Elucidating the behavior of trophectoderm derivatives in mouse implantation

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https://doi.org/10.1016/j.devcel.2022.01.010

Studying mammalian implantation in utero is difficult, but many in vitro models of peri-implantation development lack contributions from extra-embryonic tissues. Two recently published Developmental Cell papers present biomimetic systems for culturing peri-implantation mouse blastocysts ex vivo. These papers reveal dynamics and developmental impacts of two essential trophectoderm derivatives: extra-embryonic ectoderm and trophoblast.

Implantation of an embryo into maternal uterine tissue is a critical step in mammalian embryonic development, and understanding the mechanics of implantation is essential to ensure safe and healthy pregnancies in humans. However, even when using animal models such as mice, studying implantation presents a considerable challenge; delivering spatio-temporally controlled perturbations to and obtaining data from embryos is exceptionally difficult when the embryos are embedded in the uterine wall (Spiteri et al., 2020). Excitingly, advancements are being made in the field of ex vivo culturing systems that enable researchers to take pre-implantation embryos in utero and continue culturing them in biomimetic environments. Two papers from Developmental Cell demonstrate exciting and innovative applications of ex vivo culture systems to study the complex dynamics of implantation for mouse embryos (Iichikawa et al., 2022; Govindasamy et al., 2021).

Mammalian implantation occurs several days after fertilization, by which time the zygote has undergone rapid cell division and developed into a blastocyst. In mice, the inner cell mass housed within the trophectoderm segregates into primitive endoderm cells and pluripotent epiblast cells prior to implantation. Upon implantation, the epiblast undergoes lineage specification to further develop into the embryo proper in contrast to the trophectoderm and primitive endoderm, which are considered to be extra-embryonic tissues. The trophectoderm differentiates into extra-embryonic ectoderm (ExE), which remains adjacent to the epiblast, and trophoblast, which continues to invade the uterine wall to restructure maternal vasculature and begins to form the placenta. These extra-embryonic tissues are essential components of healthy embryonic development in mammals, especially at the implantation stage (Hiramatsu et al., 2013; Christodoulou et al., 2019).

Current models of implantation have provided important insights but suffer from critical drawbacks that hinder their in vivo relevance. Mouse embryos fully embed themselves in the uterine wall during implantation, but many ex vivo culturing systems rely on using 2D substrates to facilitate observation and data collection at the expense of in vivo relevance and accurate mechanical cues (van den Brink et al., 2014; Shahbazi et al., 2016; Bedzhov and Zernicka-Goetz, 2014). Similarly, many 3D culture systems for examining mammalian embryonic development lack extra-embryonic tissues and their contributions to developmental events (van den Brink et al., 2014; Zheng et al., 2019).

In their recent publications, Ichikawa et al. and Govindasamy et al. showcase innovative and efficient ex vivo culturing protocols that embed mouse embryos from in utero (complete with extra-embryonic tissues) in biomimetic gel-based 3D culturing environments that allow for controlled perturbation and observation of embryonic development over the course of 48 h. Ichikawa et al. submerged pre-implantation blastocysts in a Matrigel collagen gel and used inverted light sheet microscopy to achieve in toto live imaging of the developing blastocyst (Figure 1A). Subsequent embryonic development over 48 h of ex vivo culture in this system closely resembled developmental events from E4.5 to E6. This faithful implantation environment readily enabled examination and disruption of tissue-to-tissue interactions and was used to study the effect of ExE signals on epiblast development. In contrast, Govindasamy et al. created a microfluidic chip with a degradable 3D gel environment wherein blastocysts were cultured in the proximity of vasculature that was also embedded in the gel (Figure 1B). The gel was tailored to possess similar mechanical properties to the decidua, and fluorescent imaging and used to examine the invasion dynamics of trophoblast toward the vasculature in this biomimetic environment.

The authors of both papers leveraged these methods to uncover valuable information regarding the interactions of the various tissues involved in mammalian implantation. Ichikawa et al. found that the ExE plays an essential role, via chemical and mechanical signaling cues, in the growth, patterned morphogenesis, and even cavity formation of the epiblast during implantation. Meanwhile, Govindasamy et al. discovered that trophoblast invasion occurs through collective cell migration and that the cells leading the invasion communicate with maternal blood vessels by exhibiting certain vascular traits that are essential for intervascular signaling. These
traits allow the trophoblast to form connections with maternal blood vessels for subsequent remodeling through the signaling pathways used to create new blood vessels.

Although both groups have made considerable contributions toward understanding mammalian embryonic development during implantation, the full potential of such ex vivo cultures has yet to be realized. Both groups recognize that their ex vivo culture systems cause a delay in embryonic development, which is likely caused by the disruptive nature of removing the embryo from in utero and preparing the tissues for ex vivo culture. Optimizing protocols for embryo transfer to be less disruptive might make it easier to translate the timeline of developmental events observed ex vivo to the developmental timeline in utero. However, developing minimally disruptive techniques for extracting and handling embryos in utero remains difficult.

Minimizing human interference in these culture methods is also essential to achieve optimal in vivo relevance. In order to facilitate ExE formation ex vivo, Ichikawa et al. found it is necessary to release tension in the trophoderm by removing the mural trophectoderm (Figure 1A), which according to Govindasamy et al. is the region from which trophoblast invasion originates. Ichikawa et al. believe maternal tissues induce tension release in utero, but Govindasamy et al. make no mention of ExE formation in their system, so it is unknown if their decidua-like environment resolves this issue. Integrating uterine tissues into the Ichikawa et al. system dramatically increases its complexity, and preserving in toto imaging and perturbation controllability presents a considerable challenge.

Similarly, maternal tissues will likely hold the key to extending the duration of ex vivo culture beyond the E4.5 to E6 window. Ichikawa et al. found that in utero development up to E4.5 was necessary for successful ex vivo culture; the authors believe that their ex vivo system lacks unknown but essential contributions from maternal tissues leading up to E4.5 that help ensure embryo viability in utero. Discovering and replicating those contributions may enable ex vivo culture for earlier embryos and could even facilitate reliably extending ex vivo culture past E6. Although studying the early interactions between the uterus and the embryo is difficult, new methods for imaging mouse embryos in utero past E9.5 show promise for observing implantation in the future (Huang et al., 2020).

Lastly, although mouse models can only tell us so much about human development, the scarcity of available human embryos and the ethical and legal considerations of ex vivo human embryo culture pose significant obstacles. Now that these ex vivo methods have been established and characterized, the next step is to apply them to non-human primate embryos, which are more difficult to obtain but offer greater human relevance. By applying these techniques to more human-relevant models, our understanding...
of human implantation will continue to expand and deepen.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES


