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Current and future therapies for abnormal early embryogenesis with assisted reproductive technology

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Abstract: Each stage of embryonic development, including normal gamete maturation, fertilization, zygotic genome activation, and cleavage, is crucial for human reproduction. Early embryo arrest is a common phenomenon. It is estimated that about 40%–70% of human embryos are arrested at early developmental stages. However, the exact mechanism remains largely uncertain. Embryos can be investigated *in vitro* by way of the development of *in vitro* fertilization/intracytoplasmic sperm injection. In addition to iatrogenic factors related to abnormal oocyte/embryo development, multiple gene mutations have been found to be involved in such phenotypes. Based on the knowledge of known etiological factors, several therapies are proposed to improve clinical outcomes. Here, we shed light on current and potential therapies for treating these conditions through reviewing articles and combining with our clinical and research experience.

According to reported data, infertility affects 15% of couples of reproductive age, and recurrent pregnancy loss affects approximately 5% of women of childbearing age globally (Sun *et al.*, 2019; Zhou *et al.*, 2021). Infertility and recurrent pregnancy loss are common disorders. However, their exact mechanisms remain unknown. The stages of embryonic development, including normal gamete maturation, fertilization, zygotic genome activation (ZGA), and cleavage, are of vital importance for uterine embryonic implantation and the development of a baby. It has been difficult to directly study human oocytes/embryos previously due to ethical challenges and the scarcity of research materials. However, with the help of *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI), investigators are now able to observe developmentally abnormal human oocytes/embryos *in vitro*.

Oocyte/embryo developmental abnormality include internal (e.g., aneuploidy) and morphological factors that can be optimally addressed in the following stepwise fashion. Firstly, when it occurs in an IVF/ICSI cycle, a clinical doctor or embryologist must assess the patients' condition. Normal oocyte/embryonic development is impacted by body mass index (BMI), age, endometriosis, ovarian reserve, male sperm quality, and the couple's chromosomal makeup (Demko *et al.*, 2016; Esteves *et al.*, 2019; Lin *et al.*, 2019; Le *et al.*, 2021). Secondly, iatrogenic factors must be excluded. Noting the personalized treatment that doctors choose a controlled ovarian stimulation protocol according to clients is therefore important. It has been reported that a mild stimulation protocol such as progestin-primed ovarian stimulation (PPOS) improves the chances of attaining euploid embryos (La Marca *et al.*, 2020). Furthermore, starting and maintenance dosage of gonadotropin (Gn) should be monitored and adjusted for each individual case. Sometimes, even the timing of human chorionic gonadotropin triggering influences oocyte maturation and embryo development. Thirdly, it is important to note that some adjuvant drugs, such as growth hormones, dehydroepiandrosterone, calcium, and vitamin D, have been implicated in improving oocyte and embryo quality (Nardo and Chouliaras, 2020). Finally, it is useful to consider that, in addition to these drugs, several modifications in *in vitro* maturation (IVM) media, such as melatonin supplementation, can improve levels of oocyte/embryo maturation, although the effects are subtle (Nardo and Chouliaras, 2020; Li *et al.*, 2021b).

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Notably, not all oocytes/embryos lead to live birth events. In fact, 40%–70% of human embryos are estimated to arrest at early developmental stages (Gardner and Lane, 1997). To complicate things even further, some infertile patients experience multiple IVF/ICSI cycles due to either the complete oocyte maturation arrest, preimplantation embryonic lethality, or recurrent implantation failures not attributable to any of the aforementioned factors. This leads to serious physiological and economic stress for patients.

A number of genes influencing early embryonic development have been identified in animal models, whereas the etiology in humans has remained largely elusive until recently. Due to advances in whole exome sequencing (WES), some abnormal oocyte/embryo development is considered as one of Mendelian disorders. Twenty-three genes have been confirmed to be related to abnormal human oocyte/embryo development. One gene links to oocyte death (*PANX1* (Sang *et al.*, 2019)). Another is tied to oocyte maturation arrest (*TUBB8* (Feng *et al.*, 2016), *PALT2* (Chen *et al.*, 2017b; Maddirevula *et al.*, 2017a), *TRIP3* (Zhang *et al.*, 2020), *CDC20* (Zhao *et al.*, 2020) and *MEI1* (Ben Khelifa *et al.*, 2018)). Four links to genuine empty follicle syndrome (*ZP1* (Zhou *et al.*, 2019), *ZP2* (Yang *et al.*, 2021), *ZP3* (Chen *et al.*, 2017c) and *LHCGR* (Yariz *et al.*, 2011)). Three are associated with an oocyte's lack of a zona pellucida (*ZP1*, *ZP2* and *ZP3* (Huang *et al.*, 2014)). One gene has been implicated for thin zona pellucida (*ZP2* (Dai *et al.*, 2019)). Eight link to fertilization failure (*ZP2* (Dai *et al.*, 2019), *PLCZ1* (Heytens *et al.*, 2009), *WEE2* (Sang *et al.*, 2018), *PALT2* (Wu *et al.*, 2019), *TUBB8* (Chen *et al.*, 2017a), *TLE6* (Alazami *et al.*, 2015), *CDC20* (Zhao *et al.*, 2020) and *NLRP5* (Li *et al.*, 2021a)). Two have been implicated in zygotic cleavage failure (*PADI6* (Maddirevula *et al.*, 2017b) and *BTG4* (Zheng *et al.*, 2020)). Nine link to early embryo arrest (*TLE6* (Alazami *et al.*, 2015), *NLRP2* (Mu *et al.*, 2019), *NLRP5* (Mu *et al.*, 2019), *PATL2* (Chen *et al.*, 2017b), *TUBB8* (Chen *et al.*, 2017a), *CDC20* (Zhao *et al.*, 2020), *KHDC3L* (Wang *et al.*, 2018), *REC114* (Wang *et al.*, 2020), *MEI1* (Dong *et al.*, 2021) and *MOS* (Zhang *et al.*, 2021b)). One has been associated with pronuclear fusion failure (*CHK1* (Zhang *et al.*, 2021a)). Five have been tied to recurrent hydatidiform moles (*KDHC3L* (Wang *et al.*, 2018), *PADI6* (Maddirevula *et al.*, 2017b), *NLRP5* (Rezaei *et al.*, 2021), *NLRP7* (Rezaei *et al.*, 2021) and *MEI1* (Nguyen *et al.*, 2018)), and so on. As a result of these new insights, an expanding spectrum of mutation sites and phenotypes has been identified. It seems that different phenotypes of infertility can be linked to one gene, and one causative gene may induce several phenotypes. This indicates that the pathways of oocyte/embryo development are very interconnected and complex. Reproductive doctors and genetic counselors must be aware of the links among infertility, miscarriages, and aberrant chromosomal makeup. For patients with multiple unexplained IVF/ICSI failures, WES and Sanger sequencing combined with family history must be ascertained to confirm whether they carry any of the reported pathogenic genes. In cases where pathogenic variants are identified, doctors can offer genetic and reproductive counselling options to affected couples. Sperm-induced oocyte activation failure has been successfully treated by way of artificial oocyte activation (AOA) with no increase in birth defect rate (Long *et al.*, 2020). However, for other conditions, oocyte/sperm donation should be suggested because patients carrying these mutations rarely give birth, yet it might still be a feasible option to enable carriers to have their own children (Sang *et al.*, 2021). Moreover, it should be mentioned that abnormal oocyte/embryo development cannot be completely explained by the aforementioned genes. Thus, WES for these patients can help identify novel pathological genes, which would thus pave the way for future diagnosis and treatment.

Gene therapy may soon become a promising treatment option. In 2020, Zhao *et al.* (2020) injected CDC20 complementary RNAs (cRNAs) into oocytes in maturation arrest (Zhao *et al.*, 2020). All injected oocytes were successfully fertilized, and developed into embryos, in which case the preimplantation genetic screening showed that one of the blastocytes was normal (Zhao *et al.*, 2020). In addition, injections of human *PLCZ1* cRNAs or recombinant protein were able to trigger Ca^{2+} oscillation, which aided oocytes' development to the blastocyte stage (Rogers *et al.*, 2004; Yoon *et al.*, 2012). Exogenous cRNAs and protein complements can help early embryonic transition to the next development phase. However, it remains a big question whether such cRNAs or protein injections will ultimately adversely impact progeny, despite their rapid rate of metabolization.

For embryos experiencing causally unknown recurrent implantation failure, preimplantation genetic testing-aneuploidy (PGT-A) will soon be applicable for screening euploid embryos to increase their chances of implantation. PGT is an invasive technology that requires the suction of 5–8 trophectoderm (TE) cells for effective detection. Furthermore, it does not lend to a direct means of assessing the inner cell mass (ICM). Recently, noninvasive PGT-A (niPGT-A), a new technology, has been proposed for analyzing cell-free DNA in the spent culture media of human blastocytes to directly assess for ICM (Huang *et al.*, 2019). However, it includes expelled abnormal chromosomes from TE/ICM, which reduces the reliability of the technology. Fluorescence lifetime imaging microscopy (FLIM) is another technology used to measure metabolic characteristics of embryos to identify the ploidy of an embryo (Sanchez *et al.*, 2019). Nonetheless, presently the researches is still solely focused on mouse oocytes/embryos and is yet to be applied to human embryos.

In conclusion, oocyte/embryonic development is influenced by both the personal chromosomal status of patients and iatrogenic factors. To date, a series of genetic contributors has been identified by the way of combing clinical phenotypes and WES. These genes can be potential biomarkers for diagnosing patients with embryo developmental abnormalities, and may thus be a viable direction for future therapy. Although NiPGT-A and FLIM are noninvasive technologies for identifying euploidies, they have their disadvantages. We anticipate that new noninvasive technologies will be developed to replace PGT-A for direct assessment of ICM. By these means, future reproductive physicians will not be confined to simply optimizing clinical treatments so as to avoid iatrogenic factors. Rather they will also be equipped to diagnose such patients and thereby point them towards a new range of helpful avenues for assisting them.

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