Refresher Course

Factors Affecting Preimplantation Embryonic Development

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INTRODUCTION

Reproductive medicine has progressed very rapidly since in vitro fertilisation (IVF) has provided the opportunity for studying human embryos. However, the facts concerning early embryogenesis, including the preimplantation period, remains uncertain in many aspects. Many difficulties such as the lack of sufficient embryo samples and laboratory techniques, as well as ethical issues, have limited this work. Many studies in the preimplantation period, therefore, have had to be established in other mammals, including mice. Studies in mice have been accepted as a practicalmodel even in IVF laboratories[1].

After the long arrest in the dictyate stage, the granulosa cells surrounding the oocyte are triggered by luteinizing hormone to activate oocyte maturation[2]. When the first meiotic division is completed, the first polar body is expelled. At ovulation, the oocyte arrests at the second metaphase stage of meiosis until the time of fertilisation, which normally takes place in the ampular part of the fallopian tube[2]. Spermatozoa undergo capacitation before fertilisation in the female genital tract. Completion of capacitation permits the occurrence of the acrosome reaction[3]. Zona pellucida 3 (ZP3) and zona pellucida 2 (ZP2), the primary and secondary sperm receptors of the oocyte, bind specifically to the sperm head and also induce the acrosome reaction that allows the sperm to penetrate the zona pellucida of the oocyte[4]. When the sperm penetrates and reaches the oocyte, fusion of the sperm cell membrane and the oocyte membrane occurs and causes the maturation of the oocyte. After fertilisation, the second polar body is expelled when the oocyte completes its second meiotic division. An oocyte-specific protein kinase, c-mos, has been thought to regulate the activity of M-phase promoting factor (MPF) at various stages of the final oocyte maturation[5]. Within 24 hours after fusion of the female and male pronuclei, the zygote embarks on a regulated series of mitotic cell division called cleavage[2]. Many cleavage divisions are carried out until blastocyst formation. At the 8-cell stage the embryo undergoes compaction and forms a morula. A blastocoele cavity eventually develops at the 32-cell stage[2]. The blastocyst hatches from the zona pellucida and implants into the endometrium. During this preimplantation period, many factors influence and control the development of the embryo. These factors arise from both embryo and the surrounding environment. Many studies have been carried out to try to understand this period.

Factors affecting the cleavage stage of the preimplantation embryo

After fertilisation, several mitotic divisions occur. In both mouse and human, 15-50% of preimplantation embryos do not survive during this preimplantation period[6]. The rate of development and the degree of fragmentation that are found in the embryo are two major factors determining the quality of the embryo[7-9].

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Rate of embryonic development

The ped gene has been reported as a factor that controls the developmental rate of mouse embryos as well as their survival[10]. The ped gene was found to correlate with the major histocompatibility complex (MHC) haplotype of mouse strains. With genetic linkage analysis, the ped gene was localised to the Q region of the MHC[11]. It is composed of two functional alleles, fast and slow. The number of cells per embryo at 89 hours after HCG administration for induction of ovulation depends on the functional alleles[10,12]. In MHC Class Ib four genes, namely Q6, Q7, Q8 and Q9, encode protein. Only Q7 and Q9 have been specified as the genes responsible for the Ped gene phenotype[13]. In the ped fast phenotype, the embryo may express either Q7 or Q9 gene, or both. Mouse embryos with the ped slow phenotype are found to have a deletion of both Q7 and Q9 genes.

Degree of fragmentation in cleavage stage embryo

The degree of fragmentation found in embryos has been thought to be controlled by the genes that mediate apoptosis[14]. Cellular fragmentation with apoptotic nuclei was found in arrested embryos with poor morphology and unequally sized blastomeres.

Using a terminal transferase-mediated DNA end labelling (TUNEL) assay in arrested and fragmented human embryos, apoptosis was triggered before blastocyst formation[15]. Approximately 30% of the arrested embryos were positive for the TUNEL assay and all of them revealed fragmentation. In contrast, normal embryos were found to give negative results[16].

By using RT-PCR, there are two gene families, which control apoptosis; Bcl-2 and the caspase gene families[17]. In the Bcl-2 family, Bax and bcl-2 are transcribed in both mouse and human preimplantation embryos[18]. Bax is responsible for promoting apoptosis, whereas bcl-2 inhibits apoptosis. The ratio of these two expressed proteins influences the survival of blastomeres[17,19].

Factors affecting morula and blastocyst formation

After 3 to 4 mitotic divisions, the embryo has a mulberry-like appearance, namely a morula. Compaction commences at this stage. Gap junctions, which are established during compaction, play a role in mammalian embryo development[20]. Analysis of the gene expression of a gap junction suggests that the function of gap junctions may be mediated by the different expression of the connexin gene family[20]. At least four connexins, namely cx 31, cx 31.1, cx 43 and cx 45, contribute to gap junction formation in preimplantation development[21]. Connexin 43 (cx 43) has been thought to contribute to gap junction formation because embryos homozygous for a cx 43 null mutation develop normally until implantation. During blastocyst formation, gap junctions containing cx 32 or cx 26 have been found in the trophectoderm cells of blastocyst[22]. Variable levels of gap junctions may appear in preimplantation embryos. The multinucleated, apoptotic and decompacting cells might firstly appear in embryos which are morphologically normal but which contain abnormal gap junctions. Normal embryo morphology, therefore, may not be a reliable predictor of embryo survival.

Autocrine and paracrine factors affecting the preimplantation embryo

During mitotic division, the embryo is transferred from the fertilisation site into the uterine cavity. Many substances, which are secreted by the cells of the fallopian tube and uterus as well as the embryo itself, have been studied to determine their influence on preimplantation development.

Growth factors have been found to be produced by the uterine epithelium and the embryo[23,24]. The presence and expression of growth factor transcripts have been reported to be necessary for enhancing early development through the preimplantation period[25]. Many growth factors are thought to influence the development of cultured preimplantation embryos[26]. The improvement in embryonic development in high concentration culture media might be the result of increased concentration of endogenously produced growth factors[27].

Insulin-like growth factor-I (IGF-I) and insulin-like growth factor-II (IGF-II)

Preimplantation development might be controlled by IGF from both maternal (IGF-I) and

embryonic (IGF-II) sources[28]. In mouse blastocysts, IGF-I has been found to influence preimplantation development through the insulin receptor[29]. This growth factor has been identified in the fallopian tube and its expression is controlled by the level of maternal oestradiol[30]. Using RT-PCR, a decreasing level of IGF-I was detected from the oocyte to the 8-cell stage, whereas an increasing level was detected from the 8-cell to the blastocyst stage[31].

Epidermal growth factor (EGF)

EGF-receptor has been found to be expressed in mouse embryos from the 4-cell stage[32]. The action of this growth factor may be directed towards cavitation and blastocyst formation. EGF caused a more rapid accumulation of blastocoele fluid during mouse blastocyst formation[33]. Adding EGF to the IVF culture medium was found to increase protein synthesis, cell number per blastocyst and hatching rate[27,34]. A significantly higher percentage of expanded and hatching mouse blastocysts at 72 hours was found with a concentration of EGF of 2-100 ng/mL in the incubating system[35].

Transforming growth factor-alpha (TGF-α)

TGF-α has been proposed as one of the epidermal growth factor (EGF) family. Peptide growth factors have been shown to be necessary in regulating cell proliferation and differentiation during mammalian preimplantation development[36]. TGF-α has been found to be present in the mouse oocyte and later at the 8-cell stage[37]. TGF-α may regulate apoptosis in the inner cell mass (ICM) of mouse blastocysts. This growth factor, therefore, may act as a survival factor in the preimplantation embryo by activating receptors on the ICM in order to produce cavitation during blastocyst formation[38].

Activin and inhibin

Activin and inhibin, members of the transforming growth factor-β (TGF-β) family, are polypeptides capable of respectively enhancing or suppressing FSH released from the pituitary gland[39]. The expression of activin/inhibin βa, βb and the α subunit, and active receptor-IIB gene have been detected in both oocytes and preimplantation mouse embryos[40]. These growth factors affect the cleavage stage embryo. Activin-A has been considered as a necessary factor for releasing the arrest of the cleavage embryo at the 2-cell stage[41].

Platelet activating factor (PAF)

PAF has been reported to be present in human embryos[42]. In mouse embryos, the oxidative metabolism of glucose and lactate is directly affected by PAF[43]. PAF may bind to cell surface receptors on preimplantation embryos and then stimulate the inositol triphosphate (IP3) pathway to increase intracellular calcium[44]. PAF was found to increase during the preimplantation period and reach a maximum level at the expanded blastocyst stage[45]. PAF has been proposed to enhance both fertilisation ability and embryo viability[46]. Adding PAF to the IVF culture medium can improve the development from the 2-cell stage to blastocyst in both mouse and human[47,48]. In contrast, adding PAF antibodies to the culture medium can inhibit embryonic development as well as implantation[49,50].

Colony stimulating factor-1 (CSF-1)

CSF-1 was originally thought to produce harmful effects on embryo development[51]. CSF-1, which is produced by activated lymphocytes and macrophages, has been shown to inhibit embryo development to the blastocyst stage[51]. However, subsequent studies have reported that CSF-1 enhanced the development of 2-cell to blastocyst stage mouse embryos[23]. Moreover, adding CSF-1 to IVF medium revealed a significant increase in the number of trophoblast cells at the blastocyst stage[52].

Tumour necrotic factor-alpha (TNF-α)

TNF-α has been identified in the IVF medium of human embryos cultured up until the morula stage[53]. TNF-α is not normally found in the blastocyst[53]. Further studies in mouse embryos have demonstrated the specific inhibition of TNF-α in the cell proliferation that contributes to the inner cell mass (ICM) of the blastocyst. Therefore, the occurrence of TNF-α in the blastocyst can result in the reduction of the ICM[54].

With regard to paracrine and autocrine factors affecting preimplantation development, there are still more substances that influence this period, for instance, early pregnancy factor and leukaemia inhibiting factor[55,56].

Factors affecting the genetic pattern in the preimplantation embryo

The first cleavage division of the embryo has been thought to be controlled by the maternal genome[57]. The switching of genomic control from maternal to embryonic is thought to occur at the 2-cell stage[58]. This hypothesis has been supported in many species as most of the embryos do not develop to the blastocyst stage but arrest at the 2-cell stage in IVF[59,60]. In the human, this process may happen at the 4-cell to 8-cell stage[61,62]. The maximal incidence of embryonic arrest in the human, therefore, correlates with the period of transition from maternal to embryonic regulation of development[7].

In untransferred cleavage-stage embryos from fertile patients, using multicolour fluorescent in situ hybridisation (FISH) with DNA probes for chromosome X, Y and 1, the chromosome patterns of the embryos could be classified into four groups; normal, abnormal, mosaic and chaotic[63]. The chromosomally abnormal and mosaic embryos may progress beyond implantation, but the chaotic embryos are unlikely to develop further[64]. Up to 46% of human embryos from infertile patients were found to have a chromosomal abnormality[65]. In IVF, chromosomally abnormal embryos were found to be unable to develop further after the freezing and thawing process[66]. Furthermore, the higher incidence of chromosomal abnormalities was found to be related to a higher incidence of embryo with abnormal morphology[67-69].

Miscellaneous factors affecting preimplantation embryo development

During the early cleavage stage, pyruvate has been accepted as the most important energy substrate for the embryo[70]. Glucose, which is one of the components of conventional IVF culture medium, has been shown to produce an adverse effect on embryo development[71,72]. However, during compaction and blastocyst formation, which need high levels of ATP, the embryo utilises glucose in both oxidative and glycolytic metabolism[73,74].

Amino acids have been accepted as necessary factors in human embryonic development. Many kinds of amino acids have been found within the fluid of the female reproductive tract[75]. Most of them have shown to be important for the cleavage stage embryo[76].

The physical environment has also been found to affect the preimplantation period. A relative oxygen tension has been reported to be harmful for embryo development and viability because of the production of oxygen radicals[77]. Increased oxygen radicals may arrest the embryonic development at the 2-cell stage in mouse embryos[78].

With regard to IVF, to maintain intracellular function and cell volume, the embryo must be under the same osmotic pressure as in a natural environment. The osmotic pressure must be greater than 360mOsm[79]. Adding some amino acids to the culture medium and adjusting the osmotic pressure have been suggested in order to enhance embryonic development[80,81].

CONCLUSION

Many factors have been shown to influence preimplantation embryo development. Using recently developed techniques, such as TUNEL, FISH and RT-PCR, it has been discovered that several factors affect preimplantation development. However, not only the ethical issues surrounding the use of human embryos for experiments, but also a debate over the present concept of homology in developmental genetics, which has resulted in questioning the reliability of the correlation between experimental results in some species of animals and human, has resulted in not fully exploiting the use of embryos in scientific and medical researches. The effectiveness of the currently used techniques has been doubtful[82]. Therefore, more co-operation between scientists who are interested in this field would enhance our understanding in this complicated developmental period. The knowledge obtained might be utilised in order to improve embryo culture systems and consequently the success of IVF.

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