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Calcium Signaling in Human Preimplantation Development: A Review

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Purpose: *Cell cycle-related calcium signals, bearing some similarity to those previously described in other animal species, have also been observed in human preimplantation embryos. These signals follow those occurring in both gametes during the period preceding fertilization and those induced by the fertilizing spermatozoon in the oocyte after gamete fusion. Even though the signals occurring during each of these distinct developmetal periods have different temporal and spatial characteristics, there may be a relationship between them; in fact, abnormalities of calcium signals occurring in an earlier developmental period may be at the origin of abnormal signals during later developmental periods.*

Methods: *Possible mechanisms by which inadequate or truncated calcium signals can impair embryo development are discussed.*

Results: *These mechanisms include complete failure of the second meiotic division, leading to triploidy; incomplete failure of the second meiotic division, leading to de novo chromosomal numerical abnormalities; abnormal pronuclear development and function; abnormalities of the blastomere cell cycle, possibly leading to embryo cleavage arrest; and problems with blastomere allocation to embryonic cell lineages, leading to disproportionate development of the inner cell mass and trophectoderm derivatives, which can be the origin of implantation failure or miscarriage.*

Conclusions: *Future research should make it possible to decipher the nature of normal developmental signals, to determine the key checkpoints at which these signals are required to prevent the switch to apoptosis, and to examine the possibilities of therapeutic action at these checkpoints to rescue the endangered embryo for normal development.*

KEY WORDS: calcium signaling; preimplantation development; human.

INTRODUCTION

As in other animal species, calcium signaling is involved in a number of important regulatory processes during human gamete maturation, fertilization, and early embryonic development. Most of the present knowledge about the role of calcium in these processes comes from relatively recent studies that made use of the improved resolving power of cell imaging systems and benefited from the availability of novel fluorescent indicators with which calcium can be visualized in single living cells. This short review brings together the most relevant data generated by these studies. These data are presented with regard to their significance for the current assisted reproduction technologies. Hypotheses for future research, hopefully leading to further improvements of assisted reproduction efficacy, are also formulated.

SURVEY OF PRINCIPAL DATA

Calcium signals have been reported to regulate cell function in gametes shortly before fertilization, in freshly fertilized oocytes, in two-pronucleated zygotes, and in cleaving embryos. Calcium influx, occurring in human spermatozoa in response to progesterone added at concentrations similar to those present in the human cumulus oophorus, was first observed by analyzing a global cellular response in sperm suspensions (1). More recently, when analyzed in single sperm cells, the progesterone-induced calcium increase in human spermatozoa has been shown to have a repetitive character (2).

Human germinal vesicle oocytes produce typical calcium oscillations in response to 17β-estradiol (3), and this response can be abolished by androstenedione (4).

During human in vitro fertilization, the fertilizing spermatozoon induces calcium oscillations in the oocyte (5). Calcium oscillations were also observed when human oocytes were fertilized after subzonal insemination (6) or after intracytoplasmic sperm injection (ICSI) (6,7). The fertilization-related calcium oscillations of human oocytes have a unique pattern

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of spatial propagation (8), are likely to involve calcium release from both inositol trisphosphate-sensitive and ryanodine-sensitive calcium stores (9,10), and can be modulated by sustained activation or inhibition of protein kinase C (11).

Calcium signals were also detected later during pronuclear zygote development and in blastomeres of cleaving human embryos, where they appear to be related to the phase of the cell cycle (12). Cytochemical visualization of calcium in electron microscopic preparations of cleaving human embryos revealed regional differences in the calcium load of endoplasmic reticulum but also of mitochondria, suggesting that, in addition to endoplasmic reticulum, mitochondria may also serve a store of mobilizable calcium in human embryonic cells (13). Patterns of calcium signals occurring at different stages of preimplantation development have been reviewed recently (14). Briefly, three distinct phases of calcium signaling can be distinguished in human preimplantation development: the early postfertilization phase, the pronuclear zygote phase, and the cleaving embryo phase. Calcium signals in each of these three phases are unique and differ from the other phases in the temporal and spatial patterns of calcium wave propagation as well as in the sensitivity to drugs such as ryanodine.

Studies conducted in other mammalian and nonmammalian species (reviewed in Ref. 15) indicate that inappropriate calcium signaling during preimplantation development can be the origin of developmental failure. Even though direct experimental evidence for a similar relationship in human assisted reproduction still remains to be provided, circumstantial evidence suggests the involvement of calcium signaling disturbances in several developmental abnormalities leading to assisted reproduction failure, including the failure of meiosis completion, deficiencies of pronuclear development and function, anbormalities of the blastomere cell cycle, and irregularities of blastomere allocation to embryonic cell lineages. These questions are discussed below, along with the possible linkage between calcium signaling in gametes during fertilization and in the embryos resulting from the interaction of the respective gametes.

CALCIUM SIGNALING AND COMPLETION OF MEIOSIS

Experimental data obtained in protostome worms (16,17) have shown unambiguously that the truncation of calcium signals produced in oocytes at fertilization can intervene with the completion of meiosis and the extrusion of the second polar body. It must be noted that meiosis is still incomplete in human oocytes at the moment of sperm–oocyte fusion. Truncated calcium signals were demonstrated in some human oocytes fertilized by injecting mature spermatozoa (7) or round spermatids (18) into the ooplasm. By analogy with the protostome worm model, such calcium signal abnormalities can be expected to lead, in some cases, to anaphase II failure (Fig. 1) and, thus, to the retention of an extra female haploid set of chromosomes in the oocyte. After metaphase chromosome decondensation, this set is thus included in an extra pronucleus. The development of tripronucleate, digynic zygotes with a single polar body, which represents one of the most frequent fertilization abnormalities after ICSI, can therefore, in all probability, be attributed to an abnormal calcium signal at fertilization.

In other cases, the anaphase failure resulting from truncation of the sperm-induced calcium signal may be only partial, concerning one or a few chromosomes. This would lead to chromosome nondisjunction and numerous abnormalities (Fig. 1). This mechanism has been proposed to explain the de novo chromosomal abnormalities occurring after ICSI (19), but it may also be responsible for similar defects after the use of other assisted reproduction techniques.

CALCIUM SIGNALING AND PRONUCLEAR FUNCTION

Pronuclei have long been believed merely to provide a structural basis for the chromatin rearrangements required for syngamy and to support the first embryonic S phase. This concept was challenged by the demonstration of limited transcriptional activity in male pronuclei developing in polyspermically pene-

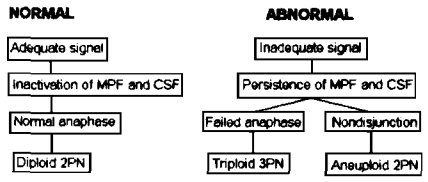

Fig. 1. Schematic representation of the possible developmental consequences of inadequate calcium signals at fertilization. Unlike the adequate signal, leading to complete inactivation of the metaphase promoting factor (MPF) and cytostatic factor (CSF) and thus driving the fertilized oocyte to anaphase, the incomplete inactivation of these factors, resulting from inadequate calcium signals, can either lead to complete anaphase failure and the development of triploid tripronuclear zygotes (3PN) or cause anaphase abnormalities at the single-chromosome level, leading to nondisjunction and aneuploidy.

trated human zygotes (20,21). Later, this finding was confirmed by the demonstration of paternal transcripts in human pronuclear zygotes (22). Moreover, male pronuclei of mouse zygotes have been shown to have four to five times greater transcriptional activity compared to female pronuclei (23).

The parent-specific pattern of this early zygotic transcription was suggested to reflect differential histone contents and chromatin acetylation, leading to unequal abilities of transcription factors to bind to DNA (reviewed in Ref. 24). The involvement of the zygote calcium signals, detectable in human zygotes throughout the pronuclear stage (11), in the control of this early transcriptional activity is a tempting hypothesis.

In fact, several lines of evidence suggest that the proper expression of gamete-derived genomes during preimplantation development is more dependent on intact calcium signaling compared to somatic cell-derived genomes. For instance, nuclear transplantation experiments showed that embryos resulting from artificially activated enucleated oocytes having received a nucleus from a cell descended from a conventionally fertilized egg can develop into normal embryos (reviewed in Ref. 25). In this system, somatic cell nuclei are remodeled to a pronucleus-like structure even though the underlying calcium signals are likely to be different from those occurring during normal fertilization. It remains to be determined which events in the remodeling of gamete nuclei into somatic cell nuclei are critically dependent on intact fertilization-associated calcium signals and whether the insufficiency of these signals can be overcome by artificial means. This might be of help in the current efforts to improve the efficacy of conception with spermatids and other sperm precursor cells, where both truncated calcium signals (18) and abnormal pronuclear development (26) are frequent findings.

CALCIUM SIGNALING AND THE BLASTOMERE CELL CYCLE

We recently observed an association of typical high calcium spikes with cell division in early human embryos (11). Cell cycle-related calcium signals were previously reported in the sea urchin (27) and starfish (28). It is also known that early postfertilization signaling events can influence later signaling events and thus determine the embryo quality and viability. The mechanisms by which signaling events during different developmental periods are linked with each other remain to be elucidated.

CALCIUM SIGNALING AND EMBRYONIC CELL LINEAGE DIFFERENTIATION

Even though early mammalian blastomeres can revert to totipotency at least to the four-cell stage (29), the unequal tendency to contribute daughter cells preferentially to one of the three distinct cell lines, inner cell mass, germline, and trophectoderm, may already be developed for each of the two first blastomeres (24). Distinct patterns of calcium signals in individual blastomeres of human preimplantation embryos have been observed from the two-cell stage onward (11). It was suggested that this inequality of calcium signals in the first two human blastomeres may be an essential step in the primary differentiation and formation of a cell line differentiating into the germline and trophectoderm (24). It was also experimentally demonstrated that epigenetic factors acting on early mouse zygotes can influence cell allocation to individual cell lineages, thus generating differences that can be manifest only after implantation (30). Further research is needed to determine the exact role of calcium signaling in these early embryonic epigenetic regulatory events.

POSSIBLE LINKS BETWEEN CALCIUM SIGNALING IN GAMETES AND EMBRYOS

Since both the fertilizing spermatozoon and the maturing oocyte generate characteristic calcium signals, one may ask whether there is a relationship between these prefertilization gametic signals, on the one hand, and the signals controlling zygote and embryo development, on the other hand. Calcium oscillations in human germinal vesicle oocytes are facilitated by 17β-estradiol (3) and inhibited by androstenedione (4), both steroids being fully active at physiological concentrations that can be attained in human follicular fluid. Interestingly, the ability of in vitro matured human oocytes to develop into cleaving embryos after fertilization is potentiated by the presence of 17β-estradiol in the maturation medium (3). It is not known whether a similar relationship exists between the sperm prefertilization calcium signal and future embryo development. If this is the case, treatment of spermatozoa with acrosome reaction-inducing stimuli that provoke calcium influx, such as ionophores and progesterone, can be expected to improve embryo development after ICSI even in species with small acrosomes in which the intra-oocyte toxicity of acrosomal components is negligible.

PRACTICAL CONCLUSIONS

The relatively high pregnancy and birth rates in the current practice of human assisted reproduction require multiple embryo transfer and the resulting risk of multiple pregnancy. Although the implantation rate can be improved by various methods of embryo selection before transfer, based on evaluation of embryo morphology, developmental failure is frequent even for "good-looking" embryos. Recent data have revealed a marked heterogeneity of calcium signaling patterns among individual blastomeres from the same embryo. Because, in some species, calcium signals generated during and immediately after fertilization can influence further development for many subsequent cell cycles, further research should address the problem of the possible role of abortive calcium signaling in developmental failure at different preimplantation and postimplantation stages. This research should define the critical checkpoints at which inadequate signaling events can be the origin of embryonic cell switch from the developmental to the apoptotic pathway. It may also be possible to intervene therapeutically at these checkpoints so as to rescue embryos from such switches. Searching for easily detectable morphological correlates of abnormal signaling events (e.g., morphology of pronuclei, position of cell blastomeres) will hopefully enable better selection of developmentally competent zygotes and embryos and thus limit the tendency toward multiple embryo transfers.

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