Chapter

Introductory Chapter: New Theory and Technology in Early Clinical Embryogenesis

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1. Introduction

Embryology has a long history. Since early last century, many studies focused on revealing the mechanism of the fertilization and embryo formation and development, which formed a branch discipline of biology science, that is, embryology. Embryology is a vast discipline concerned with the study of embryogenesis, and it is the branch of biology that studies the prenatal development of gametes, fertilization, and development of embryos and fetuses. Additionally, embryology encompasses the study of congenital disorders that occur before birth, known as teratology [1, 2]. Findings in embryology have helped in the understanding of congenital abnormalities and developing assisted reproduction procedures.

To date, embryology has been enriched and developed greatly in the terms of its contents and forms. It not only includes oogenesis, spermiogenesis, embryogenesis, implantation, and fetal formation mechanism, but also involves pharmacology, basic scientific research, and regenerative medicine. Although this subject has been studied for more than a century, it is still a pioneering field with many alternative aspects such as embryonic stem cell, somatic cell cloning, and many novel discoveries that appear continuously. Particularly, some novel embryo biotechnologies have initiated a new era in the fields of medical science and agriculture owing to their enormous biomedical and commercial potential.

In the last four decades, the assisted reproductive technology (ART) has created some new observations and novel discoveries in the early stage of embryogenesis, especially in preimplantation from gametogenesis to blastocyst embryo formation in vitro (**Figure 1**).

Thus, this book contains some novel discoveries and theories on the embryology field in last the decade.

The key technique of the assisted reproductive technology is in vitro fertilization (IVF). Since this technique's creation, the current developed methods have been widely used for the treatment of infertile couples to make them realize their dream to

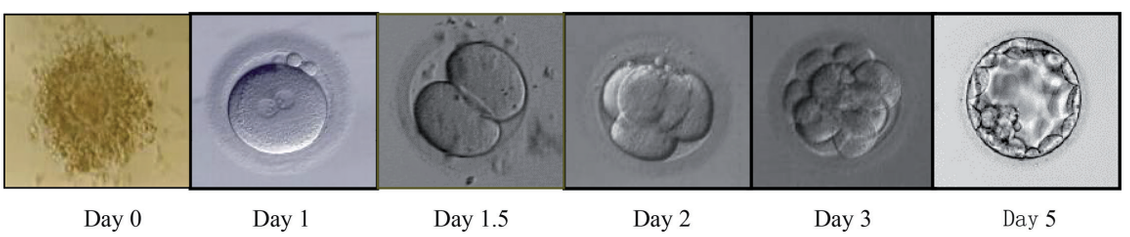

Figure 1.
Human embryogenesis from oocyte to blastocyst stage during preimplantation.

have a baby. Currently, these technologies mainly contain in vitro fertilization (IVF) and its related procedures—intracytoplasmic sperm injection (ICSI), frozen embryo transfer (FET), and preimplantation genetic testing (PGT) for aneuploidies (PGT-A, previously known as PGS), for monogenic/single-gene defects (PGT-M, previously known as PGD), and for chromosomal structural rearrangements (PGT-SR, previously known as PGD Screen (PGS)). [Note: New PGT nomenclature announced by the International Committee Monitoring Assisted Reproductive Technologies (ICMART) in collaboration with the American Society for Reproductive Medicine (ASRM), European Society of Human Reproduction and Embryology (ESHRE), and other professional medical societies, 2018] [3]. So far, more than 10 million IVF/ICSI and frozen embryo transfer babies have been born throughout the world. This technique indeed gives many infertile families to bring happy life. Although this technology has been widely used in human infertile treatment and animal industry, it also faces some problems which need to be resolved, such as low embryo development rate, low pregnant rate, as well as birth baby health. Thus, some continuous improvements on this technology have been happening now. Firstly, in order to obtain good quality eggs, ovarian stimulation needs to use some high-quality medicines such as gonadotropins, including human chorionic gonadotropin (hCG), which is the most important reproductive hormone for embryogenesis and embryo development and implantation in the uterus [4–6]. Thus, we should concern the total hCG protein content varied from batch to batch and a large number of contaminant urinary proteins identified in all analyzed samples except for the recombinant product. The good quality hCG application will improve embryo quality and pregnant rate.

In vitro maturation (IVM) is a technique used to induce in vitro maturation of immature oocytes collected from ovarian follicles without any medication stimulation. In a routine IVF practice, several kinds of gonadotropins are used to produce more mature eggs for fertilization every egg retrieval cycle. This procedure is widely used in most of the IVF centers, but the application of these drugs obviously increases fertility treatment cost, and patients also suffer many times from drug injections. In IVF practice, another pathway is oocyte in vitro maturation; that is, without ovarian stimulation, immature oocytes are retrieved from ovary and conducted for maturation under the laboratory condition for about 24 h in vitro, subsequent for normal insemination. Currently this technology has achieved some success, but it needs to be further improved to obtain a higher pregnant rate. Thus, IVM application significantly reduces IVF cost, patient emotional address and side effects, and frequent hospital visits [7].

At beginning, IVM technique was designed as an alternative to conventional IVF for minimizing the risk of the ovarian hyperstimulation syndrome (OHSS) in patients with the polycystic ovarian syndrome (PCOS). As an effective treatment method, IVM can be used to treat patients with polycystic ovary syndrome, ovarian hyperresponsiveness, and hyporesponsiveness, as well as to preserve the fertility of cancer patients [8]. This technology has been used worldwide for the birth of thousands of healthy babies. The improvement in clinical IVM technology mainly focuses on the IVM medium and the optimization of the culture environment and operation process. Recently many research groups have started to combine clinical application of IVM with a natural cycle or mild stimulation in IVF practice, especially for PCOS and age women. In particular, the combination of mild stimulation IVF with IVM is not only expected to become a viable alternative to current standard treatments, but may also become a potential option of first-line treatment.

After obtaining MII oocytes, a key technique is oocyte insemination with sperm including in vitro fertilization and intracytoplasmic sperm injection in order to obtain normal fertilized egg, that is, zygote. ICSI technique has greatly improved normal IVF fertilization failure and increased oocyte fertilization rate and solved

severe male infertility problem [9], which has made many infertile males realize their dream to have children in their family. However, some fertilization failures still appear on good quality oocytes. Thus, oocyte activation is also very important for fertilization. The incidence and etiology of total fertilization failure after ICSI have led embryologists to search new activation studies including physiology of oocyte activation, electrical properties of gametes' membranes, and ion currents. It is very important that calcium oscillation is an essential process during oocyte activation. Many researches have shown that oocyte activation is triggered by sperm factor [10, 11]. Artificial oocyte activation (AOA) also imitates sperm factor function to stimulate oocyte activation for successful fertilization. Artificial activation can be induced by the use of electrical, mechanical, or chemical stimuli to elevate intracellular concentrations of calcium ions [12]. Thus, discussion on the effect of different AOA methods on the success and safety will help embryologists to improve fertilization rate and reduce fertilization failure accident.

As shown in **Figure 1**, after fertilization, the embryo experiences a series of events including the formation of the maternal and paternal pronuclei (2PN) on day 1 and cleavage stage on day 1.5 to day 3, reaching the morula (compaction) on day 4 and the blastocyst on day 5, until it arrives at the uterus, where it "hatches" from the zona pellucida to implant into the endometrium. During this period, some important findings are obtained from many IVF practices. Thus, understanding human and animal blastocyst formation and development and their physiology, morphology, and gene expression of blastocyst will help us to reveal the reason for high rate of embryo implantation failure. All these processes for embryonic development need neuroendocrine regulation. Neuroendocrine, an integration of the nervous system and endocrine system as its name implies, plays a critical role in the reproductive system. However, less progress has been made in the particular effects of neuroendocrine on embryo implantation. Despite these barriers, significant knowledge has been gained through recent studies and shows that neuroendocrine is tightly involved in embryo implantation. Therefore, the current state of knowledge about the impaction of neuroendocrine on embryo implantation could make us consider a potential strategy to get higher pregnancy rate after in vitro fertilization and embryo transfer (IVF-ET) in order to decrease recurrent implantation failure (RIF) possibility through modulating the neuroendocrine systems [13, 14].Another important branch in IVF practice is embryo cryopreservation. Embryo freezing technologies have widely been developed and used in human IVF practice and animal industry [15, 16]. In the last decade, the cryopreservation of reproductive cells including stem cells, embryos, gametes, tissues, and organs has become a routine work in many IVF centers and animal industry [17]. As the embryo freezing number increases, some new techniques for cryopreservation have been developed, especially oocyte and embryo vitrification, which have greatly improved oocyte and embryo survival rate after thawing or warming [16]. It is very interesting that a new "Theory About the Embryo-Cryo Treatment" has been formed. It should be considered that the method of cryopreservation is not only a technology for storing embryos but also a method of embryo treatment that can potentially improve the success rates in infertile couples. This theory believes that freezing and thawing process could activate endogenous survival and repair mechanisms in preimplantation embryos. We think that the embryonic thawing process induces low levels of stress, which results in hormesis and could repair mitochondrial damage and protein misfolding. Thus, this theory may explain the higher success rate of frozen-thawing embryo transfer than fresh embryo transfer for age women and the higher miscarriage case. Thus, the "treatment" effect of freezing an embryo may explain the higher success rates than fresh embryo transfer. The analysis of facts and suggestions should enable researchers to rethink the position of cryobiology in reproductive medicine.

During the last couple of decades, the assisted reproductive technologies have become one of the fastest developing branches of medicine. Assisted reproductive technology has been widely used for the treatment of infertile couples to realize their dream to have a baby. However, many people are worried about ART baby health outcome. Although many reviews of ART effect on birth babies have been reported, it is very difficult to find a system data about ART outcome on sex ratio and frozen embryo transfer. Our study provides some detailed data about the effect of ART on human birth babies by two country IVF center data. The result showed that ART patient age is significantly older than normal delivered women; the gestation of fresh and frozen embryo transfer has the same as normal deliver baby gestation days, but multi-baby birth women have shorter gestation days; there is no significant difference on early birth between fresh embryo transfer single babies and normal delivered babies, but multi-babies have a higher early birth rate, and frozen embryo transfer has lower early birth rate; there is no significant difference between male and female babies although fresh embryo transfer looks like more male babies and frozen embryo transfer has more female babies; there is no significant difference on the baby weight between ART singleton babies and normal delivered babies, but male baby weight is more than female babies, and multi-baby birth weights have significant lower singletons, while frozen embryo transfer babies have significant heavier birth weight than fresh embryo transfer. These results showed that the singleton with ART treatment does not have any significant difference from normal single babies on gestation days, early birth rate, and baby birth weight, but multi-baby birth often has high early birth rate with shorter gestation day and lower birth weight. The frozen embryo transfer may significantly reduce early birth rate. Thus, frozen embryo transfer may be recommended as a very healthy strategy in ART.

In fine, some new technologies, theories, and methods in early embryogenesis from ovarian stimulation to final ART outcome have been developed in the last decade. The systemic understanding of these new knowledge containing some basic theories and technologies will be helpful for animal scientists and human clinical physicians and embryologists to improve their ART outcome.

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