In human in-vitro fertilization (IVF) embryos are routinely transferred to the uterus on day 2 or day 3 of development. Resultant implantation and pregnancy rates are disappointingly low, with only ~10% of embryos transferred leading to a live birth. The ability to culture embryos to the blastocyst stage should help to resolve this problem by synchronizing the embryo with the female reproductive tract, and by identifying those embryos with little developmental potential. Co-culture has offered a possible means of producing blastocysts capable of high implantation rates. However, recent developments in the field of embryo physiology and metabolism have led to the formulation of new sequential serum-free culture media capable of supporting the development of viable blastocysts in several mammalian species, including the human. It is therefore proposed that blastocyst transfer should be considered for routine use in human IVF. The high viability of blastocysts cultured in the appropriate sequential media means that fewer embryos are required for transfer to achieve a pregnancy, culminating in fewer multiple births. Furthermore, the development of suitable non-invasive tests of embryo viability should further increase the overall success of human IVF by the ability to select before transfer those blastocysts most able to establish a pregnancy.

**Key words:** blastocyst transfer/implantation rate/metabolism/viability

### Introduction

This paper aims to highlight the recent developments in embryo culture systems which have resulted in an ability to produce highly viable blastocysts from the zygotes of several mammalian species, including the human. Furthermore, a non-invasive method to quantify blastocyst viability prior to transfer is proposed. It is envisaged that blastocyst transfer in human in-vitro fertilization (IVF) will result in an increase in implantation and pregnancy rates and decrease the number of embryos required for transfer in order to achieve a pregnancy.

It is beyond the scope of this paper to review the history of embryo physiology and culture. Several in-depth accounts of this have been published over the past decade (Biggers, 1987; Biggers et al., 1989; Leese, 1991; Rieger, 1992; Gardner and Lane, 1993a; Bavister 1995).

**Why transfer embryos at the blastocyst stage?**

It is an accepted global practice in human IVF to transfer embryos on day 2 (around the 4-cell stage) or on day 3 (around the 8-cell stage) of development. However, it is...
important to note that, in vivo, such cleavage stage embryos reside in the Fallopian tube and not in the uterus. The significance of this observation is that in other mammalian species the transfer of cleavage stage embryos to the uterus does not result in high pregnancy rates when compared with embryos transferred post-compaction or at the blastocyst stage (Bavister, 1995). Indeed, the premature replacement of the human embryo to the uterus may account in part for the low implantation rates associated with human IVF. Implantation rates of 10–15% are routinely reported in the literature, with only ~10% of embryos transferred proceeding to term. Data to date on the replacement of human cavitating morulae and/or blastocysts on day 4 or 5 of development indicate that such embryos have a higher implantation rate (Huisman et al., 1994; Olivennes et al., 1994; Ménézo and Ben Khalifa, 1995). In the case of the blastocyst, implantation rates twice those of cleavage stage embryos have been reported (Scholtes and Zeilmaker, 1996).

Such data therefore support the hypothesis that the transfer of later stage embryos will increase implantation and pregnancy rates per embryo transferred. In support of this hypothesis is the study by Buster et al. (1985), in which human blastocysts developed in vivo and flushed from the uterus were transferred singly to recipient patients. In this case an implantation and pregnancy rate of 60% per blastocyst transferred was reported.

Potential advantages of blastocyst culture and transfer in human IVF therefore include: (i) synchronization of the embryo with the female tract leading to increased implantation rates, thereby reducing the need for multiple embryo transfers; (ii) assessment of viability of an embryo before transfer. This can be achieved by both the identification of those embryos with little developmental potential, as manifest by slow development or degeneration in culture (Dawson et al., 1995; Ménézo and Ben Khalifa, 1995), and by the introduction of non-invasive tests of developmental potential to select the most viable embryos from within a cohort for transfer (Gardner and Leese, 1987, 1993; Lane and Gardner, 1996). Furthermore, culture of the human embryo beyond the 4–8-cell stage, the time at which the genome is activated (Braude et al., 1988), will facilitate the quantification of true embryonic markers as opposed to those inherited from the oocyte, i.e. after the 8-cell stage one is assessing embryo physiology, while prior to this the physiology of the cleavage stage embryo reflects that of the oocyte; (iii) an increase in the time available between cleavage stage embryo biopsy and embryo transfer. This is of particular importance where the biopsied material has to be sent to a separate locale for analysis; and (iv) facilitation of the introduction of trophectoderm biopsy for the screening of genetic diseases. Trophectoderm biopsy represents the earliest form of genetic diagnosis of non-embryonic material.

The question is, therefore, why are embryos not routinely transferred at the cavitating morula or blastocyst stages? The answer stems in part from an inability to maintain the mammalian embryo in culture for more than a couple of days without compromising its viability. It is important here to differentiate between the ability of a particular culture medium to support blastocyst development in vitro and the ability of the said culture medium to give rise to a viable blastocyst. For example, although rates of blastocyst development of 40% have been reported (Bolton et al., 1991), the resultant blastocysts only gave rise to an implantation rate and live birth rate of 7%. Other groups have reported similar rates of blastocyst development in culture (42% by Hardy et al., 1989a; and 26% by Dokras et al., 1993). These studies used either Earle’s balanced salt solution, supplemented with pyruvate, or medium T6, both supplemented with 10% maternal serum. Clearly then, there are deficiencies in such simple culture media. Interestingly, when Hardy et al. (1989b) replaced serum with bovine serum albumin (BSA), and cultured embryos in reduced volumes, the resultant blastocyst formation was 59%. This apparent increase in blastocyst development could be due to either the removal of serum and/or the culture of embryos in a reduced volume (see below). Subsequently Noda et al. (1994) demonstrated that, using a more complex tissue culture medium, α-minimal essential medium (MEM), it was possible to achieve blastocyst formation rates of 47.4% on day 5 after insemination. However, the resultant pregnancy rates from day 5 transfers was 28.2%, lower than those obtained for day 2 (33.3%) and day 3 (37.5%) transfers in the same programme. It is also important to acknowledge that relatively high rates of blastocyst development of >60%, have been achieved using Vero cell co-culture systems (Ménézo et al., 1990, 1995; Turner and Lenton, 1996). However, the efficacy of co-culture in human IVF in prospective randomized trials has been challenged (Van Blerkom, 1993; Sakkas et al., 1994), and further studies are required to validate the suitability or otherwise of co-culture systems. However, analysis of the modifications made to culture medium by the somatic cells used in co-culture system, may help in the formulation of defined culture media (Moore and Bondioli, 1993; Edwards et al., 1997).

So, even though good rates of blastocyst development are attainable in culture, the viability of such blastocysts appears to be <60% implantation and live birth rate reported by Buster et al. (1985) for in-vivo developed blastocysts. Significantly, the ability of a given set of culture conditions to give rise to a high percentage of blastocysts does not necessarily mean that such blastocysts are viable. Different
culture media which support equivalent rates of blastocyst development can result in very different pregnancy rates (Gardner and Sakkas, 1993; Lane and Gardner, 1997a).

Furthermore, it is not conceivable that all pronuclear embryos conceived through IVF will be able to reach the blastocyst stage, as a significant number will be abnormal. The majority of such abnormalities are chromosomal. It has been determined that ~25% of oocytes are aneuploid (Kola et al., 1993; Van Blerkom, 1994), and that this problem is exacerbated with maternal age (Janny and Ménézo, 1996).

Although the culture to the blastocyst stage prior to transfer will eliminate the grossly abnormal embryos, some aneuploids will form blastocysts. Given a particular case history of repeated spontaneous abortions, such patients would be candidates for trophectoderm biopsy prior to transfer to identify possible affected embryos. Other factors contributing to embryonic attrition include an insufficiency of stored oocyte coded gene products, and a failure to activate the embryonic genome (Tesarik, 1994). Therefore, the culture of embryos to the blastocyst stage in vitro can be considered as part of the natural selection that embryos undergo during development. One could then conclude that embryos that reach the blastocyst stage have a higher inherent developmental potential.

There have been numerous developments in the field of embryo culture media over the past decade which lead us now to propose that the time has come to re-evaluate our approach to human embryo culture and consider the routine transfer of the blastocyst stage in human IVF. The data presented in this review will show that not only is it possible to culture the zygotes of several mammalian species to the blastocyst stage at high frequencies in defined culture media in the absence of serum and somatic cell support through co-culture, but that resultant blastocysts have a high developmental potential after transfer, close to reported values for in-vivo developed blastocysts.

A key component in the evolution of the new generation of embryo culture media has been the determination of both the changes that occur in embryo physiology as development proceeds, and the parallel changes in the environment of the female reproductive tract. In the past, media designed specifically for embryo culture have tended to be rather simplistic, lacking important regulators of cellular function and homeostasis such as amino acids. This problem has been compounded by the routine employment of a single medium to support the embryo during its highly dynamic period of preimplantation development. Data will be presented here which support the hypothesis that media for embryo culture need to be more complex and reflect the environment of the female reproductive tract. Furthermore, the composition of such media should change as embryo development proceeds. The concept of developing an optimal medium is therefore challenged, optimal embryo growth requiring the use of two or more media formulated to fulfil the changing requirements of the embryo, the composition of which needs to increase in complexity as development proceeds.

**Embryo physiology**

An important, yet apparently overlooked, consideration when formulating culture media for the development of the preimplantation mammalian embryo is that the starting point of culture, the zygote, shares few physiological similarities with the blastocyst, the end point of the preimplantation period. The cleavage stage embryo is characterized by relatively low levels of biosynthesis (Epstein and Smith, 1973), low respiratory rates (Mills and Brinster, 1967; Houghton et al., 1996; Thompson et al., 1996) and limited capacity to utilize glucose as an energy source (Brinster and Thomson, 1966; Biggers et al., 1967, 1989; Whittingham, 1971; Leese and Barton, 1984; Gardner and Leese, 1986; Leese, 1991). In contrast, post-compaction, biosynthetic rates are increasing, along with an increased respiratory capacity and an ability to utilize glucose. This has significant implications for the production of energy by the embryo and the relative activities of the pathways used to do so (Rieger, 1984; 1992; Gardner, 1998). It is important to note that any perturbations in the relative activities of specific energy generating pathways during development, induced by the culture conditions, has dire consequences for subsequent development. Prior to the morula stage, individual blastomerses of the embryo remain unattached to each other and are subsequently all exposed to the external environment to an equal degree. Before compaction each blastomere can be considered as an independent entity, lacking the sophisticated regulatory mechanisms present in a multicellular tissue. Indeed, the homeostatic mechanisms employed by the individual blastomeres of the cleavage stage embryo are somewhat analogous to those used by unicellular organisms (Gardner 1998). It is therefore important to take this into account when designing media for the early embryo. Of significance to embryo physiology is that compaction represents the formation of the first transporting epithelium of the conceptus (Biggers et al., 1987). Once the embryo is capable of actively controlling ionic gradients etc, it is able to regulate its internal environment. Therefore, with regard to embryo physiology, it is appropriate to consider the preimplantation period in at least two phases: pre- and post-compaction. Such a breakdown of the preimplantation period is of importance when one considers changes to medium formulations. Other considerations include the time at which the embryonic...
genome is activated, when the embryo appears to be increasingly susceptible to its external environment. However, with the exception of the mouse, this usually occurs just prior to the time of compaction (Telford et al., 1990).

**Influence of culture volume and embryo grouping**

Before considering the formulation of embryo culture media, it is worth considering the physical dimensions in which we attempt to culture embryos. In general there is a tendency to culture human embryos in tubes or 4-well plates, in relatively large volumes of culture media, up to 1 ml. However, it has been demonstrated in several species that the culture of embryos in reduced volumes of medium and/or in groups, significantly increases blastocyst development (Wiley et al., 1986; Paria and Dey, 1990; Lane and Gardner, 1992), as well as increasing blastocyst cell number (Paria and Dey, 1990; Lane and Gardner, 1992; Gardner et al., 1994). More importantly, culturing embryos in reduced volumes increases subsequent viability after transfer (Lane and Gardner, 1992). It is proposed that the beneficial effects of growing embryos in small volumes and groups is due to the production of autocrine/paracrine factor(s) by the embryos that stimulate their own or surrounding embryo development. Therefore culture in large volumes results in a dilution of the factor so that it becomes ineffectual (Gardner, 1994). This phenomenon is not confined to the mouse, in which several embryos reside in the female tract at one time, but has also been reported for the sheep, which like the human is monovular (Gardner et al., 1994). Therefore, such data have implications for human IVF. It has recently been demonstrated that decreasing the incubation volume:embryo ratio specifically stimulates the development of the inner cell mass (ICM), as blastocysts cultured in a reduced incubation volume:embryo ratio had significantly more ICM cells than those cultured in large volumes, whilst the number of trophectoderm cells was unaffected (Gardner et al., 1997a; Figure 1). This would explain the increased viability of embryos cultured in reduced volumes or in groups (Lane and Gardner, 1992). It is therefore plausible that this effect is manifest through a specific embryo derived factor(s), and that such factor(s) are produced throughout development as embryos cultured in a simple medium that was changed every 48 h, also had a significantly reduced viability after transfer (Lane and Gardner, 1994). Possible candidates for such autocrine/paracrine factor(s) include platelet activating factor and insulin like growth factor II (O’Neill, 1997). The identification of such factors will be of greatest significance when there are only a limited number of embryos available for culture.

![Figure 1. Effect of incubation volume:embryo ratio on cell allocation in the mouse blastocyst. Two-cell embryos from CF1 mice were cultured in mMTF medium (Gardner and Lane, 1993c), in increasing volumes. After 72 h of culture total blastocyst cell number (solid bars) and cell allocation to the trophectoderm (hatched bars) and inner cell mass (open bars) were determined. Significantly different from 1:2 µl; *, P < 0.05; **, P < 0.01.](image)

**Types of embryo culture media**

In spite of the evident changes the embryo undergoes during the preimplantation period, attempts to optimize culture conditions have focused on the development of a single medium to support all stages prior to implantation. In general, the types of media presently used for human IVF fall into two categories; simple and complex (Gardner and Lane, 1993a). Simple media are those such as Earle’s (Edwards, 1981), T6 (used by Dokras et al., 1993) and human tubal fluid (HTF) (used by Quinn et al., 1985), which are balanced salt solutions with added carbohydrate energy sources such as pyruvate, lactate and glucose. Complex media, such as Ham’s F-10 and α-MEM, are those designed specifically for the culture of somatic cells in vitro. It is not uncommon for such media to be supplemented with serum, usually in the form of the patient’s own or fetal cord serum. The composition of the two most commonly used culture media for human IVF, HTF and Ham’s F-10 are shown in Table I. What is perhaps most surprising from this comparison is that equivalent rates of embryo development and pregnancy have been reported for both media, despite the obvious differences in composition. This can perhaps best be explained by the fact that resultant embryos are transferred very early in development, before true differences in embryo development can be assessed. Furthermore, although equivalent rates of success have been reported for both types of media, the key observation is that the success in both is depressingly low, with only around 10% of the
embryos transferred in human IVF resulting in a live birth. Consequently, multiple embryos are transferred in order to achieve an acceptable pregnancy rate of 20–30% per embryo transfer procedure.

So what are the new developments in embryo culture media which are proposed to help address this situation? The answer lies with a series of studies which demonstrate the changing requirements of the developing mammalian embryo, which supports the hypothesis that to obtain high rates of viable embryo development in culture, one must alter media composition at key times during the preimplantation period in order to meet the needs of the embryo and minimize cellular trauma in the embryo. Examples of such cellular trauma are given in the following sections.

**Carbohydrates**

Unlike most somatic cells, the cleavage stage embryos of several mammalian species do not appear to utilize glucose as an energy source to any great extent (Biggers et al., 1967; 1989; Leese, 1991). Indeed, in simple culture media, glucose is responsible for the retardation or developmental arrest of the cleavage stage embryo from several mammalian species (Schini and Bavister, 1988a, Chatot et al., 1989; Thompson et al., 1992; Conaghan et al., 1993a; Gardner and Lane, 1993b; Rosenkranz et al., 1993; Matsuyama et al., 1993; Quinn, 1995). Rather than using glucose, the zygote and subsequent cleavage stages utilize carboxylic and amino acids (Leese, 1991; Gardner and Lane, 1993a; Bavister, 1995). The premature utilization of glucose by the cleavage stage embryo is associated with an impairment of oxidative capacity, culminating in reduced embryo development (discussed below). Parallel with the changes in the embryo’s physiology and energy metabolism, are changes in the composition of fluids of the female reproductive tract. Analysis of oviduct and uterine fluids throughout the menstrual cycle in the human revealed changes in carbohydrate concentrations both within the cycle and between the oviduct and uterus (Gardner et al., 1996a). Specifically, the levels of glucose in the oviduct were lowest at the time when the oocyte/early embryo would be present (0.5 mM), whilst pyruvate was at its highest (0.32 mM). Conversely, glucose reached its peak concentration in the uterus (3.15 mM) and pyruvate its lowest (0.1 mM; Table II). Importantly, the cumulus cells surrounding the oocyte and early embryo are themselves very active metabolically and readily consume glucose and release lactate and pyruvate (Leese and Barton, 1985; Gardner et al., 1996a). It is plausible therefore that, in vivo, the oocyte and zygote are exposed to an essentially glucose free environment.

In the light of its detrimental effect on embryos when present in simple culture media, there is a growing tendency to remove glucose from media for the development of the human embryo (Quinn, 1995; Pool et al., 1997). Although it may be considered physiological to culture the zygote and possibly the following couple of cleavage stages in the absence of glucose, the complete removal of glucose from culture media designed to support embryo development beyond the first few cleavage divisions cannot be considered as physiological. When the cumulus cells have dispersed, the embryo will be exposed to glucose in the female tract, albeit at low concentrations in the oviduct. Before removing glucose from embryo culture media it is important to consider three things; firstly, the embryo possesses a specific transporter for glucose from at least the 2-cell stage onwards, indicating some physiological function for this hexose (Gardner and Leese, 1988). Secondly, in the presence of suitable regulators (such as amino acids; discussed below) glucose does not impair embryo development, and thirdly, glucose has important cellular functions other than as an energy source. Glucose is a key anabolic precursor and is required for the synthesis of triacylglycerols and phospholipids, and as a precursor for complex sugars of mucopolysaccharides and glycoproteins. Glucose metabolized by the pentose phosphate pathway (PPP) generates ribose moieties required for nucleic acid synthesis and the reduced nicotinamide dinucleotide phosphate (NADPH) required for the biosynthesis of lipids and other complex molecules (Reitzer et al., 1980; Morgan and Faik, 1981). NADPH is also required for the reduction of intracellular glutathione, an important antioxidant for the embryo (Rieger, 1992). Therefore, glucose will become increasingly important once the embryonic genome is activated and biosynthetic levels increase. In support of these roles for glucose in the embryo, although blastocysts can be obtained in the absence of glucose in the culture medium (Quinn, 1995, Gardner and Lane, 1996), resultant blastocysts have significantly impaired developmental competence after transfer compared with blastocysts which developed in the presence of glucose (Gardner and Lane, 1996).

**Amino acids**

The role of amino acids in mammalian embryo development has been somewhat overlooked, and only recently has their significance been highlighted (Bavister and McKiernan, 1993; Gardner and Lane, 1993a,c; Bavister, 1995; Gardner 1998). It is proposed that amino acids are amongst the most important regulators of mammalian preimplantation development, and therefore amongst the key constituents of embryo culture media. Not only do amino acids maintain cellular function, but they are capable of stimulating both development and differentiation of the embryo in vitro and of increasing developmental potential in vivo. Indeed, the
inclusion of specific amino acids in embryo culture media has been shown to alleviate the so-called 'culture blocks' to mammalian embryo development in all species studied to date (rabbit; Kane and Foote, 1970; hamster; Bavister et al., 1983; Schini and Bavister, 1988a,b; Bavister and McKiernan, 1993; mouse, Gardner and Lane, 1996; rats, Kishi et al., 1991; sheep, Gardner et al., 1994; cow, Gardner, 1994).

Table I. Composition of human tubal fluid (HTF) and Ham’s F-10 media

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (mM)</th>
<th>Component</th>
<th>Concentration (mM)</th>
<th>Component</th>
<th>Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>101.60</td>
<td>NaCl</td>
<td>126.60</td>
<td>Lysine</td>
<td>0.20</td>
</tr>
<tr>
<td>KCl</td>
<td>4.69</td>
<td>KCl</td>
<td>3.82</td>
<td>Methionine</td>
<td>0.03</td>
</tr>
<tr>
<td>MgSO4.7H2O</td>
<td>0.20</td>
<td>MgSO4.7H2O</td>
<td>0.62</td>
<td>Phenylalanine</td>
<td>0.03</td>
</tr>
<tr>
<td>Na2HPO4</td>
<td>1.31</td>
<td>Proline</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KH2PO4</td>
<td>0.37</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaCl2.2H2O</td>
<td>2.02</td>
<td>CaCl2.2H2O</td>
<td>0.30</td>
<td>Threonine</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CuSO4.5H2O</td>
<td>0.00001</td>
<td>Tryptophan</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FeSO4.7H2O</td>
<td>0.0030</td>
<td>Tyrosine</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZnSO4.7H2O</td>
<td>0.0001</td>
<td>Vaine</td>
<td>0.03</td>
</tr>
<tr>
<td>NaHCO3</td>
<td>25.00</td>
<td>NaHCO3</td>
<td>14.28</td>
<td>Biotin</td>
<td>0.0001</td>
</tr>
<tr>
<td>sodium pyruvate</td>
<td>0.33</td>
<td>Sodium pyruvate</td>
<td>1.00</td>
<td>Ca pantothenate</td>
<td>0.0015</td>
</tr>
<tr>
<td>sodium lactate</td>
<td>21.40</td>
<td>Calcium lactate</td>
<td>2.23</td>
<td>Choline chloride</td>
<td>0.005</td>
</tr>
<tr>
<td>glucose</td>
<td>2.78</td>
<td>Glucose</td>
<td>6.11</td>
<td>Cyanocobalamine</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Alanine</td>
<td>0.10</td>
<td>Folic acid</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arginine</td>
<td>1.21</td>
<td>Inositol</td>
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<tr>
<td></td>
<td></td>
<td>Asparagine</td>
<td>0.11</td>
<td>Nicotinamide</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aspartic acid</td>
<td>0.10</td>
<td>Pyridoxine</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cysteine</td>
<td>0.26</td>
<td>Riboflavin</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glutamate</td>
<td>0.1</td>
<td>Thiamine</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glutamine</td>
<td>1.0</td>
<td>Hypoxanthine</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Glycine</td>
<td>0.1</td>
<td>Lipic acid</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Histidine</td>
<td>0.14</td>
<td>Thymidine</td>
<td>3.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Isoleucine</td>
<td>0.02</td>
<td>Leucine</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Table II. Concentration of carbohydrates in the human oviduct and uterus (from Gardner et al., 1996a)

<table>
<thead>
<tr>
<th></th>
<th>Pyruvate (mM)</th>
<th>Lactate (mM)</th>
<th>Glucose (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oviduct (midcycle)</td>
<td>0.32</td>
<td>10.5</td>
<td>0.50</td>
</tr>
<tr>
<td>Uterus</td>
<td>0.10</td>
<td>5.87</td>
<td>3.15</td>
</tr>
</tbody>
</table>

aLactate measured as the biologically active L-isoform.

The absence of amino acids from conventional embryo culture media has stemmed from the earliest attempts to culture the mammalian embryo. The pioneering research in the laboratories of Wes Whitten (Whitten, 1956; 1957) and John Biggers (Brinster, 1965; Biggers et al., 1967; Whitten and Biggers, 1968) in the late 1950s and the 1960s, led to the formulation of media which could support the development of 2-cell mouse embryos, and zygotes from F1 hybrid parents, to the blastocyst stage. Such media were based on balanced salt solutions supplemented with carbohydrates and BSA, but lacked amino acids. Resultant blastocysts were subsequently shown to be capable of giving rise to live offspring after transfer to recipient females. However, once this had been shown, few changes were made in culture conditions for mouse embryos for nearly 20 years. The impetus for a resurgence in embryo culture research came from the increasing number of human IVF programmes and the realization that embryo development in culture was not only far from optimal, but contributed significantly to the observed embryonic loss after transfer. Interest in the role of amino acids in mammalian embryo development was rekindled by work on the hamster embryo by Bavister’s laboratory, who showed that specific amino acids were essential for development of the zygote past the 2- and 4-cell block in culture (Schini and Bavister, 1988a,b; McKiernan et al., 1991). Parallel studies on the mouse embryo concentrated on the effects of those amino acids present at high levels in the fluids of the female reproductive tract (Miller and Schultz, 1987; Moses et al., 1997). Interestingly, this group of amino acids, with the exception of glutamine, shared a striking homology with those present in Eagle's non-essential amino acids (Eagle, 1959), i.e. those amino acids not required for the development of somatic cells in culture (Table III). Initial studies revealed that the
non-essential group of amino acids and glutamine stimulated the development of F1 mouse zygotes to the blastocyst stage within 72 h of culture (Gardner and Lane, 1993c), the time at which the mouse blastocyst is formed in vivo. Subsequently, it was shown that these amino acids significantly reduced the duration of the first three cell cycles, resulting in increased rates of development and viability (Lane and Gardner, 1994, 1997a). Quite remarkably, the essential group of amino acids (which are present at relatively low levels within the female tract) not only did not confer any benefit to the embryo in culture prior to the 8-cell stage, but exposure of the mouse embryo to such amino acids at the concentration in Eagle’s medium before compaction resulted in loss of viability (Lane and Gardner, 1994) and impaired development to the blastocyst stage in culture (Gardner and Lane, 1993c; Lane and Gardner, 1994, 1997b). However, studies on mouse embryos collected from the oviduct/uterine junction at the 8-cell stage and then placed in culture showed that the exposure of such later stage embryos to the essential group of amino acids was actually stimulatory, specifically increasing the cleavage rate of the ICM and subsequent fetal development. Concomitantly, the non-essential amino acids and glutamine were found to stimulate the formation of the blastocoel, increase trophoderm cell number and increase hatching rates (Lane and Gardner, 1997b). Therefore, the highest percentage of blastocyst development and hatching, the highest total cell and ICM number, along with the highest implantation rates and fetal development occurred when mouse zygotes were cultured for the first 48 h in the presence of non-essential amino acids and glutamine, followed by culture for a further 48 h in the presence of all 20 of Eagle’s amino acids (i.e. both non-essential and essential groups). One of the more significant observations from these studies was that the implantation rates were equivalent to those of embryos developed in vivo. The importance of a culture system that stimulates ICM proliferation is that ICM development is positively correlated with subsequent fetal development after transfer (Lane and Gardner, 1997b). Furthermore, these studies question the suitability of embryo morphology as a marker of embryo development when comparing the suitability of different culture systems.

In summary, it is evident that along with the major changes that occur in the energy metabolism of the embryo during the preimplantation period (Biggers et al., 1989; Leese, 1991; Rieger, 1992), there are specific changes in the nitrogen requirements of the embryo. That such a change exists infers different roles for the two groups of amino acids. In terms of the embryo’s physiology, it is important to consider the different functions and thereby mechanisms by which amino acids confer their benefit to the embryo as it develops and differentiates. Importantly, an exposure as short as 5 min to medium lacking amino acids at the zygote stage results in a significant impairment of subsequent development (Gardner and Lane, 1996). It is proposed that during this time the embryos loses its intracellular pool of amino acids to the surrounding medium through mass action, resulting in intracellular trauma. This would suggest that it is important for oocytes and embryos to be maintained and cultured in the presence of amino acids.

Why then are non-essential amino acids and glutamine beneficial to the embryo prior to compaction? Three possible modes of action for amino acids are proposed, on top of their function as anabolic precursors for proteins, all of which have been shown to operate in the cleavage stage embryo. These mechanisms are: (i) regulators of energy metabolism; (ii) osmolytes; and (iii) buffers of [pH]i.

<table>
<thead>
<tr>
<th>Table III. Concentration of amino acids in Eagle’s medium</th>
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<tr>
<td>Non-essential amino acids</td>
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<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>Alanine</td>
</tr>
<tr>
<td>Asparagine</td>
</tr>
<tr>
<td>Aspartate</td>
</tr>
<tr>
<td>Glutamate</td>
</tr>
<tr>
<td>Glycine</td>
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<tr>
<td>Proline</td>
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<tr>
<td>Serine</td>
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aGlutamine, an essential amino acid as defined by Eagle (1959), is used at 1.0 mM.
Regulators of energy metabolism

The first explanation for the beneficial effects of amino acids is their ability to help regulate the activities of the energy generating pathways of the embryo. Prior to compaction, the mammalian embryo does not utilize glucose as the main energy source, but rather uses pyruvate, lactate and/or amino acids to generate relatively low levels of energy required through oxidation. Utilization of glucose through glycolysis by cleavage stage embryos is associated with impaired energy production and embryo development. The mechanism by which glucose affects the embryo has yet to be fully elucidated; however, it would appear that some form of the Crabtree effect (Koobs, 1972) is in operation, resulting in reduced respiratory capacity. In support of this hypothesis, Menke and McLaren (1970) reported that culture of 8-cell mouse embryos to the blastocyst stage in a simple culture medium resulted in an impairment of oxidative capacity. Subsequently, Gardner and Leese (1990) demonstrated that culture of morulae overnight in a simple medium, M16 (Whittingham, 1971), resulted in a significant increase in lactate production by the resultant blastocyst, with no concomitant changes in glucose uptake. Seshagiri and Bavister (1991) went on to show that both respiration and oxidative activity in the hamster embryo were inhibited by glucose and phosphate. A relative abundance of glycolytic enzymes in the preimplantation mammalian embryo (Biggers and Stern, 1973) may predispose the embryo to this type of in-vitro induced effect. It is evident, however, that the inclusion of specific amino acids, namely those in the non-essential group and glutamine, can reduce the detrimental effects of glucose on embryo development by inhibiting glycolytic activity (Gardner and Lane, 1993b). Similarly, Barnett and Bavister (1996) demonstrated that the inhibitory effect of glucose and phosphate on the development of the 2-cell hamster embryo in culture is significantly diminished in the presence of specific amino acids. Amino acids act possibly through their own oxidation, which in turn will lead to the allosteric inhibition of the glycolytic enzyme phosphofructokinase through an increase in the levels of ATP and/or citrate, or by the direct inhibition of a glycolytic enzyme, such as pyruvate kinase by alanine.

Osmolytes

A wide variety of organs and organisms employ extracellular amino acids and polyamines in order to maintain intracellular function and cell volume (Somero, 1986; Nakanishi et al., 1991; Yancey, 1995). As such these compounds are referred to as osmolytes. In support of the use of osmolytes by embryos is the observation that the osmotic pressure of oviduct fluid is >360 mOsmol (Borland et al., 1977; 1980), far greater than that of any culture medium. When embryos are incubated in such high osmolarities in the absence of known osmolytes, development is significantly impaired. In contrast, when osmolytes such as glycine are included in the culture medium increased embryo development is observed (Van Winkle et al., 1990; Van Winkle and Campione, 1996). Subsequent studies have shown that glutamine, taurine and betaine are also used by the cleavage stage embryo as osmolytes (Lawitts and Biggers, 1992; Biggers et al., 1993; Dumoulin et al., 1997). Importantly, the work of Ho et al. (1995) showed that the addition of amino acids to the mouse embryo culture medium KSOM enabled resultant blastocysts to synthesize mRNA of several types of proteins at levels found in in-vivo developed blastocysts. Such observations are consistent with amino acids maintaining intracellular physiology.

Buffers of pH

A further explanation for the beneficial effects of amino acids may lie with the physical arrangement of the blastomeres prior to compaction, i.e. they lack the transporting capabilities of an epithelium. Indeed the 2-cell mouse embryo appears to lack some of the regulatory mechanisms for controlling pH (Baltz et al., 1990, 1991). Bavister and McKiernan (1993) were the first to propose that specific amino acids might act as intracellular pH buffers. As the non-essential amino acids are present at relatively high concentrations within the female tract, amino acids such as glycine, which is present at concentrations >2 mM, could act as an intracellular zwitterionic buffer, helping to minimize pH fluctuations. Analysis of intracellular pH in individual mouse zygotes and morulae has revealed that prior to compaction the presence of non-essential amino acids and glutamine in the culture medium helps to minimize intracellular pH shifts when the embryo is exposed to an increasing acid load (D.K.Gardner and L.J.Edwards, unpublished observations; Figure 2). However, after compaction amino acids have little effect upon the embryo’s ability to regulate an acid load. Importantly, if the morula is de-compacted experimentally by incubation in calcium/magnesium free medium, then the embryo loses its ability to regulate pH. (D.K.Gardner and L.J.Edwards, unpublished observations). This data suggests that after compaction, the blastomeres start to acquire a more somatic cell-like physiology with the formation of an epithelium (Biggers et al., 1967). Therefore the embryo’s dependence upon extracellular buffers becomes diminished at compaction.

It has not yet been determined which particular amino acid(s) fulfil the above niches in cleavage stage embryo physiology, nor what their optimum concentrations are.
However, it would appear that the embryo exhibits a degree of plasticity regarding the use of such amino acids, in a way similar to that by which it can compensate for the lack of one particular carbohydrate in the culture medium by increasing the uptake of another (Gardner and Leese, 1988). For example, Dumoulin et al. (1992a,b) showed that when taurine is present as the sole amino acid in embryo culture media, it confers benefit upon the developing mouse embryo. However, when taurine is added to culture medium already containing non-essential amino acids and glutamine it has no effect on embryo development (Gardner and Lane, 1993c). The mechanism by which essential amino acids stimulate the development of the ICM has yet to be elucidated.

Importantly, the beneficial effects of amino acids have not been confined to one species. On the contrary, in all mammalian species studied to date, amino acids have been shown to be important regulators of embryo development (Bavister and McKiernan, 1993; Gardner and Lane, 1993c; Gardner, 1994; Gardner et al., 1994; Lui and Foote, 1995). In the case of the sheep, resultant blastocysts had equivalent viability to blastocysts developed in vivo (Gardner et al., 1994), further highlighting the significance of amino acids in regulating mammalian preimplantation development.

**Amino acids and ammonium: a catch 22?**

Although amino acids are important regulators of mammalian embryo development in culture, they are both metabolized by the embryo and also spontaneously undergo breakdown to release ammonium into the culture medium, the concentration of which increases with time (Gardner and Lane, 1993c). Unfortunately, ammonium is embryo toxic, not only impairing development in culture, but also significantly reducing viability (Gardner and Lane, 1993c; Lane and Gardner, 1994, 1997b). Furthermore, in the mouse embryo ammonium has been shown to induce the neural tube birth defect, exencephaly (Lane and Gardner, 1994). Therefore, in order to alleviate this toxicity, the culture medium has to be replaced every 48 h for the amino acids present to confer maximal benefit. This finding also has significant implication for the storage of any culture medium containing amino acids. However the renewal of culture media to prevent the toxic build up of ammonium also removes any potential autocrine factor(s) that stimulate embryo development. A solution to this paradox was the development of a method to transaminate ammonium in the culture medium to the amino acid glutamate eliminating the need to renew the medium.

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**Figure 2.** Effect of amino acids on regulating intracellular pH (pH_i) in mouse zygotes. Mouse zygotes were collected and maintained in modified mouse tubal fluid (mMTF) medium (Gardner and Lane, 1993c), with (solid circles) or without (open circles) non-essential amino acids and glutamine. Their baseline pH_i was then quantified using the pH-sensitive probe seminaphthorhodafluor (SNARF) and confocal microscopy. Groups of embryos were subsequently exposed to one of four concentrations of the non-metabolizable acid dimethadione (DMO). In the presence of amino acids there was a significant decrease in pH_i at concentrations of DMO of ≥ 5 mM. However, in the absence of amino acids, pH_i fell significantly at 1 mM DMO. At all concentrations of DMO, the fall in pH_i was significantly greater in the absence of amino acids (P < 0.01). Significantly different from 0 mM DMO; *, P < 0.05; **, P < 0.01.
(Lane and Gardner, 1995). After 48 h of culture the enzyme glutamate dehydrogenase, the substrate α-ketoglutarate and the co-factor NADH were added to the culture medium at embryo-safe concentrations, the enzyme subsequently converting the ammonium present to glutamate. Blastocysts cultured in the presence of amino acids when ammonium was transaminated from the medium had a significantly increased viability after transfer compared to blastocysts cultured with the medium renewed after 48 h, indicating that the continual exposure of embryos to these factors stimulates development (Lane and Gardner, 1995).

**Chelators**

The most commonly employed chelator in embryo culture media is EDTA, which sequesters divalent cations. Although EDTA was initially shown to alleviate the 2-cell block in mice embryos in 1977 by Abramczuk *et al.* (1977) it has not routinely been included in embryo culture media until relatively recently (Chatot *et al.*, 1989; Mehta and Kiessling, 1990; Lawitts and Biggers, 1993; Barnes *et al.*, 1995; Quinn, 1995; Summers *et al.*, 1995; Gardner and Lane, 1996; Gardner *et al.*, 1997b). It was proposed that EDTA in the culture media increases embryo development by the chelation of toxic heavy metal ions that might be present in the media. However, it has recently been determined that the presence of EDTA in the medium also inhibits premature utilization of glycolysis by cleavage stage embryos thereby preventing any Crabtree-like effect that is associated with developmental arrest in culture (Gardner and Lane, 1993a). In practical terms, it represents extra work in the busy IVF laboratory and also introduces the potential for disease transmission.

**Table IV.** Composition of human embryo culture media G1 and G2

<table>
<thead>
<tr>
<th>Component (mM)</th>
<th>G1</th>
<th>G2</th>
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<tbody>
<tr>
<td>NaCl</td>
<td>85.16</td>
<td>85.16</td>
</tr>
<tr>
<td>KCl</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>NaH2PO4.2H2O</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>CaCl2.2H2O</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>MgSO4.7H2O</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>NaHCO3</td>
<td>25.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Na pyruvate</td>
<td>0.32</td>
<td>0.10</td>
</tr>
<tr>
<td>Na lactate (L-isofrom)</td>
<td>10.5</td>
<td>5.87</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.50</td>
<td>3.15</td>
</tr>
<tr>
<td>Glutamine</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Taurine</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Non-essential amino acids</td>
<td>All</td>
<td>All</td>
</tr>
<tr>
<td>Essential amino acids</td>
<td>None</td>
<td>All</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.01</td>
<td>0.0</td>
</tr>
<tr>
<td>Human serum albumin</td>
<td>2 g/l</td>
<td>2 g/l</td>
</tr>
</tbody>
</table>

aNon-essential and essential amino acids present at the concentration specified by Eagle (1959; Table II). Antibiotics and Phenol Red are optional. Human serum albumin is preferred over bovine serum albumin. Osmolarity of both media is 260–265 mOsmol, (from Barnes *et al.*, 1995).

bAs sperm velocity and fertilization rates are reduced when glucose is omitted from the culture medium (Quinn, 1995), it is advisable to either increase the glucose concentration of G1 for fertilization, or simply increase the number of spermatozoa or reduce the duration of incubation of spermatozoa and oocytes (Giarnaroli *et al.*, 1996).

However, such details are not the major concern about the use of serum in embryo culture. Rather, recent data on the sheep embryo have shown that serum can adversely affect the development of embryos at several levels: (i) precocious blastocoel formation (Walker *et al.*, 1992; Thompson *et al.*, 1995); (ii) sequestration of lipid (Dorland *et al.*, 1994; Thompson *et al.*, 1995); (iii) abnormal mitochondrial ultrastructure (Dorland *et al.*, 1994; Thompson *et al.*, 1995); (iv) perturbations in metabolism (Gardner *et al.*, 1994); and

**Serum: friend or foe?**

Although there are several types of culture medium presently used in IVF, a common supplement to any embryo culture medium is serum. However, how suitable is serum as the protein/fixed nitrogen source for embryos? Oviduct fluid is not a serum transudate but is formed by the epithelium of the oviduct (Leese, 1988). In contrast, serum is formed by the pathological clotting of blood. One may therefore question the suitability of serum for embryo development. Furthermore, the composition of serum varies with many factors including patient aetiology, day of cycle, state of fasting/diet, so that each patient’s embryos are actually cultured in unique conditions making valid comparisons about embryo development very difficult if not impossible (Gardner and Lane, 1993a). In practical terms, it represents extra work in the busy IVF laboratory and also introduces the potential for disease transmission.
(v) association with abnormally large offspring in sheep (Thompson et al., 1995).

The mechanism by which serum induces these aberrations remains to be resolved. However, the role of growth factors in serum in inducing altered patterns of development cannot be overlooked. If serum does perturb embryo development so dramatically, why is it still used in human IVF? The main reason is that conventionally, embryos are transferred on days 2 or 3 of development, i.e. before compaction and possibly before the expression of growth factor receptors. Therefore the many adverse effects of serum observed in the blastocysts of other mammalian species are avoided. Should one wish to move to blastocyst transfers in the human, then the inclusion of serum in the culture system used should be considered very carefully. Although serum would seem a rather artificial inclusion in an embryo culture system, it does confer a major advantage in that it is a chelator of potential embryo toxins. Serum also acts as a pH buffer. As such, serum confers a certain degree of protection to the cleavage stage embryo. This has helped perpetuate the inclusion of serum in media. Fortunately, contrary to popular belief, serum is not required for the successful culture of the mammalian zygote to the fully expanded and hatching blastocyst stage. Rather serum can be replaced with an appropriate form of serum albumin (Pool and Martin, 1994) or suitable physiological macromolecule such as hyaluronate (Gardner et al., 1997c) together with more complex media designed to cater for the changing requirements of the embryo. In the case of hyaluronate, it was found that the inclusion of this glycosaminoglycan in the transfer medium alone resulted in an almost doubling of implantation rates of mouse blastocysts (Gardner et al., 1997c). The role of such glycosaminoglycans in human embryo development in culture and in implantation after transfer is therefore warranted.

**Formulation of physiological culture media**

From the data obtained from the studies outlined above it is evident that conventional embryo culture media are not suitable for the extended culture of embryos to the blastocyst stage, especially when such media are supplemented with serum. In the light of the data presented, two media have been formulated for the culture of the human zygote to the blastocyst stage (Barnes et al., 1995). These media have been designated G1 and G2, the composition of which is shown in Table IV. Medium G1 was formulated to support the development of the zygote to the 8-cell stage, whilst medium G2 was formulated to support development from the 8-cell stage to the blastocyst. Using these sequential media it was possible to obtain >75% blastocyst formation from 24 cryopreserved human pronuclear embryos after 5 days of culture (Gardner et al., 1997d). In a pilot clinical trial using these media, the mean blastocyst development on day 5 post-insemination was 66% in the eight patients involved (Figure 3). When a mean of 2.75 blastocysts were transferred (a total of 22 blastocysts) an implantation rate of 45% was obtained, culminating in an ongoing pregnancy rate of 63% (Gardner et al., 1998). In the following 10 patients, an implantation rate of 50% and ongoing pregnancy rate of >60% were established (D.K.Gardner and W.B.Schoolcraft, unpublished observations). Although these preliminary studies are encouraging, extensive prospective clinical trials are now required to validate the effectiveness of blastocyst transfer after development in such serum-free media.

**Requirement for quality control**

It is important to reiterate that the inclusion of serum, and somatic cells in co-culture, confers a degree of protection to the embryo from potential toxins. Therefore, when moving to a serum/somatic cell free system, it is imperative that adequate quality control procedures are set in place. All components of the culture media have to be screened individually using some form of bioassay. Unfortunately there is no guaranteed assay, save for human IVF itself. However, mouse embryos cultured from the pronuclear stage provide quite a sensitive bioassay, providing conditions are set to maximize the sensitivity of the embryos. Therefore, embryo culture should take place in the absence of any protein, at least from the 2-cell stage forward (Weiss et al., 1992; Gardner and Lane, 1993a). The culture period should be defined and development quantified at this point only. For example, >85% of F1 mouse zygotes should reach the fully expanded blastocyst stage after 96 h of culture. The majority of embryos will reach the blastocyst stage if the culture period is extended for a further 24 h, but this would make the results of the bioassay meaningless. Ideally blastocyst cell number should be determined, as it is a more sensitive measure of culture conditions. Alternatively, mouse IVF or intracytoplasmic sperm injection (ICSI) could be performed, although the extra time, cost and logistics may make this impractical for most IVF programmes.

**Assessment of embryo viability**

The assessment of embryo viability prior to transfer is notoriously subjective, morphology being the most commonly used parameter for selection. The development of more quantitative methods of assessing the developmental potential of an embryo will further increase the pregnancy rate following IVF. Several methods have been proposed and
tested, and their merits or otherwise have been reviewed extensively elsewhere (Rieger, 1984; Gardner and Leese, 1993; Overstom, 1996). However, two techniques do appear as possible candidates for both the predicting and quantification of embryo viability. The ability to predict whether an embryo will develop in culture has been associated with rate of cleavage (McKiernan and Bavister, 1994; Gonzales et al., 1995). In these studies it was observed that the speed at which an embryo developed was related to its ability to form a blastocyst. Furthermore, morulae and blastocysts developed from slowly dividing embryos gave rise to lower fetal development after transfer than those embryos which developed from more rapidly dividing cleavage stages (McKiernan and Bavister, 1994). Together with extended culture to the blastocyst, such analysis of embryo development may prove to be of significance in human IVF. A promising criterion for the quantification of embryo viability is energy metabolism, which can be readily quantified non-invasively for individual embryos in culture (Leese and Barton, 1984; Gardner and Leese, 1986). This approach provides the opportunity to identify the most viable embryos from a cohort at a given time. The first indication that embryo metabolism was linked to viability came from Renard et al. (1980) who showed in day 10 cattle blastocysts that those blastocysts with a glucose uptake of >5 µg/h developed better in culture and gave rise to more pregnancies than those blastocysts with a glucose uptake <5 µg/h. Due to the insensitivity of the spectrophotometric method used, it was not possible to analyse blastocysts before this advanced stage. Rieger (1984) went on to demonstrate that morphologically normal day 7 cattle blastocysts utilized significantly more glucose than degenerating embryos. Rieger therefore proposed that quantification of embryonic metabolism may provide a suitable marker of embryo viability. Gardner and Leese (1987) used the non-invasive technique of ultramicrofluorescence to measure glucose uptake by individual day 4 mouse blastocysts prior to the transfer to recipient females. Those blastocysts that went to term had a significantly higher glucose uptake in culture than

Figure 3. Human blastocysts developed in media G1 and G2. Expanded blastocyst on day 5 (4 days of culture from the pronuclear stage) (original magnification ×300). Hatched blastocyst on day 6 (5 days of culture from the pronuclear stage) (original magnification ×300).

Figure 4. Fetal development of mouse blastocysts selected for transfer using glycolytic activity as the selection criterion. ‘Viable’ blastocysts were classified as those with a glycolytic rate similar to that of in-vivo developed embryos, whilst ‘non-viable’ blastocysts were classified as those with excessive lactate production, beyond that which could be accounted for by glucose uptake alone. On each day of experiment a selection of blastocysts was transferred at random from a morphologically similar cohort, i.e. their metabolism was not used to select for transfer. Different superscripts indicate significantly different populations \((P < 0.01)\). For further details see Lane and Gardner (1996).
those embryos that failed to develop after transfer. However, such studies were retrospective, and so it was not evident whether metabolism could be used to select prospectively those embryos with the highest developmental potential. Subsequently it was found that the metabolism of frozen–thawed day 7 cattle blastocysts could be used to identify those embryos capable of re-expansion in the hours immediately after thawing and those embryos which subsequently died (Gardner et al., 1996b). Furthermore, there was no overlap between the viable and nonviable populations of blastocysts with regard to their glucose uptake and lactate production, indicating that such parameters could be used for the prospective selection of embryos for transfer. Lane and Gardner (1996) therefore used both glucose uptake and lactate production (to estimate glycolytic activity) to select prospectively individual day 4 mouse blastocysts for transfer. It was also proposed that the quantification of the activity of a metabolic pathway, as opposed to measuring nutrient uptake alone, would better reflect cellular function/viability. Prior to any transfers a distribution of glycolytic activity in individual blastocysts was determined and the hypothesis proposed that the most viable embryos would be the ones with a glycolytic activity closest to that observed for embryos developed in vivo (~50%; Gardner and Leese, 1990). Therefore those embryos exhibiting excessive lactate production were proposed to be non-viable, i.e. they had lost control over their energy metabolism. When morphologically identical blastocysts were selected at random for transfer, resultant fetal development was 20% per blastocyst transferred. However, when blastocysts were prospectively selected for with a glycolytic activity similar to in vivo developed blastocysts, the resultant fetal development rate was 80%, a 4-fold increase over random selection. In contrast, those blastocysts with an abnormally elevated glycolytic activity had a fetal development rate of only 6% (Figure 4). Such data therefore support the hypothesis that it is possible to select embryos for transfer using metabolic criteria. Now that culture systems exist which can maintain the human embryo to the blastocyst stage, it is proposed to use non-invasive assessment of physiology of the later stage embryo. Analysis of nutrient uptake/metabolism at the blastocyst stage will more accurately reflect embryonic function.

Conclusions and future scenarios

In this review, data have been presented to support the move to blastocyst culture and transfer in human IVF. Not only is it possible to achieve blastocyst development in defined culture media at rates equivalent to those obtained using co-culture and serum, but most importantly, the resultant blastocysts have a high viability as determined by their implantation and resultant pregnancy rates. This in itself will improve the overall efficiency of human IVF and lead to a reduction in the number of embryos required to achieve a given pregnancy, thereby reducing the number of embryos transferred and minimizing multiple births. The ability to select the most viable blastocysts for transfer by the non-invasive quantification of metabolism should culminate in further increases in implantation and pregnancy rates per blastocyst transferred.

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