Role of Autocrine Mediators in the Regulation of Embryo Viability: Lessons from Animal Models

INTRODUCTION

Recent research has resulted in an impressive adaptation of assisted reproductive technology to the treatment of a wide range of infertility etiologies. Yet there has been limited progress in the improvement of the viability of embryos produced by assisted reproductive technologies. Although it is difficult to obtain reliable data, anecdotal evidence suggests that overall only around 10% of embryos produced by in vitro fertilization (IVF) and related techniques survive to produce a neonate.

While therapies for human infertility pioneered the development of these techniques, they are now being widely used in other mammalian species. It is surprising to note that the poor viability observed for human embryos produced by IVF also occurs for embryos of other species. This is of interest because in many other species (rodents in research, agricultural animals), the animals used are selected for their high fertility. This indicates that poor viability of embryos after human assisted reproduction is not due solely to the poor fertility of the treatment population—the infertile.

Cell biologists typically consider viability as an instantaneous characteristic (i.e., the cell is viable, alive, at the instant of testing). For the clinical embryologist, however, viability refers to the pregnancy potential or survivability of the embryo. A workable definition of embryo viability is the embryo's ability to complete differentiation and development to form a grossly normal fetus by 8 weeks of gestation. By analogy, fetal viability can be defined as the ability of the conceptus to survive the fetal period of development, from 8 weeks of gestation till term, to produce a normal neonate.

This definition of embryo viability becomes functional only in retrospect; we cannot predict the fate of an individual embryo at a given point in time. It also has the limitation that survival of the embryo over this period may be limited (or possibly enhanced) by factors external to the embryo. Thus, it is conceivable that an embryo which is not viable in one mother may be in another. While some measurable parameters of preimplantation embryos show statistically positive associations with embryo viability [e.g., platelet-activating factor (PAF) release (1), utilization of carbohydrates (2)], these are not absolute measures of the viability of individual embryos. We can therefore discuss embryo viability only on a population basis. We can conclude, however, that as a population, embryos produced by IVF and related technologies have markedly impaired viability.

ANIMAL MODELS

The logistics and ethics of procuring a sufficiently large number of human embryos produced by IVF for research purposes, the validity of using "spare" embryos unsuitable for transfer, and the prohibitive cost of nonhuman primates ensure that much of the modeling for this problem is performed in laboratory species. A series of elegant studies (3–5) has been important in defining the causes of the loss of embryo viability following IVF. Using a sophisticated reciprocal embryo transfer protocol in rats, the major components in the IVF procedure were isolated, allowing their contribution to the loss of embryo viability to be defined. Loss of embryo viability was not due to the insult of oocyte collection and manipulation in vitro. Embryos fertilized in vitro had rates of development to the two-cell stage similar to those of embryos fertilized in vivo, but devel-

opment rates of IVF embryos declined thereafter and the late-stage preimplantation embryos possessed fewer cells per embryo than controls. A large proportion of the loss of embryo viability occurred during late preimplantation and immediate postimplantation period. The authors concluded that a cause of the loss of viability may have been the absence in the culture of factors normally provided in vivo during or immediately after (16 hr) fertilization.

The concept that it is the nature of fertilization in vitro itself which retards embryo development and reduces viability is confronting. One interpretation is that the process of fertilization in vitro may be fundamentally different from that in vivo, resulting in long-term downstream aberrations in the growth of the resulting embryo.

It has long been suspected that the poor viability of IVF embryos is caused largely by the production of embryos with a high incidence of chromosomal abnormalities. In a recent study (6) using fluorescent in situ hybridization (FISH) of 93 spare cleavage-stage human embryos, it was shown that approximately half were normal for chromosomes X, Y, and 1. Only two embryos were aneuploid while 30% were considered to be chromosomal mosaics, the majority of these were ploidy mosaics, with haploidy being the most common. An earlier study (7) showed that the early embryo can partially compensate for polyploidy by the creation of mosaics. However, using a mouse model for IVF, there was only a minor incidence of chromosomal aneuploidy or mosaicism (8). Interestingly, this model shows levels of reduced embryo viability after IVF similar to those in humans (9). It seems likely therefore that chromosomal aberrations are a contributing factor to reduced embryo viability in the human. The observation that mouse embryos also show reduced viability even though gross chromosomal aberrations are few suggests that there are other factors at play.

HOW CAN THE PROCESS OF FERTILIZATION EXERT LONG-TERM EFFECTS ON EMBRYO GROWTH?

Growth regulation of the early mammalian embryo is not well understood. During the preimplantation phase, regulation of mitosis appears to be largely autonomous. It is initially triggered by oocyte activation that is induced by fertilization.

The factors that trigger subsequent mitoses in mammals are not well defined. A widely accepted paradigm in cell biology states that mitosis is contingent on stimulation by cell- or tissue-specific growth factors. The exposure of sparse cell cultures to tissue-specific growth factors causes an exponential increase in cell numbers. The exponential increase in cell numbers in the embryo (10) from the zygote to the blastocyst stage suggests that these cells are also under growth factor-induced stimulation. However, the ability of preimplantation embryos to develop in entirely defined media without the requirement for exogenous growth factors questions this assumption. In this respect, the early embryo more closely resembles the phenotype of neoplastically transformed cells, which in general have a markedly reduced or no absolute requirement for exogenous growth factors. This phenotype has been largely explained by observations that transformation is often accompanied by ectopic overexpression of tissue growth factors, their receptors, and/or elements of their signal transduction pathways, resulting in autocrine or endogenous stimulation of mitosis. Recent evidence suggests that the growth phenotype in the early embryo may be under similar autocrine regulation (11). Furthermore, evidence in the mouse suggests that the release of some autocrine growth factors is deranged by fertilization of oocytes in vitro (9).

Early evidence for a role of autocrine growth factors in embryo development was the observation (12) that embryos cultured in groups developed better than those cultured alone and that embryos cultured in large volumes of media have poorer viability than those cultured in microdrops, inferring the release of diffusible factors which are limited by dilution. In mice, the retarded rate of preimplantation development under conditions of a low embryo concentration results in poor viability following embryo transfer (13). Many growth-factor ligands and their receptors are expressed and exert trophic actions on the early embryo. These include PAF (14,15), insulin-like growth factor (IGF)-I (16,17), IGF-II (18), transforming growth factor (TGF)- α /epidermal growth factor (EGF) (19), and growth hormone (GH) (20). It is likely that the precise panoply of autocrine growth factors and their temporal profile of expression will vary between species. It also seems likely that while autocrine stimulation may be sufficient for embryo development, in vivo this

stimulation will be supplemented by the same ligands of maternal origin and also paracrine mediators of maternal origin [e.g., insulin (21)].

THE EFFECT OF IVF ON PRODUCTION OF AUTOCRINE GROWTH FACTORS

The process of fertilization of oocytes in vitro apparently compromises the production of autocrine growth factors by the resulting embryos. This was first recognized (9) by the observation that IVF further exacerbated the adverse effects observed when embryos were cultured in limiting dilution. This adverse effect could be at least partially reversed by the supplementation of media with a variety of putative embryonic autocrine factors. The most extensively studied example is PAF. PAF is an ether-linked phospholipid, the synthesis of which is initiated by fertilization, by the activation of calcium-dependent biosynthetic enzymes (22). The early embryo possesses a receptor for PAF, while binding of the ligand activates an intracellular calcium transient (23). The mechanisms by which PAF exerts its growth promoting effect are not currently known, although it stimulates oxidative metabolism in embryos. Fertilization in vitro caused the resulting embryos to release seven-fold less PAF into media than embryos cultured under identical conditions but fertilized in the reproductive tract. Of interest, there was no difference in the amount of PAF remaining associated with the embryo following IVF, suggesting that the cause of the reduced PAF release was not the inability of the embryo to synthesize PAF. This conclusion was supported by the observation that putative rate-limiting enzymes in the biosynthetic pathway of PAF (lysoPAF acetylhydrolase and CDP choline) were not decreased following IVF (9). Human embryos produced by IVF released low but variable quantities of PAF (1,24), while there was a broad correlation between the amount of PAF released and parameters of the embryo's development potential and viability. Evidence of a direct effect of IVF on the release of other putative autocrine mediators is not yet available, although the observation (9) that IGF-I and -II could partially compensate for the adverse effects of culture of IVF zygotes at a low embryo density suggests that their synthesis or release may also be compromised by IVF.

It remains to be determined how fertilization in vitro causes PAF release to be disrupted while biosynthesis is apparently unaffected. The mechanisms by which PAF is released from cells is not well understood. The lipid (hydrophobic) structure of PAF requires that it is released onto a hydrophilic acceptor protein. Albumin apparently normally serves this function (25). PAF release onto albumin is accompanied by cell (embryo)-dependent conformational change to the albumin acceptor molecule, involving the cysteine-cysteine disulfide bonds (26,27). An interesting feature of PAF release onto albumin in this configuration is that it binds exclusively to domain II of albumin (amino acids 240–386). However, when PAF was added as a supplement to media, it did not bind at this location. The mechanisms by which the conformational change to albumin, and localization of PAF to domain II, is catalyzed is not known. It is conceivable that IVF disrupts this reaction, causing the reduction in PAF release observed following IVF.

An important observation was that the embryo-released PAF bound exclusively at domain II of albumin. At this site it was resistant to degradation by PAF acetylhydrolase to the inactive lysoPAF (26). This resistance to metabolism presumably means that PAF has a prolonged half-life in vivo and accumulates to sufficient concentrations to act on the embryo and also to exert effects on maternal physiology (28–30). This observation potentially resolves a long-standing enigma of PAF's actions; How could such a labile molecule as PAF, released by the single cell of the zygote, achieve concentrations high enough to act as a mediator?

Another unexpected consequence of PAF's binding to albumin in this manner is that it becomes very difficult to extract with conventional organic extraction procedures. This probably contributes to the conflicting reports in the literature on the ability of embryos to release PAF (see Ref. 26 for discussion) when unconventional extraction procedures were used.

It was shown that the adverse effects of IVF were partially reversed by supplementing media with exogenous PAF [human (31), mouse (9)]. This supplementation is a relatively simple matter for a defined medium such as that used in mouse embryo culture. Human IVF medium, however, is often undefined, containing complex protein sources such as serum. Almost all complex protein sources of mammalian origin contain PAF acetylhy-

drolase, which can rapidly degrade PAF. While PAF released by embryos binds to domain II and is protected from PAF acetylhydrolase, exogenous PAF added as a supplement is rapidly degraded (32). A requirement therefore, is the deactivation of the enzyme by acid treatment (pH 2.5) (31,32). Under such conditions it was shown that PAF supplementation caused improved embryo metabolism and viability as assessed by clinical pregnancy rate and live birth (31). Attempts to confirm such findings by a multicentre trial gave encouraging preliminary results (33). These could not be extended, however, due to resistance by clinics to the brief treatment of serum with acid, followed by neutralization. It has not been possible to overcome these concerns. It seems that alternative methods of preparation that do not involve reversible acidification of protein supplements are required to achieve acceptance for the use of PAF as a supplement. Possibilities include the use of entirely defined media without PAF acetylhydrolase and the development of methods to duplicate the localization of PAF to domain II of albumin together with the conformational changes which cause embryo-derived PAF to be protected from hydrolysis by PAF acetylhydrolase. To date, it has not been possible to mimic this configuration in vitro. Another possibility will be to use analogues of PAF that are not hydrolyzable by PAF acetylhydrolase. The use of non-natural analogues, however, will entail onerous proof of safety.

An alternative strategy is to use the release of autocrine mediators as an outcome measure, in the design of optimized media. It will be of interest to determine the effects of recently proposed new-generation media such as KSOM (34), and the effects of supplementation of media with mixed amino acids (35), on mediator synthesis and release.

It is likely that the production or release of other autocrine mediators is also compromised by IVF. It was found (9) in a mouse IVF model that PAF and IGF-II supplementation of media were equally beneficial in stimulating embryo development. However, there was no additive effect of having both factors in media. This lack of additivity suggests that both mediators are limiting and both act on the embryo along parallel pathways. Furthermore, IGF-I alone promoted the development of IVF embryos but this was not the case for EGF. These results are consistent with the concept that there may be several autocrine mediators limited

by dilution, which act on the early embryo development. Each of these may display a significant degree of redundancy in their actions.

The accumulation of empirical evidence for the beneficial effect of autocrine growth factors on the viability of embryos does not illustrate how such growth factors act in the early phases of preimplantation development exert their effects across the span of the embryonic phase of development. One recent study (36) showed that the incidence of apoptosis in cells of the inner cell mass of mouse blastocysts increased following culture in vitro throughout the preimplantation phase. The incidence of apoptosis was higher when embryos were cultured individually in 25- μ L drops compared with the incidence observed when groups of 30 embryos were cultured in the same volume. Supplementation of media with TGF- α significantly reduced the incidence of apoptosis in inner cell masses. It was concluded that endogenously produced growth factors acted on the blastocyst to act as specific cell survival factors for the inner cell mass. A well-accepted model (37) for the regulation of apoptosis requires that cells require exposure to tissue-specific survival factors. In their absence, apoptosis is a default response. If this model applies to the early embryo, an important cause of the loss of embryo viability may be the deprivation of autocrine factors, acting as survival factors. The relatively high incidence of apoptosis observed in human embryos produced by IVF (38) is consistent with this hypothesis.

CONCLUSIONS

In conclusion, embryo viability is adversely affected by fertilization in vitro. In the human, chromosomal aberrations account for some loss of viability. However, in mice this is a less common occurrence, yet mouse embryos produced by IVF also have poor viability. One important consequence of the impact of fertilization in vitro is the reduced synthesis or release of some autocrine growth factors. The relative deprivation of these leads to retarded development to the blastocyst stage and reduced potential for implantation following embryo development. The consequences of this deprivation can be significantly ameliorated by culture in microdroplets with groups of embryos, and potential further improvements can be achieved by supplementation of media with defined growth factors such as PAF and members of the

IGF family. In the long term, an understanding of the mechanisms by which fertilization in vitro causes these aberrations in mediator release will allow more effective therapies to be devised. The deficiencies caused by IVF also serve as a form of functional ablation experiment which will serve to define the processes of normal embryo developmental physiology.

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