervised training are absolute requirements to achieve an effective embryo transfer.

Relevant morphology of the reproductive tract

The uterus is a pear-shaped, thick-walled and hollow muscular organ situated between the bladder and the rectum: the fundus is the dome-shaped portion above the level of entrance of the two Fallopian tubes; while the body, or corpus, lies below, separated from the cervix by a slight constriction termed the isthmus. The cavity of the uterine body is flattened and triangular in shape, with the Fallopian tubes entering at its basal angles. Its apex is continuous with the cervical canal at the internal os. The uterine wall is composed of an outer serosal layer (the peritoneum), a firm, thick intermediate coat of smooth muscle (the myometrium) and an inner mucosal lining (the endometrium).

The cervix is cylindrical, slightly expanded in its middle. Its canal is spindle-shaped and opens into the vagina through the external os. On the anterior and posterior walls, the endocervical mucus is raised in a series of palmate folds. Some pathologies, such as isthmical or cervical synequia, stenosis of the internal os, and unspecific or specific cervicitis, may develop in this area. These pathologies can further complicate transcervical transfers of the embryo.

The endometrial cavity may be considered as a potential space, which can be developed after introduction of a strange body, or an injection of fluid. For that reason, the development of the space where the embryos are placed will in part depend on the principles governing fluid dynamics, but also on the physiology of the preimplantation endometrium and myometrium.

Factors affecting success of transcervical embryo transfer

The probability that pregnancy occurs during a cycle of assisted reproduction is a function of the number of embryos transferred. Multiple embryo transfers result in a higher likelihood of pregnancy and probability that any one individual embryo can implant. In an attempt to explain on a quantitative base the reasons for success or failure of embryo transfer, many investigators have produced models on the relative efficiency of the embryos or uterus in sustaining implantation. One such model, by Paulson *et al*., (1990), identifies three variables that are important for achieving pregnancy: transfer efficiency, embryo quality and endometrial receptivity. They conclude that an inherent inefficiency is associated with mechanical transfers of embryos into the uterine cavity. This imposes a limit to the maximal embryo implantation rate that can be attained. They suggest that the quality of embryos produced by ovarian stimulation, follicle aspiration and IVF could be very high and equal to that of embryos produced *in vivo* during natural cycles. By contrast, endometrial receptivity is markedly diminished in stimulated cycles and currently this is the rate-limiting step to pregnancy success during IVF.

To explain failures in mechanical transfers of embryos, Knutzen *et al*. (1992) measured the uterine retention of a bolus of radiopaque dye when mimicking embryo transfer in the early luteal phase before an IVF procedure. Two patient groups

were assessed, displaying either optimal or suboptimal uterine positioning. The authors reported that dye remained in the uterine cavity in 68% of cases with optimal uterine positioning, but in only 48% of those in which uterine position was suboptimal. In the remaining cases, dye moved into the Fallopian tubes, cervix and/or vagina. The authors concluded that if mock procedure had been the actual transfer, at least 32% of the patients with optimal uterine positioning and 52% of those with suboptimal positioning would have lost their chance of pregnancy. They also reported that patients with an intrauterine length of <70 mm had lower pregnancy rates than those with greater intrauterine size.

Knutzen *et al*. (1992) assumed that the intrauterine space could accommodate at least 30–40 µl of fluid during the transfer process. Empirically, intrauterine inseminations involved a reflux of the specimen only when volumes of 200 µl or more were transferred. This assumption is complicated by the highly viscous seromucous material secreted by endometrial glands during the secretory phase. These are hydrophobic in nature, so injected fluid does not mix easily with mucus and may remain as a spherical drop on the endometrial mucous layer. This would result in the displacement of the injected bolus. Egbase *et al*. (2000) reported that highest implantation and clinical pregnancy rates were obtained in women with cavity lengths of 70–90 mm, although the differences were not statistically significant. The same study revealed how rates of ectopic pregnancy per reported clinical pregnancy were highest in women with <70 mm cavity length, e.g. 14.9% (7/47) compared with 1.8% (5/276) in women with 70–90 mm size and 0% (0/27) with >90 mm ($P < 0.0005$). Size of the uterus is thus critical in the aetiology of ectopic pregnancy after embryo transfer.

Uterine contractions

Uterine contractions may also lead to embryo expulsion. Studies using ultrasound identified four groups of patients, taking into account contraction frequencies (Fanchin *et al*., 1998). A significant stepwise decrease in implantation and pregnancy rates was found in patients with the lowest to the highest contraction frequencies. Contraction frequency and plasma progesterone concentration were negatively correlated.

Varying approaches to embryo transfer

Several embryo transfer methods are used at present. They can be ranked into two groups, according to their complexity. Low-complexity techniques include transcervical intrauterine transfer, ultrasound-controlled transcervical intrauterine transfer and ultrasound-controlled transmyometrial transfer. High-complexity techniques include those needing a higher patient engagement, and usually performed under anaesthesia during invasive methods such as laparoscopy and hysteroscopy. Those techniques are grouped together generically as surgical embryo transfer, including zygote intra-Fallopian transfer (ZIFT), pronuclear stage transfer, embryo intra-Fallopian transfer (EIFT), and trans-myometrial embryo transfer (TMET). Some of these will now be considered separately.

Transcervical intrauterine transfer

Transcervical intrauterine transfer is the most widely used technique, due to its simplicity. It is also the least invasive. Basically, it is performed by loading embryo(s) in a catheter together with a small volume of fluid before the catheter is introduced through the endocervical canal to a position close to the uterine fundus. The embryo is then expelled there.

Catheter types

Currently, more than 50 embryo transfer catheter models are available, made from different materials, in various lengths and rigidity, and consisting of one or several parts. Catheter design can be reduced to a limited number of basic models, classified according to tip characteristics, flexibility, presence of a fixed or detachable outer sheath, malleability, shape memory of the material, gauge and length. The ideal catheter must be free of embryo toxicity, soft and flexible in order to avoid causing trauma to the endometrial lining, and must offer operators with minimal training and ability the means of introducing it easily into the endometrial space. Low cost is also important. Unfortunately, each of the current varieties of catheter models that have been designed and applied has its own advantages and disadvantages. All desirable properties cannot be found at the same time in any one catheter in existence. The situation is complicated further by individual patient variations in the configuration of the vagina–cervix–uterus and by differences in technical capabilities and training among practitioners.

A wide range of plastics are used to make transfer catheters. They include polyethylene, teflon, nylon, polyurethane and polyolefin. Each catheter batch must pass a mouse embryo development assay for toxicity, which measures growth from a 1- or 2-cell stage to blastocyst. They must also be free of bacterial endotoxin.

Figure 1 illustrates three different ‘rigid’ catheter models. Rigid catheters are still widely used, but ‘soft’ catheters have now become quite common, because they are expected to minimize trauma during transfer. In **Figure 2**, examples of standard soft catheters are shown. These catheters are not innovative, because Robert Edwards and his team developed the prototype, still valid, almost 20 years ago. Its main features are flexibility, malleability and an extremely soft end. The concept underlying soft catheter design is to avoid trauma at transfer, to the embryo and all tissues passed by the catheter. It should find its own way in, rather than being forced in.

Traditional rigid catheters, e.g. Frydman’s, allow insertion with no other aid in most cases. Soft catheters must be sometimes handled with an external sheath provided in the package. Some of these catheters are also provided with an internal plastic-covered metal inserter, which is bent to give a better fit to the curvature of the cervical canal.

Some authors preferring soft catheters state that rigid ones tend to pierce through obstacles, rather than bypass them, and that, in some cases, such a feature would cause excessive mucus to accumulate inside them. In addition, rigid catheters have extremely sharp edges, which could introduce embryos into endometrial tissue, rather than depositing them in the uterine

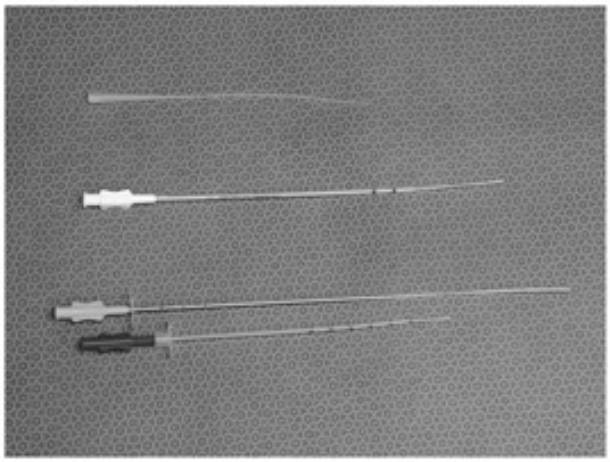

Figure 1. Some examples of 'rigid' embryo transfer catheters, from top to bottom: Frydman, Tom Cat, short and long Frydman set.

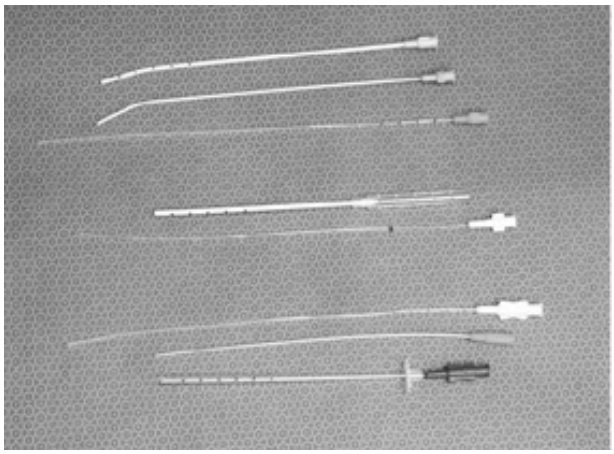

Figure 2. Some examples of 'soft' embryo transfer catheters. From top to bottom: Wallace catheter, Craft soft catheter and Frydman soft catheter.

cavity. These factors might not affect embryos with an intact zona pellucida. Previous manipulation in the zona pellucida, designed to facilitate embryo implantation could easily lead to the disaggregation of the blastomeres.

Advantages stemming from the use of one or other type of catheter are not always apparent. Comparative studies are often jeopardized by multiple factors, which are very difficult to standardize. As an example, **Table 1** depicts results obtained by three different groups when comparing a typical rigid-type catheter with a standard soft-type catheter. Results failed to show any significant difference between the devices.

Loading the catheter

Catheters are usually attached to an atoxic, tuberculin-type syringe, and the embryos are then gently drawn inside. Usually, embryos are suspended in small volumes of culture medium (0.02–0.05 ml). Dead volumes on catheter and syringe are also filled with culture medium. Since air is compressible, even a slight resistance against the catheter contents at exit could be serious. Pressure driven by an air column could be insufficient to eject embryos into the uterine cavity. In some cases, small air bubbles separate culture media containing embryos from the rest of the liquid column. Some programmes do not adopt this procedure, so after embryo loading and during transfer, the catheter must be held with its tip slightly downwards, to prevent embryos travelling through the liquid column to the end connected to the syringe. Such an effect would result in transfer failure, because embryos are significantly denser than the culture media currently used for transfer.

There is another reason for filling both catheter and syringe dead volumes with medium. This permits checks to be made, ensuring that embryos have been held in the catheter. The checks are done when the transfer is performed, by placing the catheter end on a culture plate as the syringe is detached. The remaining liquid column acts by gravity as an entraining media, washing the internal catheter out to ensure all embryos have been transferred. If this is not enough, as sometimes happens when using extremely thin catheters, this procedure is completed by refilling the syringe with medium and gently forcing it through the catheter.

Ultrasound-guided transfer

Transabdominal ultrasound-guided transfer is designed to follow the movement of the catheter into the uterus, with the aim of enhancing transfer efficiency. It may help to refine the transfer technique by guiding the position of the cannula and transfer catheter in relation to the endometrial surface and uterine fundus. The position and movement of a transfer-associated air bubble, and the impact of subendometrial myometrial contraction leading to endometrial movement, may also be observed. The method also permits the media drop containing the embryos to be inspected and to ensure that it is retained.

In a recent study, Wood *et al*. (2000) controlled transfers by ultrasound in 518 transfer cycles. Comparing transfers with and without echographic control revealed a significant difference. Pregnancy rate was 38% with echographic control and 25% without it ($P < 0.002$). Moreover, when the catheter is duly followed in its travel and the transferred drop can be seen, pregnancy rate is significantly higher (41.5%) than when it cannot be clearly followed (16.7%).

Results from other investigators (Woolcott and Stanger, 1997) indicate that a tactile assessment of the replacement catheter is unreliable compared with ultrasound. In 17.4% of transfers, the outer guiding catheter inadvertently abutted the fundal endometrium. The outer guiding cannula indented endometrium in 24.8% of cases, while the transfer catheter embedded in endometrium in 33.1%. Accidental tubal transfer was prevented by the use of ultrasound in 7.4% of procedures.

Catheters could be made more readily detectable by ultrasound to refine transfer techniques even more. Some catheters already in the market have this feature, due to an echodense sheath and tip (Letterie *et al*., 1999). At present, however, it

Table 1. Pregnancy rates (%) attained after use of two different catheter models.

Catheter used		*Data from*
Frydman	*Wallace*	
32.3	19.2	Wisanto *et al*. (1989)
36.0[a]	41.6[a]	Urman *et al*. (2000)
30.7	30.3	al-Shawaf *et al*. (1993)

[a]Not significant

seems that most programmes do not use ultrasound during embryo transfer. More controlled trials are needed to decide the value of ultrasound in this context.

Other factors related to transfer efficiency

Another strategy proposed to improve transfer is to make a previous 'dummy transfer' using an uncharged catheter. Cervical penetrability and any potential trouble related to catheter introduction can be identified. A solution can be decided on and prolonged exposure of embryos to unfavourable conditions, or their losses arising by difficulty during insertion, can be avoided.

Uterine position may be changed before transfer, modifying the angle between cervix and uterus by manipulating the speculum or using ring forceps. It was a common practice to apply a tenaculum to the cervix, but results with echographic control revealed significantly increased uterine contractions plus fundus–cervix contractions in addition to the pain caused in some women. Analyses of contraction effects on pregnancy rates (Fanchin *et al.,* 1998) showed that increased contraction frequency reduced clinic pregnancy rates in all patients in the study.

Another specific transfer-related issue concerns the total or partial retention of embryos in the catheter. This has no significant influence on results, provided it is detected and recovered embryos are retransferred. Placing air bubbles into the catheter for a dummy transfer led to higher liquid ejection rates. The presence of blood outside the catheter after transfer could also signify a decrease in implantation rates, although this does not occur when blood traces are present inside the catheter. Non-traumatic techniques are emphasized to prevent bleeding during transfer, which can also reduce implantation rates.

Bacterial contamination of catheters during passage through the cervical canal may also impair implantation, reported by several groups. A proven contamination led to a significant drop in implantation. This problem is avoided by administering preventive antibiotics during follicular puncture, which decreases positive cultures in the catheter end after transfer.

Catheter contamination with mucus must also be checked carefully. It could mechanically hinder the ejection of embryos from the catheter. Several reasons indicate the value of performing a non-traumatic mucus aspiration prior to transfer. In dummy transfers, the catheter was charged with dyed liquid and transfers were performed with and without mucus aspiration (Mansour *et al.,* 1994). A reflux occurred in 57% of the attempts without mucus suction, compared with 27% with mucus suction.

Cervical stenosis might be prevented by a test insertion of a transfer catheter during a cycle prior to stimulation. If problems are found, cervical dilatation would be prescribed before starting gonadotrophin stimulation, to facilitate embryo transfer. Alternatively, if difficult transfers are expected, surgical repair of cervical stenosis can be performed. Patients with a history of extremely difficult transfers might also benefit from a repair before new cycles are attempted.

Transmyometrial transfer

When transcervical transfer becomes difficult or impossible, a transmyometrial transfer can be performed with the help of transvaginal ultrasound. According to Kato (Kato *et al.,* 1993), this technique has been used successfully in Asia; however, only one prospective study has been published about it so far, and results do not seem outstanding (Kato *et al.,* 1993; Groutz *et al.,* 1997). Transmyometrial transfer technique may be relatively simple. Essentially, it is performed by reaching the uterine cavity through the myometrium and the endometrium with a needle similar to the one used for transvaginal oocyte retrieval, and using the same transducer and puncture guide. An 18-gauge needle is provided with a trocar, to prevent inflow of foreign material during puncture. Although the puncture needle may be inserted under general anaesthesia, experience indicates that local para-uterine anaesthesia with 5% lidocaine and epinephrine is enough.

Moreover, local anaesthesia proved to be unnecessary for transmyometrial transfer after the introduction of a modified biopsy pistol to drive the needle (Pasqualini and Quintans, unpublished results). The modification consisted in eliminating the biopsy-taking system. The instrument is shown in **Figure 3**. The needle is drawn so quickly that it is inserted with a single shot, two at the most, without causing pain to the patient or moving the uterus. Once the needle is placed within the uterine cavity, the trocar is withdrawn and a catheter inserted in its place. This catheter is used to inject a small volume of culture media (0.02–0.05 ml), in order to verify by ultrasound that the tip has been placed effectively. Then a similar catheter is inserted, now charged with embryos in a small volume of medium (0.02–0.05 ml), and transfer is performed using ultrasound to verify the location of the injected drop. Pregnancy results using this technique, used for cases of unapproachable cervix, were similar to those recorded in patients having transcervical transfer.

Number of transferred embryos, development stage and effects on pregnancy rates

Multiple pregnancies

Special attention must be paid to the number of embryos for replacement. If enough embryos are available for some selection to be done, choice is usually linked to morphological quality and developmental stage of each embryo. Although

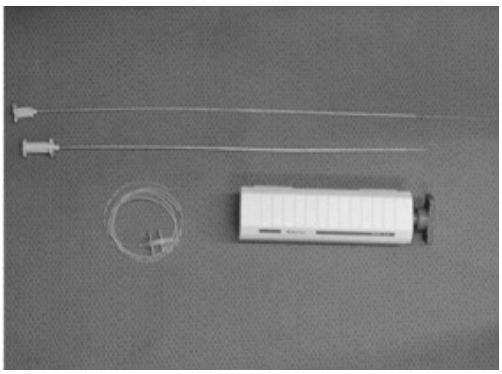

Figure 3. Instrument set for transmyometrial embryo transfer. From top to bottom: trocar, needle, catheter and modified biopsy gun.

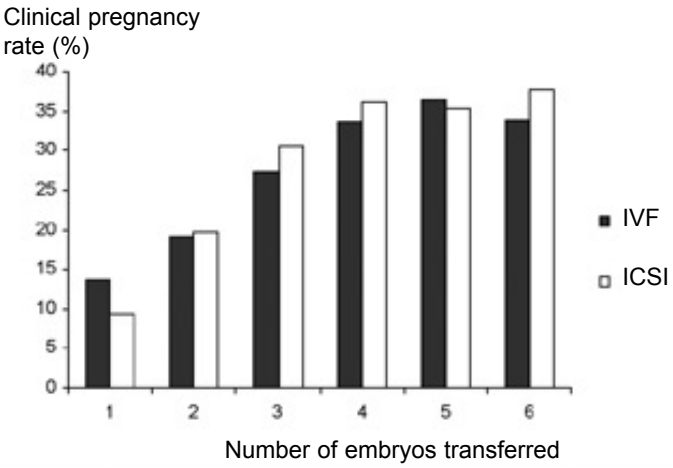

Figure 4. Clinical pregnancy rate according to the number of embryos transferred (IVF and ICSI). Data were extracted from those cases published in the 1998 annual report of the Registro Latinoamericano de Reproducción Asistida; they correspond to a total of 3501 embryo transfers from conventional IVF and 3870 transfers from ICSI.

usually transferred at the 4- to 8-cell stage (48–72 h after follicle aspiration), transfers have been made at inclusive stages between pronucleate oocytes and blastocysts. A recent method, activated oocyte transfer, involves the transfer of oocytes 1 h after intracytoplasmic sperm injection (ICSI). The incidence of pregnancy varies according to the number of embryos transferred. A trend towards the transfer of several embryos has emerged to maximize pregnancy rates, but this is accompanied by an increase in multiple pregnancies (**Figure 4**). The 1995 World Collaborative Report on IVF revealed how 24.7% of pregnancies were twins, 4.1% triplets and 0.2% quadruplets. These figures show how 44–45% of newborns came from multiple pregnancies. Data for 1998 from the Registro Latinoamericano de Reproducción Asistida on the perinatal outcome in multiple pregnancies according to number of fetuses are shown in **Table 2**.

At present, in most IVF programmes, approximately one-third of pregnancies are multiple. This is a serious problem, because multiple pregnancies present clear risks for mother and fetuses and, despite improvements in obstetric care, the risk factor remains significantly higher throughout a multiple pregnancy. The increased obstetrical complications include pre-eclampsia, eclampsia, preparturition, haemorrhagic episodes, premature parturition, retardation of intrauterine development and higher incidence of surgical delivery. The more common neonatal complications include low birth weight as a consequence of premature delivery or placental dysfunction, and congenital malformations. For single pregnancies, perinatal mortality rates are equivalent to those of the general population, nearly five times greater in twins and eight-fold higher in triplets.

Multiple pregnancies are associated with higher frequencies of brain palsy and mental retardation. The incidence of brain palsy is shown to be five times greater in twins and 17-fold higher in triplets. Prematurity and its complications are the main cause of high death rates in multiple births, as in disability.

The loss of babies from a multiple set can be particularly difficult, inevitably with enormous strain for the parents. Even in cases of normal birth, the practical difficulties of looking after three healthy babies is a major task, producing stress and complications in the standard family. Some couples would prefer twins, but few ask for triplets. Practical and emotional difficulties for a childless couple caring for two or more babies at the same time are especially problematical. Making rational decisions is hard because of stress imposed by their long and painful previous infertility. Final responsibility rests with clinicians and scientists, who must minimize the risk of a high multiple pregnancy.

Assessing embryo quality

Good embryo assessments are essential to minimize the number of replaced embryos and reduce risks of multiple births, while achieving acceptable pregnancy outcomes. Several metabolic criteria have been used to assess embryos, but currently only two practical characteristics help to evaluate embryos for transfer. They are morphology and development rate, and acceptable evaluations should consider them together. Joint decisions help to avoid gross errors. Quality can change unexpectedly, as a higher-quality 4-cell embryo on day three arrests in development, while an 8-cell embryo early on day two may indicate a developmental abnormality.

Recently, assessment criteria applicable to embryos at pronuclear stage have been described. Tesarik and Greco (1999) utilized pronuclear layout, and nucleolar number and distribution to classify embryos into six different groups. With at least one top quality embryo, pregnancies occurred in 50% of cases. All other groups combined yielded rates of 9%. Scoring nuclei and nucleoli in this manner was related to the consequent appearance of multinucleated blastomeres and morphology in cleaving embryos. The second main selection criterion is cleavage speed. Taking the occurrence of early cleavage as a starting point, early cleavage is related to the capacity to develop to the blastocyst stage.

Although there is a trend to minimize the number of observations of embryos in order to avoid potential temperature and pH changes brought about by exposure to the atmosphere, limiting observations to one or two developmental stages could produce inadequate estimates of embryo quality. Scoring perhaps twice or thrice daily could be important in assessing cleavage speed.

Perhaps the safest way to select embryos for transfer would be to leave them in culture to the blastocyst stage. This stage of development can be attained in commonly used culture media, although recent findings indicate that special culture conditions are required for blastocysts with good implantation and pregnancy rates. The use of co-cultures and development of sequential media are claimed to provide efficient culture systems until blastocysts. One retrospective study (Pasqualini *et al*., 1997) on 70 patients classified blastocysts into three groups. Group 1 included only blastocysts with optimum morphology, i.e. expanded or fully expanded by day 5. Their characteristics included a well-differentiated inner cellular mass, continuous and regular trophoblast with net cellular boundaries and no degenerative areas, cellular residues or blastomeres delayed in their divisions. Group 2 included blastocysts with slightly suboptimal morphology and one negative characteristic, e.g. lack of defined intracellular trophectodermal boundaries, a small blastocoelic cavity and poorly defined inner cell mass. Group 3 were obviously suboptimal, with two or three negative characteristics together with degenerative areas and cellular residues, or delayed blastomeres. Patients with group 1 blastocysts had a 59% implantation rate and 54% pregnancy rate. Comparable values of group 2 blastocysts were 13% and 21% respectively, while group 3 produced 4.3% and 12%. Blastocyst transfer permits morphological traits to be scored, along with other indications of embryo viability. Furthermore, blastocyst transfers provide more accurate prognoses about pregnancy success according to quality.

There is also a trend for single transfers of cleaving embryos with good prognoses. Coetsier and Dhont (1998) and Van Royen *et al*. (2000) concluded that one top quality embryo can give an acceptable pregnancy rate. The main characteristics of these top quality embryos include absence of multinucleated blastomeres, four or five blastomeres on day 2, seven or more cells on day 3 and <20% of anucleated fragments. Applying these selection criteria to 221 double transfers resulted in 106 transfers with two top embryos, giving 63% ongoing pregnancies, including 37 (57%) twins. Sixty-five transfers with a single top quality embryo gave 58% ongoing pregnancies with 21% twins (Van Royen *et al.,* 2000). The group without top embryos had 23% of ongoing singletons and no twins. Corresponding ongoing implantation rates in the three groups were 49%, 35% and 12% respectively.

Currently, transfer techniques focus on minimizing the risks of multiple pregnancy. Some authors recommend single embryo transfers with embryos having a good prognosis. This may be the case today with top quality embryos or blastocysts (Gardner *et al*., 2000). Unwanted multiple pregnancies are avoided. Moreover, transferring either two or three embryos are claimed to produce similar pregnancy rates in both groups. With double embryo transfers, triplet pregnancies declined, but the frequency of twins did not vary significantly.

Embryo cryopreservation and fertility following IVF

Human embryo cryopreservation is currently seen as necessary for any programme of assisted reproductive techniques. This is because at present no methods exist to predict accurately the fertilization rate of each particular IVF cycle, nor the chances of each or any fertilized oocytes to develop into good quality, transferable embryos. The alternative is to produce a surplus of embryos and dispose of those not transferred. This choice is, in many cases, considered unacceptable from ethical and practical points of view. Another approach, the cryopreservation of oocytes, may appear a good alternative to embryo freezing, yet at the present time it is neither reproducible nor sufficiently successful to be considered as a standard routine procedure.

A well-known but unwanted side-effect derived from cryopreservation is the chronic accumulation of embryos in IVF clinics. In general, most clinics try to keep periods of embryo storage within reasonable limits, and in some cases they must follow governmental regulations on time limits for cryopreservation. Particular cases exist where solid reasons allow cryostorage for longer than usual. In such cases, it would be useful to know for how long embryos may be cryostored without causing them harm. Cryobiologists suggest that cells can remain viable for more than 1000 years (Mazur, 1988), and other evidence shows how mouse embryos survive well after exposure to –196°C for the equivalent of about 2000 years of background radiation (Glenister and Lyon, 1986). Other experiments with mice indicate that periods up to 15 years or longer should be considered safe for embryo survival in this species (Glenister *et al*., 1990).

Very few reports are available on pregnancies achieved after the transfer of human embryos that were cryopreserved for periods close to nine years (Ben-Ozer and Vermesh, 1999; Quintans *et al*., 2000a; Go *et al*., 1998). Others are known from press reports (Go *et al*., 1998). This absence of data could be due to the fact that human embryo cryopreservation was not so widely available in previous years as it is today. In some cases, cryostored non-transferred embryos were discarded after shorter waiting periods. To date, however, in published cases of pregnancies achieved after prolonged embryo

Table 2. Perinatal outcome according to number of fetuses. (Data from: Registro Latinoamericano de Reproducción Asistida, 1998.).

	Single		*Double*		*Triple*		*Quadruple and over*	
	n	*%*	n	*%*	n	*%*	n	*%*
Born alive	1159	98.9	690	97.6	250	90.3	48	92.3
Stillbirths 20–27 weeks	5	0.4	11	1.6	15	5.4	4	7.7
Stillbirths 28 weeks	8	0.7	6	0.8	12	4.3	0	0.0

cryostorage, results indicate that normal babies are produced.

Early stage embryos are usually preserved using 1,2-propanediol as cryoprotector, while in later stages glycerol is generally used. A realistic analysis of results expected from cryopreserved embryos can be derived from data published by Mandelbaum *et al*. (1998). Within a 10-year period, these authors carried out 5032 thawing cycles involving 14 222 embryos. Survival rate in this procedure was 73% and transfer was achieved for 4590 embryos, with an average of 2.2 embryos per transfer. Clinical pregnancy rate per transfer was 16% and birth rate 12%, corresponding to a 6% birth rate per frozen embryo. After thawing and transferring 86% of available cryopreserved embryos over a 10-year period, outcomes provided an additional 8% to birth rates as compared with birth rates achieved by the transfer of fresh embryos.

Blastocyst cryopreservation seems an attractive alternative for embryos that are not transferred while fresh. From a cryobiological point of view, blastocysts offer advantages over earlier embryos. They have more cells, and these are smaller. Therefore, achieving a balance with the cryoprotective agent is reached quicker and more effectively, moreover the target for a possible cryoinjury appears much more divided.

A difficult choice must be made between cryopreservation and prolonged growth of blastocysts. An extended period of culture enhances embryo self-selection because only those embryos with the highest development potential reach blastocyst stage and are thus cryopreserved. A study of 563 transfer cycles with cryopreserved blastocysts, carried out by Kaufman *et al*. (1995), revealed how 1239 blastocysts were thawed with an 83% survival rate and 21.7% pregnancy rate. These results are lower than those attained with fresh blastocysts. Perhaps ultra-rapid vitrification, recently introduced, may offer a way to improve blastocyst cryopreservation (Lane *et al*., 1999). Other groups have improved results using standard methods of blastocyst cryopreservation by using longer after-thaw periods of incubation (Quintans *et al*., 2000b; Guerif *et al*., 2000). This approach to 24 cycles (Quintans *et al*., 2000b), involving transfers of 58 blastocysts, gave a 45.8% clinical pregnancy rate and a 25.8% implantation rate, much closer to results with fresh blastocyst transfer.

Alternate assisted reproductive techniques

Direct intra-follicular insemination (DIFI), or intra-follicular insemination (IFI)

Developed in the early 1990s, IFI can be applied in patients with at least one permeable tube. It is performed by injecting a preparation of rinsed spermatozoa in two or three pre-ovulatory follicles by means of transvaginal puncture. It is technically simple, and initial pregnancies showed it to be promising resource. However, only one pregnancy arose in a subsequent study on 50 patients published in 1995 (Nuojja-Huttunen *et al*., 1995). Assessed by the lack of bibliographical searches, interest in this technique seems to have decreased, maybe because it cannot compete with other well-established assisted reproductive techniques.

Fallopian spermatic perfusion (FSP)

FSP is an insemination technique that uses a paediatric Foley catheter to block any potential reflux from the cervix channel, thus allowing insemination with 4–5 ml of preparation of spermatozoa previously rinsed and suspended in culture media. This enables transit through the Fallopian tubes to the ovary, eventually to the peritoneum. The efficiency of this technique in comparison with traditional intrauterine insemination (IUI) is still being assessed. It is generally perceived no significant advantages accrue over traditional IUI, except in idiopathic sterility, where it is preferable to IUI.

Peritoneal oocyte stage and sperm transfer

This technique can be considered a more complex variant of IFI. It also requires permeable tubes. It is performed by depositing as many as four oocytes obtained by transvaginal puncture at the base of the pouch of the Douglas sac, together with a rinsed preparation of spermatozoa. Papers published in the late 1980s and early 1990s gave pregnancy rates of 20–25%, comparing favourably with success rates for IVF and GIFT. It was limited, however, to cases without a tubal factor.

For cases involving tubal-factor-related sterility, a variant was developed on which oocytes and spermatozoids were directly transferred into the uterine cavity. This became known as direct oocyte transfer (DOT). The first pregnancy using this method was reported in the early 1980s. A recent study (Lee *et al*., 1999) on 40 cycles in 19 couples with this technique reported the occurrence of seven clinical pregnancies.

Intra-vaginal culture (IVC)

This technique was developed in the late 1980s as a simplification of the standard IVF. The main difference is that it eliminates the incubator and conventional culture plates. It substitutes them with a tube filled with a culture medium balanced with 5% CO_2, in which oocytes and spermatozoa are placed. The tube is sealed and inserted into the patient's vagina, to act as an incubator for 40–72 h. When it is withdrawn, embryos are evaluated and transferred to the patient's uterus in the usual way. The main advantage of this technique is avoiding any need for an artificial incubator. The mother is involved in almost the whole process, which is another advantage and highly convenient from a psychological point of view. Its main drawback is the continuous presence of spermatozoa in the culture media, as they could contaminate it with products potentially toxic to the embryo. Even short exposure periods, as those used in conventional IVF procedures, can exert such harmful effects. This is why some authors recommend limiting exposure of oocytes to spermatozoa to 1-h periods. Furthermore, when media is not changed post-fertilization after exposure to spermatozoa, risks of micro-organisms from contaminated semen samples are raised. Finally, the absence of knowledge on 1-cell fertilized eggs entails the risk of implanting tripronucleate embryos into the mother's uterus, which is not recommended.

Surgical transfer techniques

Surgical transfer techniques are complex, based on invasive procedures and more demanding on the patient than methods discussed so far. They are performed under anaesthesia and require laparoscopy or hysteroscopy. Such techniques include gamete intra-Fallopian transfer (GIFT), zygote intra-Fallopian transfer (ZIFT), pronuclear stage ovum transfer and embryo intra-Fallopian transfer (EIFT).

In GIFT, laparoscopy is used to approach the Fallopian tubes with a catheter containing two or more mature oocytes and rinsed spermatozoa. Transfers can be made into one or both tubes. Current experience show how most couples who are candidates for GIFT could be treated more simply, by means of well-controlled ovarian stimulation followed by intrauterine insemination. Such cases, where no pregnancy occurs after three or four insemination cycles, are transferred to IVF. In this manner, hidden problems to achieving fertilization can be identified and then solved later by ICSI.

Tubal cannulation via the cervix was introduced as a variant of GIFT, using a less-invasive approach (Risquez *et al*., 1990). Welcomed enthusiastically at first, this method was abandoned because it required special training, and results were not outstanding compared with conventional approaches.

In the search for a tubal-transfer method useful in male-factor infertility cases, the pronuclear stage ovum transfer technique was devised. It follows almost the same technique as GIFT, but the oocytes are fertilized *in vitro* and transferred to the Fallopian tubes when in their pronuclear stages. Several variants followed this method, e.g. transferring zygotes or pre-embryos in 2- to 8-cell stages (ZIFT; EIFT). The most recently developed technique, called intra-endometrial embryo transfer, is performed under hysteroscopy. Embryos are injected directly into the endometrial stroma. Expected to favour implantation, its results were not promising because implantation rates were low, perhaps due to excessive media acidification caused by the CO_2 needed to expand the uterine cavity.

All these procedures assumed that the optimal site for early cleavage stage embryos is the Fallopian tubes. This is indicated from a physiological viewpoint, but is only absolute for certain species, e.g. cows and mice, where intrauterine transfers are unsuccessful. The procedure succeeds only with later developmental stages, e.g. morulae or blastocysts. This situation does not hold true in humans, when acceptable pregnancy rates are achieved with very early embryos, e.g. those ranging from pronuclear to blastocyst. Even injections of oocytes and spermatozoa are successful, as mentioned above. These techniques were designed and developed during a transition period when demand for new assisted reproductive techniques was so high as to exceed the capacity of most laboratories. With time, better training for embryologists, improved culture media and a more systematic quality control were established. Available laboratory techniques were refined, and implantation rate per embryo growing *in vitro* improved. These changes brought differences between Fallopian and uterine transfer in balance, so the former made no sense since the methods offered no benefit, and were more uncomfortable for patients. They also included surgical hazards – and a heavier workload for the centre.

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