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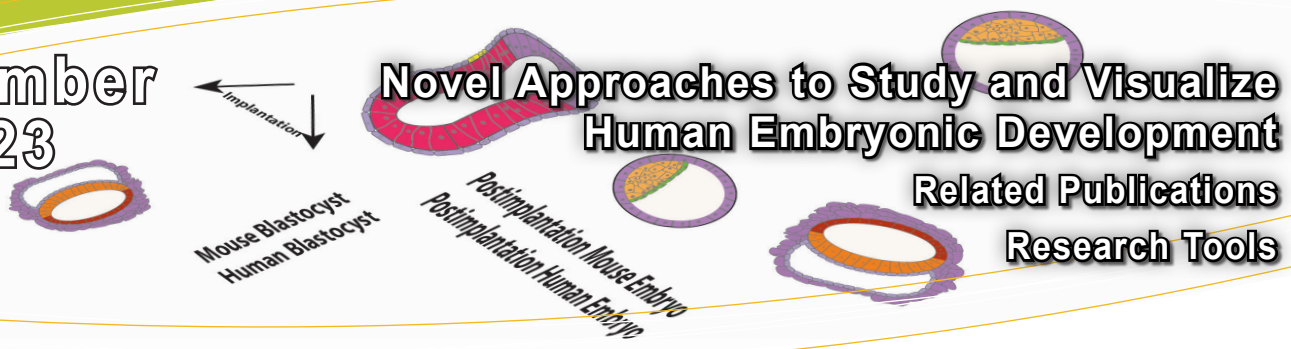

Novel Approaches to Study and Visualize Human Embryonic Development

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Novel Approaches to Study and Visualize Human Embryonic Development

Introduction

Developmental biologists have extensively studied the developmental stages of the embryo *via* mouse models and scarce human embryo data. While there is a detailed understanding of the pre-implementation stages, much less is understood between the peri-implantation to gastrulation stages due in large part because the implantation of the embryo makes it extremely challenging to visualize and study(reviewed in 1). Gaining a better understanding of how embryos develop could help researchers understand why nearly 60% of pregnancies fail between fertilization and implantation stages; furthermore, a greater understanding of the development during this period could also provide insight for treating early-stage developmental-related diseases and provide embryo-like models for testing the safety of drugs during pregnancy. There are several technical and ethical barriers that have impeded scientific progress toward understanding this "black box" in embryonic development; for example, novel approaches to growing embryos outside of the uterus have been a limiting factor. Recent advances in the tools, processes, and models have drastically enhanced scientists' ability to elucidate a more complete picture of human embryonic development, and below we discuss some of the fundamental discoveries.

Mouse Embryos Provide The Early Groundwork

Much of the elucidation of embryonic development has been achieved using mouse embryo models. These studies led to our understanding that early embryonic development requires repeated rounds of cleavage divisions that divide the single-cell zygote into the blastocyst comprised of hundreds of cells. In mouse embryo development there are several critical stages; for example, cell compaction occurs at the 8-cell stage where cells become polarized and undergo asymmetric divisions. This is essentially the prelude for the first three tissue types (pluripotent epiblast, primitive endoderm, and the trophectoderm) (see figure 1) of the embryo that are present in the blastocyst(reviewed in 2,3). The blastocyst must then implant into the uterus to continue developing, and much of the understanding of embryonic development at that point was based on snap-shot images of fixed embryos at successive stages.
The Zernicka-Goetz group published several studies where they identified media and growing conditions that allowed them to grow mouse embryos from mouse pluripotent stem cells directly on microplates that would allow for live cell microscopy[4,5]. Interestingly, with this system, the group deduced that there was no evidence of apoptosis during the cavity formation in these developing embryos, which was contrary to longstanding models. In 2021, Jacob Hanna's group further optimized the specific growing conditions needed for highly reproducible and prolonged post-implantation mouse embryo development[6]. The group used a novel *ex utero* roller culture platform that precisely controlled movement, oxygen bubbles, and media conditions, which ultimately allowed five-day-old embryos to grow outside the uterus for six additional days; equivalent to about one-third of their gestation. Based on the measurements they performed including RNA sequencing, histological, and molecular studies, the data showed that these *ex utero* embryos effectively recapitulated utero development.

Molding Human stem cells Into Embryos

One of the biggest leaps forward for the field came in 2016 when the Zernicka-Goetz and Brivanlou labs utilized the techniques that were discovered with mouse pluripotent stem cells and embryos and applied them to making human embryos from human stem cells[7,8]. The achievement was amazing and resulted in the culturing of human partial embryos to the point of gastrulation. Importantly, all experiments were stopped in compliance with the International Society for Stem Cell Research (ISSCR) guidelines on ceasing human embryo experiments

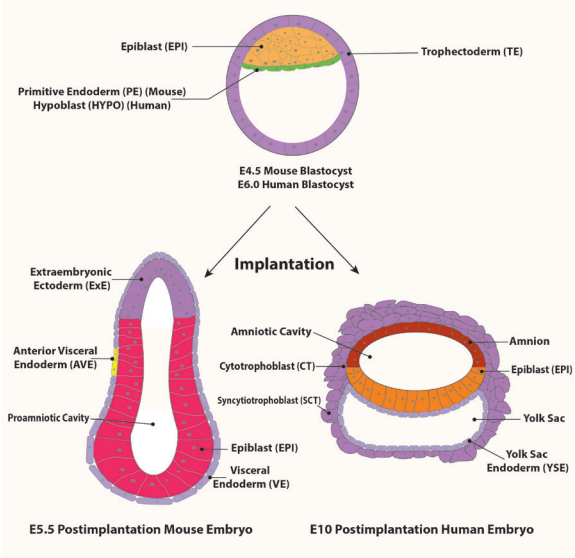

Figure 1. Schematic representation of mouse and human pre- and postimplantation embryos. Adapted from Shahbazi M. et al. Science 2019

Continued from Page 1

at 14 days post fertilization. In 2021, two additional studies were reported where scientists took naïve human pluripotent stem cells[9] or reprogrammed human fibroblasts[10] and generated human embryo models which they termed blastoids. These blastoids had the overall model of blastocysts, contained the allocated cell lineages, and had the appropriate transcriptomic profiles based on RNA analysis, but in both reports, few blastoids were capable of surviving through the post-implantation stage. Four labs (Zernicka-Goetz, Hanna, Li, and Ebrahimkhani) have published pre-prints showing that they have created human embryos that survive up to 14 days old and also produce key extra-embryonic tissues of the early post-implantation human conceptus[(reviewed in 11)]. While all the models utilized different approaches, the end results cumulatively provide the clearest picture of stem cell-derived human embryonic development thus far. One of the challenges that remain is whether the observations that were reported are real differences in human embryo development or artifacts of an *in vitro* system.

Gaining A Better Picture Of The Human Embryo

After the development of *in vitro* fertilization in the 1970s, scientists utilized excess donated embryos to build a better understanding of Embryonic development. As noted, this research occurred under strict guidelines and in very low numbers. Aside from the obvious ethical and moral issues, donated human embryos are quite challenging to study as they cannot be genetically modified or injected with DNA or RNA to assist with imaging studies. Recently, The Plachta group published the most complete picture of the developing human embryo using the minimally invasive Spirochrome live cell dyes to visualize key structures[12]. In their study, the group used SPY-Actin and SPY-DNA dyes in combination with 3D confocal microscopy to reveal the dynamics of chromosome segregation, compaction, polarization, and blastocyst formation in the developing human embryo. While doing comparative studies between mouse and human embryos, they deduced that, unlike mouse embryos where compaction happens at the 8-cell stage, human embryos undergo compaction between the 12-14 cell stage. Furthermore, they identified a novel DNA-shedding effect that occurred during blastocyst expansion, which could contribute to aneuploidy. These novel findings highlight the importance of developing a proper roadmap for human embryonic development that is based on donated human embryos as there may be key differences relative to mouse embryo development (see figure 1). Additionally, as research on donated human embryos is severely limited, more extensive drug studies or disease-based research using human embryos may need to be performed on stem cell-based human embryos; thus, it may be beneficial if these live cell imaging approaches shown here can be effectively adapted to stem cell-based human embryos.

Future Perspectives

The recent flurry of studies highlighted above shows the great progress being made toward fully understanding how human embryonic development occurs from pre-implantation through gastrulation. The tools and methodology have evolved so rapidly that the ISSCR lifted its long-standing rule stating that human embryos should not be cultured past the 14th-day post-fertilization. In the paper by Plachta and colleagues, they showed that trophectoderm biopsy, which is used to test for aneuploidy in embryos, can significantly enhance nuclear DNA shedding, which can add potential risk factors to the developing embryo[12]. Thus, it will be of interest if these minimally invasive probes like SPY-DNA are adapted for use in clinics as an alternative approach to examine the health of the developing embryo's DNA, but additional studies are needed.

References

1. Zhai, J., et al., Human embryonic development: from peri-implantation to gastrulation. Trends Cell Biol, 2022. 32(1): p. 18-29.
2. Morris, S.A., Human embryos cultured in vitro to 14 days. Open Biol, 2017. 7(1).
3. Shahbazi, M.N., E.D. Siggia, and M. Zernicka-Goetz, Self-organization of stem cells into embryos: A window on early mammalian development. Science, 2019. 364(6444): p. 948-951.
4. Morris, S.A., et al., Dynamics of anterior-posterior axis formation in the developing mouse embryo. Nat Commun, 2012. 3: p. 673.
5. Bedzhov, I. and M. Zernicka-Goetz, Self-organizing properties of mouse pluripotent cells initiate morphogenesis upon implantation. Cell, 2014. 156(5): p. 1032-44.
6. Aguilera-Castrejon, A., et al., Ex utero mouse embryogenesis from pre-gastrulation to late organogenesis. Nature, 2021. 593(7857): p. 119-124.
7. Deglincerti, A., et al., Self-organization of the in vitro attached human embryo. Nature, 2016. 533(7602): p. 251-4.
8. Shahbazi, M.N., et al., Self-organization of the human embryo in the absence of maternal tissues. Nat Cell Biol, 2016. 18(6): p. 700-708.
9. Yu, L., et al., Blastocyst-like structures generated from human pluripotent stem cells. Nature, 2021. 591(7851): p. 620-626.
10. Liu, X., et al., Modelling human blastocysts by reprogramming fibroblasts into iBlastoids. Nature, 2021. 591(7851): p. 627-632.
11. Leslie, M., Human stem cells turned into detailed lab replicas of embryos. Science, 2023. 380(6651): p. 1206-1207.
12. Domingo-Muelas, A., et al., Human embryo live imaging reveals nuclear DNA shedding during blastocyst expansion and biopsy. Cell, 2023. 186(15): p. 3166-3181 e18.

DNA Live Cell Imaging Probes

Product	Ex / Em	Amount	Cat #
SiR-DNA™ Kit Includes Verapamil	630 / 680 nm	50 nmol	CY-SC007
SiR700-DNA Kit Includes SiR700-DNA and Verapamil	690 / 720 nm	35 nmol	CY-SC015
SPY505-DNA Includes SPY505-DNA Probe	512 / 531 nm	100 stains	CY-SC101
SPY555-DNA Includes SPY555-DNA Probe	555 / 580 nm	100 stains	CY-SC201
SPY595-DNA Includes SPY595-DNA Probe	599 / 615 nm	100 stains	CY-SC301
SPY650-DNA Includes SPY650-DNA Probe	652 / 674 nm	100 stains	CY-SC501
SPY700-DNA Includes SPY700-DNA Probe	696 / 718 nm	100 stains	CY-SC601

Actin Live Cell Imaging Probes

Product	Ex / Em	Amount	Cat #
SiR-Actin Kit Includes Verapamil	652 / 674 nm	50 nmol	CY-SC001
SPY555-Actin Includes SPY555-Actin Probe	555 / 580 nm	100 stains	CY-SC202
SPY620-Actin Includes SPY620-Actin Probe	619 / 636 nm	100 stains	CY-SC402
SPY650-FastAct™ Includes SPY650-FastAct™ Probe	652 / 674 nm	100 stains	CY-SC505
SPY700-FastAct™ Includes SPY700-FastAct™ Probe	696 / 718 nm	100 stains	CY-SC605

Tubulin Live Cell Imaging Products

Product	Ex / Em	Amount	Cat #
SiR-Tubulin™ Kit Includes SiR-Tubulin, and Verapamil	630 / 680 nm	50 nmol	CY-SC002
SiR700-Tubulin Kit 35 nmol SiR700-Tubulin and 1 µmol verapamil	680 / 720 nm	50 nmol	CY-SC014
SPY555-Tubulin Kit Includes SPY555-Tubulin and Verapamil	555 / 580 nm	100 stains	CY-SC203
SPY650-Tubulin Includes SPY650-Tubulin and Verapamil	652 / 674 nm	100 stains	CY-SC503