Mammalian Embryo Quality: Is It Possible to Estimate It, and When?

EMBRYO MORPHOLOGY AND KINETICS OF DEVELOPMENT

Traditionally in in vitro fertilization (IVF) we estimate embryo quality by standard parameters of morphology and growth. For example, cytoplasmic fragmentation is usually considered a bad prognosticator for embryo quality, while a delay in embryonic development may prevent preimplantation embryos to switch successfully from maternal to zygotic gene expression. At this transition period,

the embryo has its lowest content of messenger RNA and the cell cycle is at its longest. Based on cytogenetic analysis, however, we know that morphology may not necessarily correlate with other indicators of embryo quality.

EMBRYONIC SIGNALS

Mammalian embryos can be grown from the zygote to the blastocyst stage in totally defined

media or in coculture. This suggests that factors in the upper genital tract of the female are not essential. However, in vivo, the embryo, derived from normal fertilization, emits very early specific signals in the tubal environment (1). It is now clear, from the work on rodents, that these signals do influence the oviduct metabolism by modifying the surrounding environment. Another signal regulates embryo transport, in relation to the function of the corpus luteum, for example, in the mare and hamster. In these species, the early preimplantation embryo has, at least in part, the key for opening the uterotubal junction (2). The first embryonic signals are related to the fertilization process.

The biochemical nature of these signals is not clear; they are probably not catecholamines, but may be small peptides. Kentsin (3), a tetrapeptide that has opiate properties (4,5), appears in the oviductal environment of the fertilized hamster embryo. Platelet-activating factor (PAF) could be another candidate as an early embryonic signal (6).

It is more than probable that these signals are present in the human. For example, ectopic pregnancies are sometimes related to embryonic abnormalities and so linked to abnormal signaling. For early preimplantation stages, identification and quantification of these signals are of major importance.

EMBRYONIC METABOLISM

Preimplantation embryos have two phases of metabolism: the first, from fertilization to the important waves of transcription occurring at zygotic genome activation; the second, from genomic activation to implantation.

During the first phase, corresponding to its journey in the oviduct, embryonic metabolism is rather low. At this stage development is dependent on the mRNA and protein reserves stored in the oocyte during uptake, transported into the embryo by a specific transporter. This specific type of transporter is present only in the liver. Tentative attempts to link glucose maturation in the ovary with the external milieu are low but not absent. Glycine is, for uptake, metabolic activity and embryo quality have not yielded a viable assay for use in routine IVF. Unfortunately, to date, the only way to estimate metabolism reliably is using radiolabeled material; this is detrimental to the embryo and could not be accepted in human embryology. The quantity of stored mRNA in the oocyte is fundamental and this concept is

supported indirectly from information about intracytoplasmic sperm injection. For example, developmental time gained with early spermatozoon microinjection helps in overcoming male-factor deficiencies. Delayed fertilization leads to poor-quality embryos, and microinjection performed 24 hr after retrieval rarely, if ever, leads to a pregnancy. When zygote formation and early cleavage are delayed, there is no way to overcome developmental arrest.

Before zygotic genome activation, metabolic activity cannot be used as an assay for the determination of embryo quality. We have shown in human IVF that 25% of fertilized embryos are able to reach the blastocyst stage even under borderline culture conditions. Another 25% are able to reach the blastocyst stage if culture conditions are optimal. The other 50% block at various stages of development: one half with cytogenetic problems; the other half due to gamete-related anomalies. The gamete problems may be of male origin and include centrosomal defects and poor activation due to deficiencies in soluble sperm factor (7) or of female origin such as poor mRNA and protein stores, defective signal transduction mechanisms, and suboptimal expression of cell cycle regulatory proteins.

In our opinion, there are no reliable noninvasive ways to check embryo quality before the blastocyst stage. If we consider that in human IVF, the implantation rates per cleavage-stage embryo are from 10 to 15% and those per blastocyst are from 20 to 25%, a selection of the best embryos has already taken place in vitro. There is no evidence for specific blastocyst secretions before implantation. In accordance with other authors, we have demonstrated β -human chorionic gonadotropin synthesis by the hatching blastocyst. Embryonic metabolism increases exponentially. Glucose, the major metabolic fuel used by the embryo, is necessary for hatching. We demonstrated a direct correlation between glucose uptake (during 24 hr of incubation) and bovine blastocyst viability after transfer (8). However, with a short incubation time, screening between morula and blastocysts is difficult. There is a large difference in sugar and free amino acid metabolism between mouse and bovine blastocysts. Blastocyst quality is linked to the ICM cell number, but its metabolism is related to the total cell number. Bovine embryos with 33% of the normal trophoblast cell number may implant and lead to normal calf delivery.

CONCLUSIONS

There is no objective method for determining embryo quality. A significant breakthrough may be made when we determine the specific messages released by the embryo during its dialogue with the genital tract. In vitro selection of embryos by culture to the blastocyst stage is an obvious way to improve our selection of high-quality embryos for transfer and to increase the pregnancy rate. Perhaps blastocyst transfers should be the rule, not the exception. Finally, if we compare the implantation rates per embryo in all patients to those in pregnant patients, it becomes obvious that the real stumbling block to IVF is endometrial quality. The ball is now in the court of the clinicians. The answer to whether we can check embryo viability and quality in vitro remains *no*.

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