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DEBATE

Embryo transfer and multiple gestation

How should the number of embryos transferred to the uterus following in-vitro fertilization be determined to avoid the risk of multiple gestation?

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Laboratory assisted reproduction, while aiding couples in the establishment of pregnancies, has been associated with an increased risk of multiple embryo implantation, resulting from the transfer of more than one embryo to the uterus following in-vitro fertilization (IVF) (Jones, 1995). Women carrying two or more fetuses are at greater risk of premature labour and pre-eclampsia, abruption, and their prematurely delivered infants experience greater short- and long-term morbidity (Melgar *et al.*, 1991). The increased costs associated with the care of these high order pregnancies, both in terms of the treatment of premature labour and possible need for long-term maternal hospitalization and the subsequent neonatal intensive care of their infants is also of concern. Yet how couples who have sought help in overcoming their inability to conceive, their doctors, society and their representatives should deal with these risks remains controversial. While individuals can seek multi-fetal pregnancy reduction, this procedure is not without its risks. The pregnancy loss rate reported in a recent large collaborative study varied from 7.7–22.9%, depending on the starting number of embryos. The delivery of severely premature fetuses (<24 weeks gestation) varied from 9.0–22.9% (Evans *et al.*, 1996).

In some countries, embryo transfer has been regulated through legislation at the national level. In the UK, a regulatory body has been created through an act of Parliament to oversee IVF clinics. Only three preimplantation embryos may be transferred to the uterus following IVF, irrespective of the woman's age, the couple's religious background, or their decision as regards proceeding with selective termination of pregnancy, should a multiple pregnancy occur (Lieberman *et al.*, 1994). Even more stringent rules have been promulgated through statutes in Germany, where it is not permissible to fertilize more than three eggs recovered after follicular aspiration (Beier and Beckman, 1991). This approach eliminates the ethical dilemmas associated with both embryo cryopreservation and multiple gestation. Unfortunately, it also lowers the overall chances of success for certain couples in achieving pregnancy in any individual cycle and promotes the use of recurrent cycles of hormonal stimulus and follicular aspiration, procedures not without risk.

The American system of government evolved during a climate of political confrontation, and given the diverse historic background of settlements during the colonization of North America (Quinn, 1995), it is not surprising that individual liberties and the protection of the rights of local communities from abusive forms of government were pre-eminent concerns during the founding of the US. The Federalist Papers and the debates occurring during the Constitutional Conventions eloquently bear witness to these concerns (Bailyn, 1993) which, ultimately, led to the development of a multilayered system of government, in which national, state and local governments each play significant roles. Until recently, there has been no significant government regulation of assisted reproduction, which has been left to the professional organizations. However, driven by the recent exposure of possible abuses discovered in some fertility centres, laboratory assisted reproduction has fallen under increasing scrutiny.

This small group of articles will explore the specific issues that relate to the transfer of multiple embryos following IVF. Why, in the first place, must several embryos be transferred to the uterus following IVF, to achieve clinically acceptable pregnancy rates? Strategies to reduce this number would diminish the intermittent risk of multiple implantation. Van Blerkom (1997) focuses on epi-genetic causes of the loss of oocyte competence and new approaches that may improve embryo implantation rates in the near future. Bavister and Boatman (1997) review the competence of current culture media to support the extended in-vitro culture of human embryos and the possible transfer of fewer, more developed preimplantation embryos as a means of limiting the risk of multiple implantation. Faber (1997) reviews the current social forces within the US that have predisposed to the transfer of large numbers of embryos. Bustillo (1997) explores the possible use of age-specific guidelines for embryo transfer to minimize the risk of multiple implantations without deterioration in clinical pregnancy rates, and Palmer (1997) reviews the conflicting obligations of individuals and society in reproductive health care.

First, I would like to return to the basic question of why it is necessary to replace more than one embryo within the uterus to achieve clinically reasonable pregnancy rates. Embryo quality and the receptivity of the endometrium are clearly factors in the equation. If both variables are maximized, fewer embryos need be transferred, limiting the risk of multiple implantation. Until recently, basic knowledge of the mechanism of implantation was lacking, and while this area of investigation is recently undergoing a renaissance, it is unlikely to yield information useful in the clinical arena for several years. A considerable body of evidence, however, has accumulated that human reproduction is inefficient. Early embryonic loses during implantation also appear to be common, as evidenced by transient detection of chorionic gonadotrophin elevations in

menstruating women (Edwards, 1986). The relatively low pregnancy rate of 4–7% following the transfer of single embryos recovered in natural, unstimulated cycles bears witness to this fact (ASRM/SART Registry, 1995). Against this high apparent negative biological determinism, and given our current inability to predict the likelihood of implantation for individual mid-cleavage stage embryos, it has not been unreasonable to transfer several at any one time, creating a type of reproductive roulette in which chance plays a major element. However, it should be mentioned that our ability to produce embryos capable of successfully implanting, although limited by intrinsic genetic and epigenetic factors, has increased significantly during the past 10 years. In women aged <35 years, it is not unusual to achieve clinical pregnancy rates of 30–40% following embryo transfer, although this does vary from laboratory to laboratory. Some fertility centres have unfortunately taken the approach of transferring a greater number of embryos to the uterus, to compensate for their poor quality, rather than improving their laboratory culture conditions. These clinics must take this approach to maintain their clinical pregnancy rate, the 'bottom line' statistic, at the expense of intermittently and unpredictably creating high order multiple pregnancies. Conversely, as laboratory quality improves, the number of embryos transferred will be reduced, to avoid an increasing risk of multiple implantations, but the lower limit that should be transferred is still not clear.

A woman's age is an obvious factor in diminishing embryo quality (Biggers, 1990), and many studies have documented higher rates of chromosomally abnormal embryos produced during the later reproductive years and a greater risk of spontaneous miscarriage (Richardson and Nelson, 1990). Evidence from ovum donation suggests that ovum quality plays a major role in the deteriorating success of reproduction in these women (Navot *et al.*, 1994) although there is some evidence that deterioration of the uterine environment also plays a lesser role (Meldrum, 1993). Clinical pregnancy rates following IVF deteriorate in women aged >40 years, but remain high if eggs obtained from younger women are utilized (Sauer *et al.*, 1992). While the genetic basis for this finding is apparent, eggs from older women may also be subject to age-associated oxidative stress (Tarín, 1996) and there is speculation of an increased tendency to zona hardening which might interfere with embryo hatching (Mandelbaum, 1996). Given these concerns, some individuals have made the case that a greater number of embryos should be transferred to women aged >40 years (Craft *et al.*, 1988), and some centres are performing combined gamete intra-Fallopian transfer (GIFT) and IVF in these women to increase their likelihood of conceiving. Whether this approach will increase clinical pregnancy rates without increasing the risk of multiple pregnancy is unanswered.

Another difficulty that laboratories encounter is how to judge the quality of a given preimplantation embryo. If reliable measures were available to predict the likelihood of successful implantation for individual embryos, this assessment could be utilized in determining the number of and which embryos should be transferred. Unfortunately, while it is true that cleavage rates and blastomere morphology help to identify abnormal embryos (with the proviso that these early embryos have the ability to regulate and that even the poorest appearing embryos may at times implant successfully), the converse proposition is not true; that is, all embryos of normal appearance do not have an equal chance of implanting. Indeed, many morphologically normal embryos may be aneuploid, and this frequency increases with maternal age (Munné *et al.*, 1995).

One approach to reduce the number of embryos transferred following IVF has been to increase the time of their incubation prior to transfer. This approach will 'cull' those embryos of poor quality that fail to grow over longer durations in time, leading to the transfer of only the best embryos. In theory, fewer embryos could be transferred at any one time, reducing the chances of multiple pregnancy while maintaining the clinical pregnancy rate. Preliminary evidence supporting an extended duration of embryo culture from 2 to 3 days has been conflicting, and whether culturing further to the blastocyst stage will provide further benefit remains unclear. This approach in itself will not eliminate all abnormal embryos, as it has been known that chromosomally abnormal embryos may exhibit apparently normal preimplantation development through cavitation. In addition, current culture conditions *in vitro* may yet be suboptimum to support the later preimplantation growth of genetically normal embryos. A recent report, however, in which human preimplantation embryos were co-cultured with Verro cells has confirmed both a lower rate of cavitation in embryos derived from women aged >40 years, but an equivalent liveborn rate per blastocyst transferred, irrespective of age (Janny and Ménézo, 1996).

What then should be done? My present concern is that the ability of individuals to make well informed procreative decisions in the privacy of their doctor's offices may be compromised by prematurely developed and poorly crafted legislation and place these private issues in the public arena. Witness the recent mandated large scale destruction of several thousand embryos in the UK and the inflexibility of the law to deal with this crisis (Edwards and Beard, 1996). The problem of the creation of multiple gestations has been recognized by both the medical and scientific communities. A number of approaches are currently being investigated in the laboratory that could lower the incidence of multiple gestation associated with IVF and embryo transfer in the near future, and it is difficult to believe that the problem will not be resolved. However, if this does not occur in a timely manner, control will be taken away from those best able to deal with the problem, the participants, and either market forces or legislation will be used to resolve the matter.

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The neglected human blastocyst revisited

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Indications for blastocyst culture and transfer

There are multiple clinical indications for transferring in-vitro produced human embryos at the blastocyst stage of development: patients with repeated implantation failure; patients with uterine abnormalities that preclude multiple pregnancies, thus requiring more careful selection of the single embryo transferred; patients suspected of defects in oocyte quality, thus requiring embryos to be assessed for a more extended period of in-vitro development; patients needing embryo biopsy for genetic selection; and patients undergoing replacement of supernumerary embryos frozen at the blastocyst stage (Levinson *et al.*, 1992; Ménézo *et al.*, 1992, 1995; Tarín *et al.*, 1992; Muggleton-Harris *et al.*, 1993; Olivennes *et al.*, 1994). There are also physiological and theoretical rationales for favouring transfer of human embryos cultured to the blastocyst stage. Culture to the blastocyst stage may select the most developmentally competent embryos for transfer, thereby avoiding transfer of embryos predestined to arrest. Transfer of fewer, but more competent embryos would produce a more predictable outcome with fewer multiple pregnancies. Selection by culture has an additional benefit for cryostorage of embryos, since only the most competent will be saved, resulting in fewer vials stored and fewer problems with disposal of unwanted, long-term stored embryos (Olivennes *et al.*, 1994). While it is true that in humans and non-human primates, unlike the case with most other mammals, cleavage stage embryos can successfully be transferred to the uterus (as is done for the majority of IVF cycles), it should be emphasized that this is not their natural environment. Premature exposure of embryos to the uterus may act synergistically with the stress they have already experienced in the artificial in-vitro environment. This could be a contributory component to the ~80–85% embryonic failure rate following embryo transfer. Blastocysts transferred on days 5–7, unlike the 2–6-cell stages usually transferred on day 2, should be more tolerant of the in-vivo uterine conditions, since this is their natural milieu. Recognition of both the clinical and theoretical advantages of blastocyst transfer has recently led to increased attempts to provide this service for certain categories of patients (reviewed in Bongso *et al.*, 1994; Ménézo *et al.*, 1995).

Contra-indications for blastocyst culture and transfer

What reasons might exist for not transferring embryos at the blastocyst stage? Selection for embryonic fitness *in vitro* may not parallel valid in-vivo criteria. That is, the embryo that failed to develop to the blastocyst stage *in vitro*, and was therefore not transferred, may have been perfectly capable of producing a viable fetus. This possibility is supported by the similarity in success rates for embryo transfer with embryos frozen either at the pronucleate or at the blastocyst stage, an indication that the two types of embryos had equal developmental potential (Queenan *et al.*, 1994; Kaufman *et al.*, 1995). Conversely, the embryo that survived to the blastocyst stage *in vitro*, and was therefore transferred, may still be incapable of undergoing all of the biochemical and other changes required for normal implantation: 48% of human blastocysts produced *in vitro* were incapable of secreting human chorionic gonadotrophin (HCG) (Woodward *et al.*, 1994). Such anomalies raise a key question in proposing blastocyst transfers for human IVF: that is, what non-invasive criteria can be developed to help identification of the most viable blastocysts? Peak HCG secretion occurs too late to be used as a diagnostic tool for blastocyst viability prior to transfer, but this could still be a useful parameter for identifying suitable culture media for producing viable blastocysts. Similarly, zona escape or 'hatch-

ing' might be used for this purpose, although the functional significance of this event *in vitro* is uncertain. At present, expansion and general morphology of blastocysts are the most practical selection criteria. Advances in biochemical or other noninvasive end-points for blastocyst viability would be most welcome.

Not to be overlooked is the human factor. If, for a particular patient, extended culture of pronucleate embryos produced no transferable blastocysts, the probability of pregnancy was zero. This patient may have preferred at least some chance of pregnancy, even if the probability was low. Finally, there are considerations of cost/benefit ratios. Culturing embryos to the blastocyst stage requires 3–5 additional days of incubator time, the possible introduction of cell lines for co-culture with their attendant complications such as screening for contamination, need for sub-passaging, and requirements for animal serum. These considerations alone may preclude blastocyst culture for all but either the most specialized (i.e. dealing with refractory patients) or the largest practices.

Methods for obtaining blastocysts

Clearly, the ease and reliability of producing blastocysts bears very heavily on how desirable an asset they will be in the practice of human IVF. The questions of what is the best way to obtain blastocysts and how this process can be improved will be the main focus of this discussion. Currently, when large scale studies have been reported, no single culture medium, either simple or complex, with serum albumin or with serum, reliably yields >50% blastocysts from bipronucleate embryos (Winston *et al.*, 1991; for a contrary view, see also Lopata, 1992). The fitness of these surviving embryos is also questionable (Hardy *et al.*, 1989). In one study of blastocysts produced in culture medium alone, only 39% had cell numbers ⩾30 at 120–124 h post-insemination (Winston *et al.*, 1991). Using this technology, on average, less than one normal blastocyst could be expected from the culture of five normal bipronucleate oocytes, a disheartening prospect for patient and clinician alike. As a solution to this problem, the introduction of the Vero cell line for co-culture of human embryos has become a 'means-to-the-end' of producing viable human blastocysts *in vitro* (Ménézo *et al.*, 1990, 1995). Although benefits for human embryo development have been shown with other types of co-culture cells such as autologous cumulus or granulosa cells, or human or bovine oviduct cells (reviewed in Bongso *et al.*, 1994), the use of Vero cell co-culture has become the most widely accepted due to uniformity of the cell line that has been carefully pre-screened to eliminate viral contamination (Ménézo *et al.*, 1990, 1995). In a randomized study, embryos co-cultured on Vero cells had a significantly higher blastocyst formation on or by day 6 of development compared to those in culture medium alone (77 versus 46%) and significantly more co-cultured embryos reached the expanded blastocyst stage as well (60 versus 31%; Turner and Lenton, 1996). By culture days 9–12, the geometric mean of HCG produced by co-cultured blastocysts was almost four times greater than for blastocysts cultured in medium alone (Turner and Lenton, 1996). The total cell counts per day 6 co-cultured blastocyst have been reported to be ~2.5 times greater than for the same stage blastocysts cultured in medium alone (Ménézo *et al.*, 1995). However, conclusions regarding superiority of co-culture versus culture alone for producing human blastocysts are not unanimous. In another randomized study, no significant morphological differences or cell numbers were found for cultured versus co-cultured blastocysts (Van Blerkom, 1993). Discrepancies due to differences in laboratory practice and culture components contribute to difficulties in drawing firm conclusions about relative advantages/disadvantages of co-culture (Bavister, 1995).

The means by which co-culture exerts its apparently beneficial effects on blastocyst development are unknown. The beneficial effects do not appear to depend on serum (Ménézo *et al.*, 1992, 1995). Medium conditioned by co-cultured somatic cells may also be effective (Ménézo *et al.*, 1995). Early developing embryos are sensitive to the presence of glucose and phosphate in culture media, but glucose may be required during formation of the blastocoel (Chatot *et al.*, 1989). Most media used for routine culture as well as co-culture contain glucose, a metabolite avidly consumed by co-culture cells (reviewed in Bavister, 1995). One hypothesis is that co-culture modulates the amount of glucose in the culture environment to a tolerable level (reviewed in Bavister, 1995). Specific growth factors putatively produced by the co-culture cells remain as yet unidentified (Bavister, 1995; Ménézo *et al.*, 1995).

Prognosis for the future of blastocyst culture

Do these reports imply that the quest for completely defined and reproducible culture media for human embryo development to the blastocyst stage is no longer fruitful? Not at all. Promising results obtained with co-culture show that human embryos are capable of forming blastocysts *in vitro* at high percentages, comparable with those in animal embryo studies (e.g. Pinyopummintr and Bavister, 1996; Schramm and Bavister, 1996). If, as may be suggested by co-culture studies, specific embryotrophic factors are required for optimal blastocyst development *in vitro*, it is important to find, characterize, and pharmaceutically produce (e.g. by recombinant technology) these components required by human blastocysts. The search for such components has barely begun. On the other hand, there are numerous examples of significant improvements in the outcome for both human and animal embryo culture when simple, systematic changes were made to the culture media. For example, bovine embryos produced by IVM/IVF can now reach the blastocyst stage in culture medium alone at rates equal to or exceeding those previously only achieved using co-culture (Pinyopummintr and Bavister, 1996). Our laboratory, as well as others, has found improved development with 'two-step media' formulations (Chatot *et al.*, 1989; FitzGerald and DiMattina, 1992; Pinyopummintr and Bavister, 1994, 1996; Bavister, 1995; Schramm and Bavister, 1996). With our best two-step media formulation, 61% of bipronucleate primate zygotes formed blastocysts and 44% of zygotes hatched, all without co-culture (Schramm and Bavister, 1996). Without adding further confusion to the co-culture versus non-co-culture controversy, it seems fair to say that presumptive

embryotrophic factors for blastocyst development produced by somatic cells need to be isolated and identified, and their mechanism of action established. The outcome of such work could be highly beneficial for production of viable human blastocysts *in vitro*.

In order to exploit fully the potential of human blastocyst transfer as a routine procedure, appropriate criteria for culture media need to be established. Key questions to be addressed include: Are the metabolic needs of the preimplantation embryo the same at the 1-cell and blastocyst stages? Should a single culture medium with constant ingredients be capable of producing maximal development when used throughout the entire preimplantation period? Is a medium, that initially supports excellent cleavage development with good embryo morphology, but which later fails to support the morula to blastocyst transition and/or hatching and HCG secretion, intrinsically injurious to the earlier stage embryos? Lopata (1992) eloquently argued that: (i) successful culture of human embryos to the blastocyst stage should become the 'gold standard' of a quality IVF programme, and that (ii) culture conditions which did not support all stages of preimplantation development, irrespective of results of transfer trials with cleavage stage embryos, were intrinsically injurious to embryos, which were 'rescued' only by early transfer to a more hospitable (*in vivo*) environment. We would agree with some of the important points he addressed, specifically, that a quality programme producing quality oocytes should be able to culture a high proportion of bipronucleate zygotes to the blastocyst stage, and that the choice of the culture medium is critical to formation of the blastocyst. However, increasing evidence now shows that early cleaving embryos have different requirements from those undergoing the morula and blastocyst transition (reviewed in Bavister, 1995; see also Schramm and Bavister, 1996), and that culture medium ingredients required at later stages of development can inhibit optimal development if added too early (Chatot *et al.*, 1989; Pinyopummintr and Bavister, 1994). Therefore, in our opinion, a minimalist philosophy should be applied to media formulation. Ingredients appropriate to the developmental stage of the embryo should be supplied 'just-in-time', so that media components required only at the blastocyst stage are not added to embryos destined solely for transfer at the 2–6-cell stage. Addressing the specific requirements of differentiating (blastocyst stage) embryos instead of treating them as if they were similar to cleavage stages will most likely reap ample rewards in terms of improved viability and pregnancy success rates.

Conclusions

Arguing from the intertwined viewpoints of experimental embryology and optimization of embryo culture media, we support the following propositions: (i) for human blastocyst production and transfer, cost/benefit analyses of co-culture technology should be performed, with critical examination of the advantages in performance over non-co-culture systems; (ii) renewed emphasis should be placed on the search for embryotrophic components of the natural or artificial environments, especially those required for blastocyst development and viability; (iii) for optimal blastocyst production, the benefits of 'two-step' culture systems should be explored; (iv) increased research emphasis should be placed on identifying non-invasive tests for blastocyst viability, to improve selection criteria. A relevant question is: what is the functional significance of 'hatching' *in vitro*?; (v) because of increased mean embryo viability and increased compatibility with the uterine environment, as well as possible reduction of multiple pregnancies, potential benefits of blastocyst transfers should be examined for routine human IVF practice, in addition to their value for the most refractory patients.

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Can the developmental competence of early human embryos be predicted effectively in the clinical IVF laboratory?

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Experience with clinical in-vitro fertilization (IVF) over the last two decades has profoundly changed our understanding of the developmental biology of human oocytes and preimplantation stage embryos, and has provided important insights and practical lessons concerning the proper laboratory management of gametes and embryos. The progressive increase in successful outcomes from IVF reported by most programmes can be attributed to the introduction of techniques such as intracytoplasmic sperm injection (ICSI) and to greater technical experience in the IVF laboratory. While improved success rates in infertility treatment are a tribute to the efforts of those involved in the IVF enterprise, in the US, it clearly has come at the cost of an increased frequency of higher order gestations and a marked growth in the requirement for and utilization of selective fetal reduction (Faber, 1997). It is unclear to what extent the following factors, either individually or in combination, have contributed to this increase: (i) significant and beneficial changes in laboratory procedures, including embryo culture, (ii) the persistence of the notion that the more embryos replaced the more likely it is that pregnancy will result, or (iii) a qualitative change in the population of patients for which IVF and derivative procedures are truly effective. However, if human embryos with high developmental competence are now being produced in numbers greater than those routinely obtained only several years ago, then is the 'state of the art' sufficient to predict developmental potential prior to transfer such that the number of embryos replaced will not reduce pregnancy rates but will reduce the probability of multiple gestations?

To answer this question, it is necessary to address the issue of what proportion of embryos obtained by IVF can be expected to be developmentally viable after transfer. Earlier studies that examined outcomes based on large numbers of patients and embryos concluded that each embryo had a unique developmental potential, and that for any particular IVF cycle only a relatively small proportion of cleavage stage embryos were competent to implant and develop through gestation (Acosta *et al.*, 1988; see review by Van Blerkom, 1994a). This conclusion was consistent with observations from fertile women attempting to conceive, and from IVF findings in which embryos judged morphologically normal had arrested development during the early cleavage stages with very few developing into expanded blastocysts. Upon close inspection, even those embryos that developed into blastocysts were often reported to be developmentally retarded with respect to cell numbers or allocation of cells between the inner cell mass and trophoblast (Winston *et al.*, 1991; reviewed by Van Blerkom, 1994a). Many investigators attributed the poor developmental performance of human embryos *in vitro* to conditions or media that were inadequate or inappropriate for the human embryo (see Bavister and Boatman, 1997). This was an attractive notion owing to the fact that media in common use for clinical IVF had been developed decades earlier for specific types of cell and tissue culture rather than for the maintenance of the early human embryo. However, pregnancies did occur with embryos fertilized and grown in 'older' media such as Ham's F10, and for many experienced programmes, pregnancy rates in the 30% range were routinely reported from at least the mid 1980s. Clearly, some proportion of embryos could develop normally in media considered inadequate for the human.

Developmentally lethal defects occur in the human female gamete prior to insemination

Detailed analyses of cellular and genetic characteristics of human oocytes indicate that chromosomal and cytoplasmic defects occur at a relatively high frequency. Of mature oocytes, ~25% contain numerical chromosomal disorders (aneuploidies) and structural defects (fragmentation) that arise during meiotic metaphase and predispose the fertilized egg to developmental failure (see review by Van Blerkom, 1994b). For a subset of women, substantially higher frequencies of aneuploidy have been reported (Wall *et al.*, 1996) and for some, chromosomal abnormalities may be a proximate cause of their infertility (Zenzes *et al.*, 1992). Unlike the oocytes of model systems such as the mouse, human oocytes often exhibit a pleiomorphic cytoplasm that is especially evident when high numbers of gametes are retrieved for IVF. While some of these cytoplasmic phenotypes represent developmentally lethal defects in organelle distribution or cytoplasmic organization, others are benign with respect to the implantation potential of the embryo (see review by Van Blerkom, 1994b). Some types of cytoplasmic defects are associated with very high frequencies of chromosomal disorder suggesting that subtle degenerative changes at the cellular level may also influence chromosomal normality. Indeed, recent evidence for apoptotic alterations with attendant chromosomal damage indicates another class of lethal defects that can affect the developmental competence of mature mouse and human oocytes (Fujino *et al.*, 1996). Very different levels of metabolism and ATP production have been detected in mature human oocytes from the same and different patients (Magnusson *et al.*, 1986; Van Blerkom *et al.*, 1995). Although very low levels of metabolism do not prevent meiotic maturation, cleavage stage embryos that result from such oocytes,

while normal in gross morphology, appear to have a very low developmental ability (Van Blerkom *et al.*, 1995). Several investigators have reported that sperm penetration without cytoplasmic activation or male pronuclear evolution (silent fertilization) can occur in a relatively significant fraction (20–30%) of oocytes subjected to conventional IVF (Van Blerkom *et al.*, 1994; Ash *et al.*, 1995) or assisted insemination (Urner *et al.*, 1993; Wall *et al.*, 1996). This finding further demonstrates how the developmental heterogeneity of human oocytes can be expressed during the earliest stages of the fertilization process and, with the examples cited above, illustrates why no culture medium or system will rescue from developmental failure female gametes that at retrieval or ovulation are already compromised owing to inherent biochemical, cellular or genetic defects, many of which we are just beginning to recognize.

How embryos are currently assessed for developmental competence in most clinical IVF laboratories

The practice of grading embryos, with the assignment of a numerical or letter designation based on criteria such as degree of fragmentation and uniformity of blastomeres, has become standard in the IVF laboratory. While grading schemes have undergone modification over the years and may provide an 'analytical' aspect to an IVF cycle, the subjective or empirical basis of such morphological assessments and their ability to predict subsequent developmental competence has been repeatedly questioned in the literature. However, while all IVF programmes occasionally will observe normal pregnancies after the transfer of 'poor' grade embryos, most embryos that exhibit significant fragmentation or obvious abnormalities in cytokinesis do not develop and indeed have been shown to be chromosomally abnormal (e.g. catastrophic mosaicisms: Munné *et al.*, 1993). The presence of multinucleated blastomeres at the 2-cell stage is one of the more informative indicators of subsequent developmental failure owing to the extremely high frequency of numerical disorders in the chromosomal complement that affect both blastomeres in these embryos (Kligman *et al.*, 1996). Nuclear membrane dissolution can occur in multinucleated blastomeres during the cell cycle and some of these embryos are capable of limited cleavage divisions. Consequently, the occurrence of these embryos could go unnoticed if inspections in the clinical laboratory are made only to confirm fertilization and to select embryos for replacement at the time of transfer. These findings indicate that morphological observations alone can be of some predictive value in assessing the relative developmental potential of oocytes and embryos, especially if evaluations are made during the 2-cell stage.

Recently, many IVF programmes have extended the duration of embryo culture by 24 or 48 h from the previous 'standard' of ~2 days. Embryos that fail to progress beyond the early cleavage stages are assumed to be developmentally non-viable and, depending upon the clinic's protocol, may not be considered appropriate for transfer or cryopreservation. While this is an important and useful change for programmes that inspect embryos infrequently, it is my experience that most of the embryos which do not progress after 3 or 4 days of culture can be identified during the first 48–60 h. However, with the current aggressive protocols of ovarian stimulation used in clinical IVF, a typical cycle may have eight or more morphologically equivalent and apparently developmentally progressive embryos available for transfer on days 3 or 4. It is at the mid- to latter stages of the preimplantation period that subjective morphological assessments of developmental normality become problematic for the following reasons: (i) at the late morula and expanding blastocyst stages, inspection by routine light microscopy is not an accurate approach to the determination of cell numbers, and (ii) even with optical sectioning and computer-assisted morphometry, it is often very difficult to distinguish between anuclear cytoplasts and anuclear cells in mitosis and to determine whether appropriate cell allocation between inner cell mass and trophoblast has occurred. The use of DNA specific fluorescent probes to estimate cell numbers by counting nuclei in living embryos is very effective for experimental purposes (Van Blerkom, 1993) but application to embryos destined for transfer is currently unacceptable. Findings from DNA fluorescent studies of human blastocysts demonstrate that actual cell numbers can differ significantly among embryos which appear equivalent at the light microscopic level (Hardy *et al.*, 1989; Dokras *et al.*, 1991; Van Blerkom, 1993). For many current IVF practitioners, attainment of the blastocyst stage is viewed as de-facto evidence that such embryos are developmentally normal.

Several recent studies suggest that certain co-culture systems (Feng *et al.*, 1996; Vlad *et al.*, 1996), modifications to existing media (Quinn *et al.*, 1995) or simple removal of antibiotics (Magli *et al.*, 1996) promote a pattern and rate of human embryo development that is closer to the in-vivo situation. Rarely, however, do studies of human embryo culture medium and conditions acknowledge the fact that the biochemical and cellular milieu the embryo experiences as its progresses through the Fallopian tube and uterus changes both spatially and temporally. In this respect, it is worthwhile to recall that the embryo does not travel through the reproductive tract in a fluid milieu comparable to the in-vitro condition, but rather is subjected to a complex biochemical and physically dynamic environment in which cleavage and blastocyst formation occur in very close proximity to very different cell types in the Fallopian tube and uterus respectively, that are themselves undergoing proliferation and differentiation. Because of focal and regional differences in biochemistry and cellular function and activity, what a human embryo actually sees, requires, or utilizes from its normal surroundings is largely unknown. However, successful development through the preimplantation stages *in vitro* in relatively simple medium and the fact that conventional IVF/embryo transfer bypasses the Fallopian tube entirely indicates that the biochemical requirements for early embryonic development may be rather minimal. Consequently, the detection of growth factors and cytokines in reproductive tissues may relate more to the activity of those cells than to embryotrophic influences that are absolutely required for normal development and therefore must be included in culture medium formulations. In attempts to design simple human embryo culture medium capable of supporting development from the 1-cell to the expanded blastocyst stages, Quinn *et al.*

(1995) and Gardener *et al.* (1996) have incorporated into their formulations metabolite and ionic concentrations derived from the analysis of metabolites and salts obtained from human reproductive tract flushes. Although the values obtained must be considered an approximation of what the embryo might actually experience *in vivo*, in-vitro results in which development to the expanded blastocyst stage is obtained at relatively high frequency tends to support the notion that the biochemical requirements for preimplantation human embryogenesis may not be complex.

Whether the ability of an embryo to progress to the blastocyst stage is a definitive indication of developmental potential is an issue of fundamental importance in the evolution of a standard culture medium and a set of laboratory protocols for clinical IVF. While attainment of this stage is clearly an important milestone in early embryogenesis, developmental normality cannot be assumed as both molecular and cellular studies have observed grossly normal appearing expanded blastocysts with developmentally significant molecular and cellular defects (Dokras *et al.*, 1991, 1993; Van Blerkom, 1993; Turner and Lenton, 1996). For example, Turner and Lenton (1996) compared results obtained from human embryos cultured under conventional conditions to those cultured in the presence of Vero cells. Similar to many other findings from co-culture studies (see Van Blerkom, 1993), these investigators found that a higher number of embryos reached the blastocyst stage in the presence of Vero cells. However, their results showed for the co-cultured embryos no significant improvement in the morphology of the resultant blastocysts and the occurrence of a greater number of blastocysts that seemed to be functionally incompetent, as assessed by human chorionic gonadotrophin (HCG) production. For assessments of embryonic development at the blastocyst stage to be effective in the clinical IVF laboratory, not only should efforts be made to determine total cell numbers but, more critical to outcome, whether the inner cell mass is present and normal. The importance of such determinations was demonstrated by the findings of Winston *et al.* (1991), who reported that day 5 human blastocysts often exhibited an absent or deficient inner cell mass such that if implantation occurred, the resulting embryo would be expected either not to progress or to develop as an anembryonic pregnancy (so-called blighted ovum).

If new culture systems or media formulations are shown to be unambiguously capable of supporting normal human embryogenesis to the expanded blastocyst stage, then maintenance *in vitro* for 5 days becomes a very practical approach to embryo selection on the basis of demonstrated developmental potential. An additional benefit would be a reduction in the risk of higher order gestations without compromising the probability of pregnancy. However, the clinician will be faced with the difficult task of explaining to a subset of patients why no transfer will occur owing to the failure of the embryos to develop progressively. It is in this unfortunate situation (but not uncommon even when embryos are cultured for 3–3.5 days) that difficulty is experienced in explaining to a patient how an in-vitro system can be so predictive of developmental competence that it is equivalent to her uterus in the support of her embryos. In order not to disappoint patients, many programmes will replace embryos deemed non-progressive. Clearly, confidence in any assessment system is called into question when implantation occurs with such embryos. In this respect, while new media formulations and culture conditions directed specifically to the human embryo are essential in clinical IVF, it is equally important to identify and understand the origins of those defects in human oocytes that have developmental consequences for the embryo and which cannot be rescued or corrected by an optimized in-vitro environment.

New approaches to non-invasive assessments of human oocyte and embryo developmental potential

The ability to make reasonable predictions of oocyte developmental competence requires that the methodology used be non-invasive and applicable in a clinical IVF laboratory. To be of value in the selection of oocytes for insemination or embryos for transfer, a clear understanding of the biological processes that may be defective is essential. Experimental approaches to this issue have examined whether intrafollicular influences to which the oocyte may be exposed are associated with outcome after IVF. Quantitative analysis of follicular fluid steroids, proteins, enzymes, polypeptides, and growth factors have demonstrated the extraordinary complexity of follicular fluid but have not provided a definitive insight into what constitutes a normal intrafollicular environment or one that is clearly associated with high developmental competence. The behaviour of cumulus cells with respect to three specific oocyte/embryo–autonomous patterns of attachment, proliferation, and expansion during the first 24–48h of culture has been suggested to be predictive of the implantation potential of the corresponding embryo, which is cultured separately from the cumulus cells (Gregory *et al.*, 1994). However, while the actual relevance of these phenotypes to IVF outcome remains to be determined unambiguously, they do suggest that basic cell biological processes of cumulus cells may be differentially influenced by follicle-specific conditions. If a developmentally significant relationship does indeed exist, it can provide an experimental approach to understanding the molecular basis of developmentally critical interactions between the oocyte and its supporting cells (Van Blerkom, 1996), and how differentiative events in the oocyte can be influenced by specific factors within the follicle. Although promising leads exist, no single follicular factor(s) that can be readily determined or measured in the IVF laboratory has been shown to provide the ‘magic bullet’ for a definitive prediction of developmental competence of the oocyte or the embryo. We have addressed this issue by asking whether differences in follicular fluid biochemistry, cumulus cell behaviour *in vitro*, and oocyte/embryo developmental competence are related consequences of a follicle-specific physiology that can be assessed non-invasively prior to or at ovum retrieval (Van Blerkom, 1996). Our findings from the analysis of >1000 follicles indicate that the percentage dissolved oxygen in follicular fluid measured at the time of ovum retrieval is associated with the developmental normality of the oocyte and with differences in follicular biochemistry (Van Blerkom *et al.*, 1997). The percent dissolved oxygen in follicular fluids of similar volumes and aspirated

from follicles of the same size (18–21 mm) has ranged from <1 to ~5.5%. While oxygen content appears to be unrelated to the frequency of meiotic maturation, fertilization, and cleavage, most oocytes with cytoplasmic defects and high frequencies of chromosomal and spindle disorders originate from severely hypoxic follicles, as do cleavage stage embryos with multinucleated blastomeres. Preliminary findings indicate that oocytes from follicles with oxygen contents below ~1.5% have low ATP contents (Van Blerkom *et al.*, 1995) and an acidic intracellular pH (Van Blerkom, 1996) that may be associated with abnormalities in spindle microtubules (Van Blerkom *et al.*, 1996). Both retrospective and ongoing prospective findings strongly suggest that embryos derived from follicles with oxygen contents at or above ~3% are more likely to implant than if derived from severely hypoxic follicles.

At present, the dissolved oxygen content of follicular fluid cannot be predicted by any follicular characteristic observable during routine ultrasonographic evaluation. However, the addition of colour pulsed Doppler ultrasonography provides quantitative values associated with perifollicular blood flow and microvasculature development that do correlate with the oxygen content of follicular fluid measured at aspiration (Van Blerkom *et al.*, 1997). To date, colour pulsed Doppler analysis of several hundred follicles has demonstrated that the degree of perifollicular vascularization is follicle-specific such that adjacent follicles with virtually identical characteristics on routine ultrasonographic examination exhibit very different blood flow rates and degrees of perifollicular vascular development, both of which correlate with the percentage dissolved oxygen in the corresponding follicular fluid. Our findings demonstrate that for most IVF patients in which the same protocol of follicular stimulation was used and comparable numbers of follicles were aspirated, 30–40% of the follicles present at ovum retrieval had perifollicular blood flow patterns consistent with a level of follicular oxygenation >3%. Other follicles in these cohorts displayed little or no detectable perifollicular blood flow and dissolved oxygen contents in follicular fluid at or <2%.

An association between follicular oxygen content and embryo developmental competence is suggested by our current experience in which embryos are selected for transfer at 52–60 h post-insemination (6–10-cell stage) on the basis of morphological assessments of the oocyte (Van Blerkom and Henry, 1992) and embryo (made at 10–14 h intervals after insemination), and derivation from a follicle whose perifollicular blood flow values were consistent with an intrafollicular dissolved oxygen content of at least 3%. With no change from previous years in culture system or number of embryos replaced (Van Blerkom *et al.*, 1997), the addition of colour pulsed Doppler findings to embryo selection has been associated with a significant increase in the rate of ongoing pregnancies, albeit with multiple gestations occurring at a higher frequency (>50%) than during any previous period (Faber, 1997). Very similar results have been reported recently by Nargund *et al.* (1996) and Chui *et al.* (1997) who observed that human embryos derived from follicles with high perifollicular blood flow rates showed a corresponding increase in implantation potential.

These preliminary findings suggest that the application of perifollicular blood flow characteristics to embryo selection may be of significant value in the identification of embryos with high implantation/developmental potential. However, this approach requires the ability to associate each oocyte and its corresponding follicle, a task that is often cumbersome and extremely difficult to accomplish when large numbers of follicles have been stimulated to grow. The brief application of colour pulsed Doppler to each follicle immediately preceding aspiration and the pooling of oocytes based on the degree of follicular vascularization can simplify the logistics of oocyte selection using this criterion. The relationship between vascularity and oxygen content we have observed may provide a physiological basis for the often observed differences in follicular biochemistry and cumulus cell function. Indeed, the degree of development of the perifollicular vasculature that appears to determine the level of intrafollicular oxygenation may itself be dependent upon the capacity of each follicle to produce and secrete angiogenic promoters such as vascular endothelial growth factor (Kumat *et al.*, 1995; Van Blerkom *et al.*, 1997). Continued analyses of follicular vascularity, oxygen content, and outcome will be required to determine whether this is just another 'promising lead' or one that actually provides applicable determinants of developmental competence. However, it is this type of approach that will ultimately provide critical insights into how physiological, biochemical and cellular factors interact within the follicle to produce an identifiable set of conditions that is consistent with developmental normality for the oocyte and high implantation potential for the resulting embryo. In this respect, our understanding of the complex and stage-specific nature of human follicular biochemistry, physiology, and cell biology has entered a new era of discovery in which the developing follicle is not just a source of steroid hormones, but a site of synthesis of growth factors, cytokines, and novel polypeptides whose precise function(s) in the reproductive process remains to be determined. The insights gained from these studies will not only provide fundamental knowledge related to the origins of human oocyte developmental heterogeneity, but identifiable factors that can assist in the diagnosis and treatment of infertility.

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IVF in the US: multiple gestation, economic competition, and the necessity of excess

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In the practice of advanced reproductive medicine we have progressed from the miracle baby to the day of the litter. In a few short years we have radically changed the incidence of multiple gestation, yet we have virtually no idea of the long-term impact these changes will have on our patients, their children or society. There is ample evidence of the immediate costs of treating these complicated pregnancies and an entire practice, almost a growth industry itself, of multifetal reduction has been born as a result of the practice patterns of infertility specialists in the US. Clearly, the progressive increase in pregnancy rates that has occurred in most programmes reflects the additive effects of improvements in the clinical management of patients and in laboratory methods. With increased success has come the now commonplace occurrence of higher order gestations and the 'epidemic' of multifetal reductions. I believe that the roots of this phenomenon lie not in the inability among in-vitro fertilization (IVF) practitioners to recognize this dramatic change in outcome, but rather in the nature of competition among IVF clinics with similar levels of competence, and the intentions of those providing these services to attract new infertility patients or maintain those already in their programme. It is my belief that such pregnancies represent a failure rather than a success of the IVF enterprise.

Selective intrauterine abortion is only the most obvious symptom of the larger issue of multiple gestation. I believe the rightness or wrongness of this practice has nothing to do with the abortion issue. Regardless of the pro-life feelings of a patient, she cannot ignore the poor outcome of higher order

multiple gestations. She must face the real possibility of having no healthy children if she were to continue such a pregnancy. Likewise, very few infertility patients start care with the idea of one day having to make a decision regarding abortion. Thus, in the individual case the morality of multifetal reduction has little relevance, it is a matter of making the best of a bad situation as the patient's conscience allows. The ethical dilemma of multifetal reduction relates to its role in encouraging clinicians to increase the risk of its necessity by exonerating their irresponsible practices. The ethical conflict is magnified by the fact that the clinic reports these pregnancies as a success while it is ultimately left to the patients to deal with the psychological consequences of fetal reduction and financial and emotional consequences attendant with three, four or even five babies if reduction is not elected.

The incidence of multiple gestation has continued to increase [Society for Assisted Reproductive Technology (SART) and The American Society for Reproductive Medicine (ASRM), 1996]. One might have predicted that clinicians would change their practice in order to maintain acceptable pregnancy rates while limiting the multiple pregnancy rate. In fact, every ASRM/SART report shows only higher multiple rates (SART/ASRM, 1996). The easy access and improving success of multifetal reduction allows clinicians to place embryos without enough concern about multiple pregnancies. Thus, the relationship between the infertility specialist and the one who cleans up the mistakes is a symbiotic one. By making the complicated pregnancy someone else's problem, the incidence of the problem only increases.

We cannot know the precise extent of multiple gestations that result from assisted reproductive technology (ART) procedures. The last summary from the ASRM/SART reports results from clinics in 1994 and demonstrated a 36.3% multiple pregnancy rate, with 6.7% triplets or higher (SART/ASRM, 1996). Furthermore, 55% of the children born from in-vitro fertilization (IVF) and gamete intra-Fallopian transfer (GIFT) in 1994 were from multiple gestation, with 15% part of a triplet or higher pregnancy. Of course, we have no data regarding how many pregnancies and fetuses were reduced to arrive at that number.

It would be unfair to charge that this is a problem that only occurs in programmes where there is a blatant disregard for patient safety. In our own programme we have seen a progressive rise in the percentage of higher order multiple gestation. We have transferred fewer embryos than ever, but our twinning rate hovers around 50% and triplets account for ~10% of the pregnancies in 1996. We have tried to use ultrasound Doppler flow patterns and observations of very early embryonic development to pick the best, and thereby limit the number of embryos placed. Nevertheless, along with increasing pregnancy rates we have increased the multiple pregnancy rate.

The cost of these higher order pregnancies has been documented in many studies. One study from Boston reported that just the hospital maternal and neonatal care was >US$109 000 for the average triplet gestation in 1994, or 11 times higher than a singleton pregnancy (Callahan *et al.*, 1994). This cost pales in comparison with the cost of raising three children simultaneously, especially if, as in 40% of quadruplet deliveries, one of the children has a significant developmental delay (Evans *et al.*, 1990).

Prospective patients may not appreciate the incidence of multiple gestation or the need for eventual reduction. While pregnancy rates are published and disseminated among infertility support group members, the risks of treatment may not be as well appreciated. As long as this continues, physicians will only be encouraged to place higher numbers of embryos to increase their pregnancy rates, with the obvious result of increasing multiple gestation.

A recent survey of infertility patients clearly demonstrated that they overwhelmingly desire multiple gestation (Gleicher *et al.*, 1995). Only half of these couples objected to triplets and 20% found quadruplets acceptable. To the patients, failure is defined as a negative pregnancy test. The physicians must decide their role in this conflict. Do they act as the patients' agent in providing the outcome they desire, or do they attempt to educate and even deny the service that the patient desires? As providers we must look beyond a laboratory test and to the health of the mother during the pregnancy and to the health of the children that result. Despite the fact that multiple gestation accounts for the majority of neonatal morbidity in IVF patients (Tan *et al*., 1992), some fertility care providers see their role as accommodating the patients' desire for this outcome. Rather than educate their patients about the risk of permanent disability to children of higher order gestation, these providers apparently believe that this is an issue of patient choice. The physician–patient relationship implies that the physician will act in the patient's best interest; that responsibility cannot be abdicated, regardless of the patient's desires or the possible impact on the economic health of the physician's practice. It is simply wrong to practice medicine in any way that does not minimize the risk of complications. If no one else has the perspective, we must be able to see that there are worse outcomes than not being pregnant.

The reporting system for infertility clinics and the way it is used in this country bears part of the responsibility for the continued increase in multiple pregnancy. The SART database was originally developed to protect patients from clinics where pregnancy rates were very poor. Today, however it is used as advertising and a way of comparing successful clinics. Many clinics send out annual reports of their statistics. Infertility support groups encourage its use for this purpose and the media is happy to report that Dr X has the best pregnancy rates in the city, state, country etc. It is possible, therefore, that the system devised to protect patients may increase their risk of this complication by encouraging physicians to seek and report the highest pregnancy rates.

Insurance coverage patterns in the US also contribute to the high incidence of multiple gestation among IVF patients. Typically, patients do not have insurance coverage for ART procedures, but have full benefits for pregnancy care. Thus, to the patient, the financial risk is in having an unsuccessful IVF cycle, not in the often exorbitant costs of the resultant multiple gestation. If third-party payers want to reduce the cost of IVF pregnancies, they need to become involved in the payment of IVF. This would reduce the pressure on patients and their fertility specialists to have a pregnancy at any cost.

In addition, if they are paying the bill they could reasonably ask to limit the number of embryos placed.

I believe that the incidence of multiple gestation and the need for multifetal reduction will continue to increase until infertility specialists, patients, and those paying for the results of these pregnancies agree that this is a serious concern. I also believe that we can best address these concerns in the privacy of the physician–patient relationship rather than governmental regulation, although if this does not happen, some sort of control would be an improvement over the current system. In order for this to happen, however, all parties involved need to modify their behaviour. Clinics need to stop gauging their worth by a pregnancy rate, and patients must support clinical decisions that increase the probability of having healthy children, even if the result may be a marginal decrease in pregnancy rate.

We who provide these services are entitled to celebrate our patients' successes. There are many thousands of families with children who were born as a result of the advances in reproductive medicine and the dedication of clinicians and scientists devoted to the field. I fear that we may not be as proud of the changes we have brought about in our society and the premature and subsequently damaged children that have been a result of preventable multiple gestations.

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Imposing limits on the number of oocytes and embryos transferred: is it necessary/wise or naughty/nice?

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Twins, triplets, and more! They are everywhere; in the playgrounds, schools, shopping malls and often featured in the media. As assisted reproductive technologies (ART) become more widely applied, there is a justifiably growing concern about the resulting greater percentage of multiple pregnancies. The obvious considerations include the possible maternal and perinatal morbidity and mortality of such pregnancy and premature delivery with its associated increased psychosocial and financial costs.

Practitioners have known for many years that the pregnancy rate per completed ART procedure increases when a greater number of oocytes or embryos are transferred. For instance, the pregnancy rates reported by French National IVF Registry (FIVNAT) for the years 1986–1990 ranged from 9.1–12.1% for one embryo transfers and incrementally increased for transfers of two, three and four embryos with rates of 29.5–34.9% for transfers with four embryos (Walters, 1996). However, replacing a higher number of oocytes in gamete intra-Fallopian transfer (GIFT) or embryos following in-vitro fertilization (IVF) also proportionally increases the probability of a multiple gestation of not only twins, triplets but also of higher order (more than triplets) (Walters, 1996). Therefore, the transfer of multiple oocytes or embryos is advantageous in achieving pregnancy but has concomitant increased risks (Franco, 1994). These risks can seriously affect the desired overall outcome of yielding a reasonable number of healthy babies without total financial as well as emotional bankruptcy of the treated families and/or the medical reimbursement system.

The advent of embryo cryopreservation, in the programmes where it is successful, has relieved practitioners of the necessity to inseminate only a limited number of oocytes, to transfer all the oocytes or embryos generated in one cycle, or to discard normal supernumerary embryos. Despite the availability of cryopreservation, it is not unusual for ART programmes to transfer three or more embryos in IVF (World Collaborative Report, 1993) and four or more oocytes in GIFT (Redgment *et al.*, 1994). This generally results in a better pregnancy rate than the transfer of two or more embryos or fewer than four oocytes respectively. The probability of obtaining a multiple pregnancy is related to the cumulative prospect of survival and implantation of each individual embryo transferred or generated *in vivo* in GIFT. Undoubtedly, there are many factors, both known and unknown, that may affect implantation rates. The most widely observed and important is the age of the woman providing the oocytes for IVF or GIFT (Tan *et al.*, 1990; van Kooij *et al.*, 1996). Assessment of oocyte maturity and of embryo quality by morphological appearance and rate of cleavage, although not entirely reliable, gives the clinician an approximation of a particular oocyte's prognosis for fertilization (for both GIFT and IVF) and of an embryo's chance of implantation in IVF (Steer *et al.*, 1992; Shulman *et al.*, 1993). Mature oocytes are required for normal fertilization and the faster cleaving and better looking embryo has generally a higher implantation rate. Likewise, embryos from younger women in general have a higher likelihood of implanting. This is nowhere more apparent than when glancing at the overall results for ovum donation programmes.

In general, the reported success rates for GIFT are slightly better than for IVF (Meirow and Schenker, 1995). Unquestionably, the populations subjected to these two techniques are not the same. More importantly, it appears that GIFT may be better in the older age patient especially when a high-order oocyte transfer is performed (Qasim *et al.*, 1995). It remains to be

confirmed with good randomized studies whether providing the tubal milieu for fertilization and embryo development by doing GIFT, a more invasive and costly technique, is indeed warranted in the older patient.

Whether it is because of patient characteristics/selection criteria, cycle cancellation policies and/or laboratory quality and experience, there can be an important difference in implantation rates among ART programmes. It is imperative that each ART programme analyses its own results to be able to determine the optimal number of oocytes or embryos for transfer to obtain good success with minimal risk of multiple gestation. Given the relevant clinical and laboratory observations, this analysis should take into account the age and previous ART experience of the woman who is the source of the oocytes as well as the quality of the embryos that are transferred.

From a clinical point of view, unequivocally limiting the number of oocytes or embryos for transfer without consideration of an individual programme's results, of embryo quality, of a woman's age and previous history of ART failure would no doubt compromise the overall results of IVF and GIFT. What clinicians need is a much better prognostic marker of embryo viability in order to have a reliable indicator that a particular embryo would implant. Indeed, this would give comfort that the transfer of two or more embryos would yield good pregnancy rates without the significant risk of high order multiple pregnancy. This need for a prognostic marker remains a major impetus for the support and performance of relevant high quality embryo and implantation research.

The Society for Assisted Reproductive Technology (SART) and the American Society for Reproductive Medicine (ASRM) will soon jointly publish guidelines on the number of embryos to transfer in IVF, taking into consideration the major parameters of age, programme experience, and previous failure. Couples should make informed consent in terms of both the potential of reducing the pregnancy rate if too few embryos are transferred and, when a greater number of oocytes or embryos are replaced, of the risk of multiple pregnancy with its attendant complications whether the pregnancy is subjected to a reduction procedure or carried intact. The decision on the number of oocytes or embryos to transfer should be reached with the input of the laboratory personnel, the couple, and the attending clinician. Imposing a strict limit on the number of oocytes or embryos to transfer without regard to the relevant influencing parameters would no doubt be a disservice to a significant subset of the treated population and to the field of ART in general.

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In-vitro fertilization as a social experiment

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Recent proposals to limit the number of embryos that a physician may transfer raises the larger question: which aspects of in-vitro fertilization (IVF) should be regulated by legal institutions? In some countries, such as the UK, regulatory bodies determine the number of embryos that a physician may transplant into a woman's uterus after IVF. Despite the known health risks of multiple pregnancies and births, the practice of IVF by physicians remains relatively unregulated in the highly decentralized legal system of the US where legislators and regulators operate within constitutional limitations. Those restrictions are the underpinning of a public policy framework in which potential recipients, rather than legislators, determine the number of embryos transplanted, but legislators can determine the circumstances under which physicians offer assisted reproduction services.

What to regulate?

Policy makers often forget that the individuals who use IVF have usually explored other alternatives for becoming parents. For these patients, assisted reproduction is a last resort. Policy makers also often fail to understand that assisted reproduction has its own risks, including the risk that no children will be born. More important, policy makers often ignore that becoming a parent is a deeply private and, in some cases, spiritual and religious decision, which should be made with minimal interference from the state and other public bodies. These aspects of assisted reproduction provide the social context for my assertion that limiting the number of embryos a physician can transplant in effect constricts the opportunities of some individuals to become parents.

Some aspects of the IVF process are legitimate areas for legal regulation. For instance, legislatures should decide whether agreements between private individuals who use in-vitro techniques will determine parental status when there is

a dispute among the participants. Some states, such as New Hampshire, have established a court-supervised process for enforcing such agreements. Other states, such as New York, have enacted statutes declaring such agreements to be against public policy as well as unenforceable. Prohibiting or facilitating surrogate arrangements is a legitimate legislative choice because defining who is a 'parent' after a child is born is primarily a social and legal decision (Palmer, 1994).

Before a child is born, limitations on the number of embryos that a physician may transplant is probably unconstitutional. The US Supreme Court's recent opinion on abortion (Planned Parenthood of Southeastern Pennsylvania versus Casey), limits the manner in which political institutions can exercise influence over a woman's decision about becoming a parent. The Court ruled that legislation requiring a married woman to notify her husband of her intentions to have an abortion was unconstitutional. As a result, a physician cannot be forced to require a married woman to certify that she has notified her husband of her intentions to terminate her pregnancy.

On the other hand, the Court declared that it was constitutional for legislatures to require physicians to give women specific information about fetal development prior to performing an abortion. Special legislative standards for obtaining a woman's 'informed consent' are constitutional, even though the woman is exercising her constitutional right in terminating her pregnancy. These holdings in Casey are part of a larger principle of public policy making: attempts to regulate the role of medicine in family formation must respect the right of the individual to decide whether and when to become a parent.

Maximizing individual choice about parenthood entails some social and moral costs. Firstly, some physicians may abuse the trust of their patients. Physicians, for instance, may transfer gametes without the donor's or the recipient's knowledge or consent, as is alleged to have happened recently in California. Physician-initiated gamete transfer among patients suggests that physicians are using the vulnerability of infertile couples to further their own professional or personal agendas. Existing legal tools are adequate to deal with physicians operating beyond any reasonable notion of the medical and ethical frontiers in assisted reproduction. When these types of abuses are discovered, the abused individuals should be able to recover monetary damages from the offending physician.

Secondly, patients may make requests of physicians, such as a desire for 'the perfect baby', which are trivial. The profession, particularly those physicians engaged in infertility practice, must begin to establish limits on how far it will go to meet the demands of the potential consumers of their services. On the assumption that there is no social consensus or legal doctrine to address these emerging ethical dilemmas, fertility centres would do well to develop advisory ethics groups. These groups would help physicians and their colleagues to educate themselves to the ethical pitfalls as assisted reproduction becomes part of accepted medical practice (Seibel, 1996).

Constitutional doctrines and legislation on surrogacy provide important lessons for the debate on regulating the number of embryos a physician should transfer. First, the legal definition of a parent is a legitimate sphere of public regulation, even though the desire to become a parent may be deeply private, religious or spiritual. To put it another way, just because physicians may view an individual as a 'parent' does not make that individual socially or legally a parent. When physicians, for instance, engage in ovum gamete transfer there is no guarantee that the resultant parental status of the adults will be as the physicians and parties initially intended. Legislation surrounding parental status in the US is likely to end in some type of compromise between those who view assisted reproduction as a social good and those with ethical objections to the entire practice of assisted reproduction.

Second, legislatures in the US could develop special informed consent standards for use within the physician-patient relationships in assisted reproductive technology. As an example, the kind of consent procedures established in the UK for storing and destroying frozen embryos (Edward and Beard, 1997) would be constitutional in the US. Legislatures could, therefore, provide standards for what the physician must tell the patient about risks before embryo implantation. But legislative attempts to determine the number of embryos a physician may transplant should be viewed as violations of the liberty and interests of prospective parents. In the US, it is clear that liberty can lead to imperfect choices; but that is, after all, the cost of the American form of democracy.

Conclusion

Assisted reproduction, with all of its attendant ethical questions about selective termination of pregnancy, storage of embryos, and research on embryos, is now a part of the social fabric of the US. The earlier question of whether legal institutions should allow IVF (Robertson, 1994) has become increasingly irrelevant to the policy makers who must resolve these ethical dilemmas. The practice of providing professional services to overcome various forms of infertility when many of the ethical and legal issues remain unresolved is a social experiment. Gamete donors should be allowed to be participants in this social experiment because their choice of the number of embryos transferred is protected from state interference by constitutional norms and values in the US (Palmer, 1995). Physicians and research scientists are accustomed and encouraged to explore the medical frontiers. The negative response of some American policy makers, including the President of the US, to the recent cloning of a sheep in the UK should remind practitioners of assisted reproduction that their craft and science touch on the core of the various definitions of being human.

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