OXFORD

human reproduction update

Human Reproduction Update, 2024, **00(0)**, 1–31

https://doi.org/10.1093/humupd/dmae033

The modeling of human implantation and early placentation: achievements and perspectives

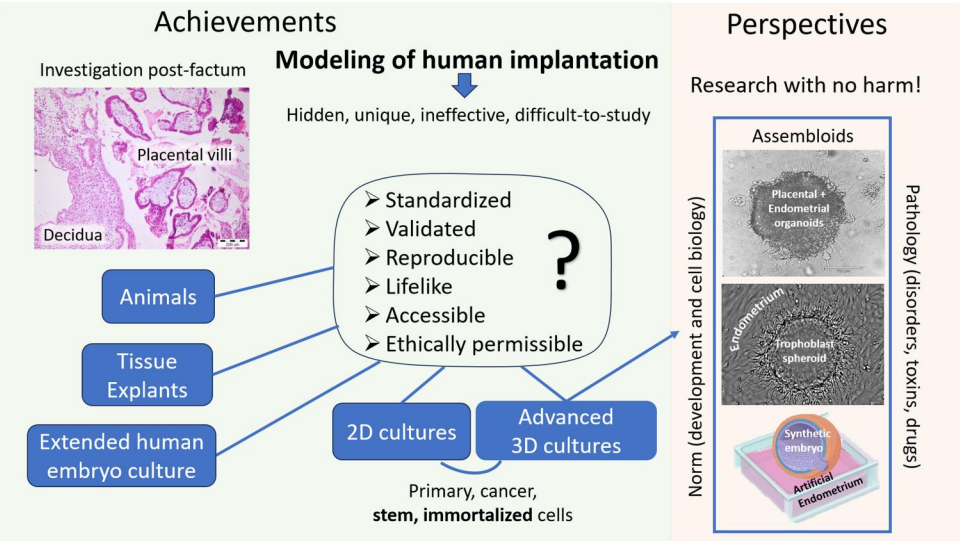
Tanya Dimova [1,*], Marina Alexandrova[1], Ivaylo Vangelov[1], Yuan You[2], and Gil Mor[2]

[1]Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]C.S. Mott Center for Human Growth and Development, Wayne State University, Detroit, MI, USA

*Correspondence address. Department of Immunobiology of reproduction, Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, 73 blvd Tzarigradsko Shosse, Sofia 1113, Bulgaria. Tel: +359-882-412742. E-mail: tanyadimova@yahoo.com
https://orcid.org/0000-0001-8665-4352

TABLE OF CONTENTS

GRAPHICAL ABSTRACT



Human implantation is a unique, ineffective, and difficult-to-model process, with a variety of *in vivo*, *ex vivo*, and *in vitro* models attempting to simulate certain phases of human implantation and related events; the advanced 3D implantation surrogates/assembloids are promising avenues for the future research of implantation.

ABSTRACT

BACKGROUND: Successful implantation is a critical step for embryo survival. The major losses in natural and assisted human reproduction appeared to occur during the peri-implantation period. Because of ethical constraints, the fascinating maternal–fetal cross-talk during human implantation is difficult to study and thus, the possibility for clinical intervention is still limited.

Received: February 1, 2024. **Revised:** October 29, 2024. **Editorial decision:** November 12, 2024.

OBJECTIVE AND RATIONALE: This review highlights some features of human implantation as a unique, ineffective and difficult-to-model process and summarizes the pros and cons of the most used *in vivo, ex vivo* and *in vitro* models. We point out the variety of cell line-derived models and how these data are corroborated by well-defined primary cells of the same nature. Important aspects related to the handling, standardization, validation, and modus operandi of the advanced 3D *in vitro* models are widely discussed. Special attention is paid to blastocyst-like models recapitulating the hybrid phenotype and HLA profile of extravillous trophoblasts, which are a unique yet poorly understood population with a major role in the successful implantation and immune mother-embryo recognition. Despite raising new ethical dilemmas, extended embryo cultures and synthetic embryo models are also in the scope of our review.

SEARCH METHODS: We searched the electronic database PubMed from inception until March 2024 by using a multi-stage search strategy of MeSH terms and keywords. In addition, we conducted a forward and backward reference search of authors mentioned in selected articles.

OUTCOMES: Primates and rodents are valuable *in vivo* models for human implantation research. However, the deep interstitial, glandular, and endovascular invasion accompanied by a range of human-specific factors responsible for the survival of the fetus determines the uniqueness of the human implantation and limits the cross-species extrapolation of the data. The *ex vivo* models are short-term cultures, not relevant to the period of implantation, and difficult to standardize. Moreover, the access to tissues from elective terminations of pregnancy raises ethical and legal concerns. Easy-to-culture cancer cell lines have many limitations such as being prone to spontaneous transformation and lacking decent tissue characteristics. The replacement of the original human explants, primary cells or cancer cell lines with cultures of immortalized cell lines with preserved stem cell characteristics appears to be superior for *in vitro* modeling of human implantation and early placentation. Remarkable advances in our understanding of the peri-implantation stages have also been made by advanced three dimensional (3D) models i.e. spheroids, organoids, and assembloids, as placental and endometrial surrogates. Much work remains to be done for the optimization and standardization of these integrated and complex models. The inclusion of immune components in these models would be an asset to delineate mechanisms of immune tolerance. Stem cell-based embryo-like models and surplus IVF embryos for research bring intriguing possibilities and are thought to be the trend for the next decade for *in vitro* modeling of human implantation and early embryogenesis. Along with this research, new ethical dilemmas such as the moral status of the human embryo and the potential exploitation of women consenting to donate their spare embryos have emerged. The careful appraisal and development of national legal and ethical frameworks are crucial for better regulation of studies using human embryos and embryoids to reach the potential benefits for human reproduction.

WIDER IMPLICATIONS: We believe that our data provide a systematization of the available information on the modeling of human implantation and early placentation and will facilitate further research in this field. A strict classification of the advanced 3D models with their pros, cons, applicability, and availability would help improve the research quality to provide reliable outputs.

Keywords: human implantation / animal models / *ex vivo* models / 2D and advanced 3D models / stem cell-based embryo-like models

Introduction

Successful interstitial implantation is a critical step for human embryo survival. There are still many gaps in our knowledge of the cell and molecular processes during implantation in humans. As a unique feature of the species, the implantation of the human embryo is characterized by deep interstitial, glandular, and endovascular invasion of the embryonic trophoblasts into specifically prepared endometrium (decidua) providing early histotrophic nutrition (Bischof and Irminger-Finger, 2005; Moser *et al.*, 2010). The subsequent formation of the villous placenta ensures sufficient hematotrophic nutrition of the human fetus until term gestation. Trophoblasts (the fetal part of the placenta) comprise the utmost border of the barrier between the fetal and the maternal blood (the villous trophoblast) and the fetus and maternal tissue (extravillous trophoblast, EVT). The unique transformation of the decidual stromal cells together with the recruited immune cells at the place of materno-fetal contact is of great importance for the recognition of the developing embryo and the establishment of immune tolerance towards its paternal alloantigens (Harris *et al.*, 2019; Muter *et al.*, 2021). During implantation and early placentation, the initially local maternal–fetal crosstalk is further extended to the entire body and the whole immune system of the mother comes into contact with fetal immunogens. The most pregnancy losses appear to occur at the peri-implantation stage or during the first week of implantation, the period which is not accessible for *in vivo* and *ex vivo* research in humans (Macklon *et al.*, 2002). Consequently, the possibility of clinical intervention is still limited. This review highlights human implantation as a unique, ineffective, and difficult-to-study process and summarizes the most used *in vivo*, *ex vivo*, and *in vitro* models. The uniqueness of the process determines the superiority of the *ex vivo* and *in vitro* models over the animal models. We discuss the models derived from primary gestational tissues and cells, cancer and immortalized cell lines, and stem cells. Their corresponding advanced three-dimensional (3D) cultures (spheroids, organoids, and assembloids) are thought to be more valuable for *in vitro* modeling of human implantation and early placentation since these recapitulate closely the *in vivo* environment. Emphasis is put on the extent to which these models succeed in representing maternal–fetal contact during human implantation and their study applications. Some aspects related to the generation, handling, standardization, validation, and modus operandi are also considered. As perspectives, the urgent need for systematization of the available 3D culture systems as well as consistency of the terminology and methodology is pointed out. The similarity of blastoid and gastruloid models to human blastocysts/gastrula provides promising avenues for implantation research, developmental biology, regenerative medicine, and drug discovery. However, these synthetic embryo models raise ethical and legal questions that affect future research and widespread adoption in industry and clinical settings, and need to be thoroughly discussed.

Human implantation and placentation in brief: a unique, ineffective, and difficult-to-study process

During the first 7 days after fertilization, the embryo develops from a zygote to an early blastocyst comprised of the inner cell mass (ICM) (embryoblast) and trophectoderm (TE) and then to a late blastocyst consisting of epiblast (precursor to the embryo proper), the hypoblast (precursor to the yolk sac) and TE (precursor of all trophoblasts) (ref. in Gerri *et al.*, 2020). During the second week of development, the TE of the blastocyst implants into the decidua basalis (transformed endometrium), turning into

trophoblast, and undergoes subsequent complex morphogenesis and placentation (Enders, 2000). Human implantation is a highly complicated and unique phenomenon consisting of three steps: an apposition phase when an unstable contact of the blastocyst to the uterine epithelium occurs, followed by firm adhesion of the embryonic trophoblast to the endometrial epithelium, and an invasion phase when the blastocyst penetrates the uterine epithelium and embeds entirely within the decidua (Norwitz *et al.*, 2001). This is the beginning of the formation of the human villous discoidal placenta. The invasive human implantation is preceded and accompanied by significant tissue remodeling and a massive endometrial reaction called decidualization. The latter prepares the tissue to accommodate the embryo and involves the development of new blood vessels, remodeling of the uterine glands, a transformation of normal fibroblasts into secreting decidual stromal cells (DSC), and recruitment of maternal immune cells (Wagner *et al.*, 2014). In humans, decidualization starts during the latter half of each menstrual cycle and is therefore independent of the conceptus (Emera *et al.*, 2012). However, the decidualization peaks when the embryo is embedding into the endometrium (Zhang *et al.*, 2013). Successful implantation supports the decidual reaction; otherwise, the decidua is shed as controlled menstrual bleeding (Cha *et al.*, 2012; Fritz *et al.*, 2014). The endometrium becomes receptive to the embryo for a short period of 2–4 days during the mid-secretory phase of each menstrual cycle (Days 19–23), commonly known as the window of implantation (WOI) (Bergh and Navot, 1992). For a long time, it was assumed that the WOI is constant in time in all women but newly published data has shown that it is personalized (Ruiz-Alonso *et al.*, 2013). The TE is the outer cell layer of the blastocyst-stage embryo, attaching to the receptive endometrial luminal epithelium to initiate implantation. The initial contact occurs via the polar TE, cells located adjacent to the ICM (Lindenberg *et al.*, 1989; Aplin and Ruane, 2017). The TE-derived trophoblast is the only fetal tissue coming into direct contact with maternal tissue and blood during implantation, becoming the fetal part of the placenta. Trophoblast consists of three types: cytotrophoblasts (CT), syncytiotrophoblasts (ST), and EVTs. The CT are proliferating mononuclear cells, that function as stem cells-like progenitors of ST and EVT. Recently, a multi-model approach showed that the invasive multinucleate ST formation from TE is promoted by attachment to the endometrial epithelium (Ruane *et al.*, 2022). Primary ST disrupts the basal lamina of the endometrial epithelium and invades the decidua, providing strong attachment to the uterus. It is important to note that the primary ST is highly invasive as compared with the secondary ST covering the definitive placental villi (Ruane *et al.*, 2022; Siriwardena and Boroviak, 2022). By Day 12, the primary placental villi are formed from CT covered with ST that invade the underlying decidual tissue providing histotrophic nourishment of the embryo under low oxygen conditions (Kojima *et al.*, 2022). These villi will eventually branch and acquire cores of mesenchyme with blood vessels and connective tissue to form the definitive placental villi (Kojima *et al.*, 2022). As a result, the vascularization of the villi associated with an initiation of the embryonic circulation will provide the effective basis for the establishment of a functional hemochorial placenta (Hamilton and Boyd, 1960; Turco and Moffett, 2019). Humans have a hemochorial type of placenta, in which the trophoblast comes into direct contact with maternal blood to establish the most intimate relationship between the developing embryo and the source of nutrition from the mother (Loke and King, 1995; Benirschke and Kaufmann, 2000). In the villi that anchor the conceptus to the decidua (anchoring villi), CT forms a continuous thick layer of trophoblast shell composed of highly proliferative CT. The formation of the shell depends on the stimulation of cytotrophoblast progenitors by histotrophs (Burton and Jauniaux, 2017). Between 11–12 and 90 days post-fertilization, the anchoring villi are numerous and closely approximated together (Hamilton and Boyd, 1960). Cells towards the outer surface of the shell undergo a partial epithelial-mesenchymal transition (EMT) to form EVT. The trophoblast shell is the source of highly invasive mononuclear EVT (Burton and Jauniaux, 2017) invading deeply into decidua basalis (maternal part of the future placenta) to reconstruct the uterine spiral arterioles into highly conductive sinusoids and adapting the blood supply to the site of implantation. At the end of pregnancy EVT in the placental bed are stationary giant cells (Moffett and Loke, 2006; Kojima *et al.*, 2022). The floating placental villi are bathed in maternal blood in intervillous spaces, where the ST participates in the mother-fetus exchange of oxygen and nutrients. ST secretes a variety of pregnancy-specific hormones such as human placental lactogen (hPL), trophoblastic protein pregnancy-specific β-glycoprotein (SP-1), and human chorionic gonadotrophin (hCG) (Tal and Taylor, 2000). The initial adhesion of the blastocyst to the uterine epithelium has never been observed *in vivo*. Most information on human implantation and the first weeks of placental development has been derived from a series of specimens collected in the 1950s, which have been described extensively (Hertig *et al.*, 1956). Some data on early human embryo development, implantation, and early pre-villous placenta (Carnegie stages 1–5) came from histological studies with archived material, and from studies with non-human primates (Boyd, 1950; Leiser and Kaufmann, 1994; Enders and Blankenship, 1999; Enders and Lopata, 1999). The available histological studies provided snapshots of events that occurred during the implantation without insights into the dynamics of the processes and possible alterations (West *et al.*, 2019). Newer atlases, based on examination of the archived specimens, examined with modern techniques continue the enrichment of our knowledge of human implantation and early embryo development (Yamada *et al.*, 2010). In Fig. 1, we show the maternal–fetal interface (MFI) in the first trimester of pregnancy (6–12 gestational weeks, gw) when both implantation and placentation are completed. Although not relevant to the implantation, the 10–12 gw period coincides with the second invasive wave of EVT and the main trophoblast subpopulations (ST, CT, EVT) could be visualized *in situ*. The decidualization of the endometrium is demonstrated by numerous decidual glands, blood vessels, immune cell recruitment, and fibroblasts transformed into DSC (Fig. 1A). Both parts of the placenta, decidua and trophoblast, are still separated (Fig. 1B). In such later specimens, the trophoblast shell becomes discontinuous, persisting only where cytotrophoblast columns are attached to the decidua basalis via anchoring villi (Fig. 1C). The distal end of the anchoring villa (in contact with the decidua) contains a CT column with highly proliferative CT giving rise to the EVT population. Floating placental villi are composed of ST, CT, and mesenchyme with numerous blood vessels (Fig. 1D). The EVT migrate as a group into early pregnancy decidua (Fig. 1E) but are the stationary giant cells in the placental bed in term pregnancy (Fig. 1F).

Human implantation and the following growth and development of the semi-allogeneic fetus in the uterus, without rejection by the mature maternal immune system, is considered an immune paradox (Medawar, 1953). Accumulating data on the immune interaction between the mother's immune system and trophoblasts showed that human implantation is probably not

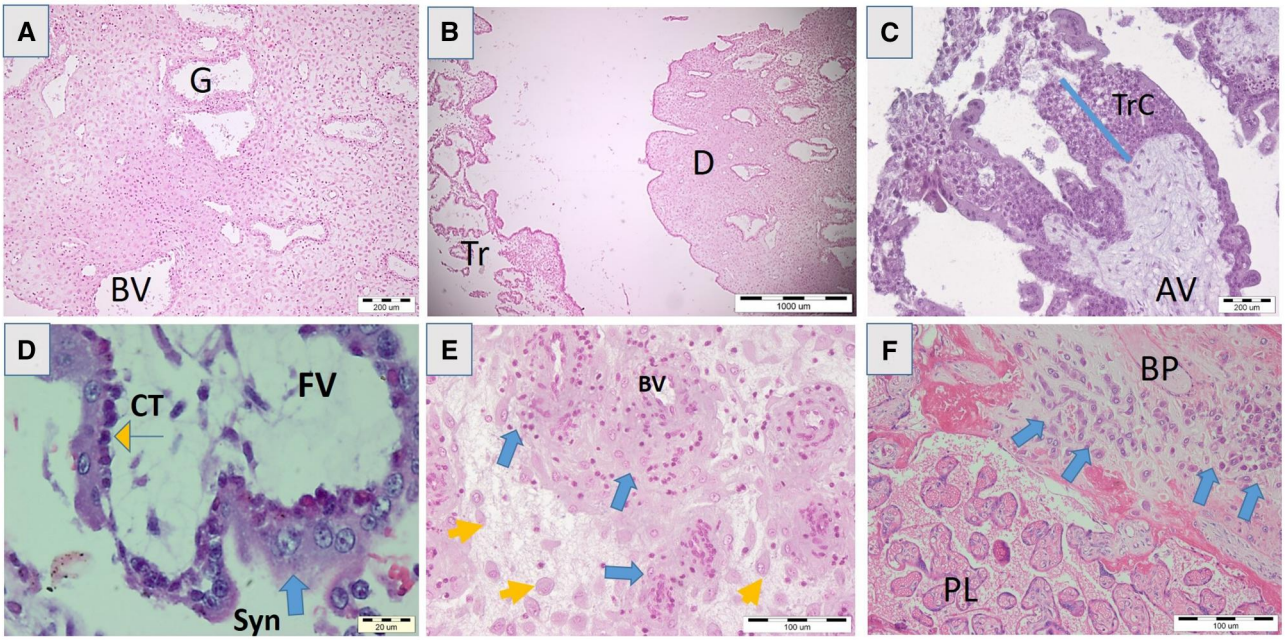


Figure 1. Maternal–fetal interface during early and term human pregnancy. Paraffin sections stained with hematoxylin and eosin were obtained according to the published protocol (Terzieva *et al.*, 2019). (**A**) decidualization of the endometrium, note numerous decidual glands and blood vessels. (**B**) Both parts of the placenta—decidua and trophoblast. (**C**) Anchoring placental villus with a column of cytotrophoblasts. (**D**) Floating placental villus with sincytio- (blue arrow) and cytotrophoblasts (yellow arrow). (**E**) Clusters of EVTs (blue arrows) remodeling blood vessel and decidual stromal cells (yellow arrows) around. (**F**) Term placental villi bordering basal plate (maternal surface) with numerous EVTs (blue arrow). G, gland; BV, blood vessel; D, decidua; Tr, trophoblasts; AV, anchoring villus; TrC, cytotrophoblast column; FV, floating villus; Syn(ST), syncytiotrophoblasts; CT, cytotrophoblasts; EVT, extravillous trophoblasts; PL, placenta; BP, basal plate.

an immune paradox but rather 'a fascinating example of a very special challenge for the maternal immune system' (Juch *et al.*, 2012). During implantation and placentation, the mother-embryo contact is always mediated by the trophoblast and is dual: (i) between maternal blood and ST of the floating placental villi and (ii) between maternal immune cells and EVT into decidua. The EVT invades deeply into decidua to the inner one-third of the myometrium to (i) establish immune tolerance via interaction with decidua-based immune cells, (ii) remodel the uterine glands to provide the early embryo with histotrophic nutrition, and (iii) remodel the uterine spiral arteries for definitive hemochorial placenta establishment. Probably different EVT populations are involved in these processes and their function is crucial for the success (or failure) of implantation. The invasion of the trophoblasts is a tightly regulated process by the maternal cells: the immune cells and DSC (Xu *et al.*, 2021). Shallow trophoblast invasion and vascular remodeling result in poor placental blood flow, causing common disorders of pregnancy, such as recurrent miscarriage, preeclampsia, and fetal growth retardation (Roberts and Escudero, 2012). Conversely, excessive invasion can lead to a life-threatening complication (placenta percreta), where trophoblast cells can rupture the uterus (Jauniaux and Jurkovic, 2012). Thus, the understanding of trophoblast differentiation and function is crucial to improving the management of implantation disorders. The successful implantation is generally associated with the downregulation of the adaptive immune system (conventional T-cell responses), T-regulatory cell enrichment (Aluvihare *et al.*, 2004; Dimova *et al.*, 2011), and specific adaptation of the innate immune cell populations at the MFI (Robertson and Moldenhauer, 2014; Alexandrova *et al.*, 2022b; Manchorova *et al.*, 2022). Hence, local maternal immunity provides an immunosuppression of specific responses towards the fetus without massively compromising the ability to fight infection and tumor transformation. The villous trophoblasts bathed in maternal blood do not express HLA class I molecules (Blaschitz *et al.*, 2001) but EVT contacting with maternal immune cells in the decidua express a unique pattern of HLA molecules non-classical (invariant) HLA class I molecules such as HLA-G, HLA-E and HLA-F (Apps *et al.*, 2009; Yang *et al.*, 2022) and the only classical polymorphic HLA-C molecule (Proll *et al.*, 1999). EVT invading decidual tissue interacts with maternal uterine natural killer (uNK) cells, macrophages, and T cells (Moffett and Loke, 2006; Hanna *et al.*, 2006). Killer immunoglobulin-like receptors (KIRs) on the maternal cytotoxic T and NK cells specifically recognize parental HLA-C allotypes (Hiby *et al.*, 2010). It seems that some maternal/fetal KIR/HLA-C combinations might be favorable to implantation/placentation, while others could initiate allo-responses or insufficient placental growth (Hiby *et al.*, 2010).

Human reproduction (native and assisted) is an ineffective process and probably this is part of the price for the intimate coexistence of two different genomes. Only around one-third of natural human conceptions progress to a live birth, with the others resulting in failed pregnancy (Chard, 1991; Clark, 2003; Koot *et al.*, 2012). Approximately half of all conception losses in healthy couples are pre-clinical (i.e. lost before registration of pregnancy) (Chard, 1991; Zinaman *et al.*, 1996; Macklon *et al.*, 2002; Clark, 2003). Of these failures, around 30% are before implantation, predominantly due to 'abnormal' embryos (e.g. chromosome abnormalities). Another 30% are lost following implantation, between the third and fourth week of gestation (Zinaman *et al.*, 1996; Macklon *et al.*, 2002). Early clinical pregnancy loss shows significant variation with age and accounts for 10% of all conceptions (Wang *et al.*, 2003; Ojosnegros *et al.*, 2021). Nowadays women tend to decide to conceive later in life and delay motherhood. However, with aging, the chance of natural conception and maintenance of pregnancy decreases, and an

assisted reproductive technology treatment might be needed. IVF-ET (*in vitro* fertilization-embryo transfer) allows the compartmentalization of the treatment process so that it becomes possible to know when an embryo was transferred and if implantation has occurred. Implantation failure or recurrent implantation failure (RIF) is one of the main reasons for the low success rate in IVF-ET (Cha *et al.*, 2012) as only 34.6% of the transferred embryos successfully implant (European IVF Monitoring Consortium, ESHRE *et al.*, 2023). Even though the embryologists are equipped with instrumentation to evaluate and choose top-quality embryos for transfer, the impact of the maternal side on implantation remains to be evaluated. There is now considerable interest in the role of the endometrium and peri-conceptus environment on implantation and subsequent embryo development. Because of ethical and practical constraints, the *in vivo* investigation of implantation in humans is not possible and the law in many EU countries also prohibits *in vitro* studies using human blastocysts (Matthews and Moralí, 2020).

In summary, the unique feature of human implantation is the deep interstitial, glandular, and endovascular invasion providing early histotrophic nutrition and subsequent formation of a single-disk hemochorial villous placenta. The majority of losses in natural and assisted human reproduction appear to occur at the peri-implantation stage or early after implantation. Unfortunately, this period is not accessible for *in vivo* and *ex vivo* research in humans. Thus, human implantation is still a considerable barrier and limiting step to natural and assisted reproduction, as the lack of detailed knowledge limits the possibilities for clinical intervention.

Modeling of the human implantation: the status quo

In the next section of this review, we discuss the primates and rodents as *in vivo* animal models for studying human implantation (Tables 1 and 2) as well as the *ex vivo* and *in vitro* models derived from primary gestational tissues and cells, cancer and immortalized cell lines, and stem cells (Table 3), as well as the corresponding advanced 3D constructs such as spheroids and organoids. The complex assembloid models (Table 4) recapitulate, to the highest extent, the MFI during human implantation. Some of the models are schematically presented in Fig. 2.

In vivo (animal) models: primates and rodents

Although primates and rodents are valuable *in vivo* models for human implantation research (Fig. 2A), one must bear in mind that the mode of implantation and placenta type in humans are different (Schmidt *et al.*, 2015; Aghajanova, 2020). The main differences in the implantation of humans, primates, and rodents are summarized in Table 1. Evolutionarily, humans are classified as one group with the great apes (gorillas, chimpanzees, orangutans, bonobos), separate from the old world monkeys (macaques, baboons) and new-world monkeys (marmosets) (Nakamura *et al.*, 2021). Primates (great apes, old and new world monkeys) have been part of placental research for many years (Myers, 1972; Enders, 1995, 2000; Carter and Pijnenborg, 2011; Carter *et al.*, 2015). Great apes have the greatest structural similarity to human beings in terms of decidualization, routes, and depth of trophoblast invasion, the timing of implantation, and placenta type (discoidal villous) (Enders, 1993; Nakamura *et al.*, 2021) (Table 1). In chimpanzees, bonobos (pigmy chimpanzees), gorillas, old-world monkeys, and humans, decidualization occurs before implantation (mid-secretory phase of each cycle), and is linked to the interstitial type of implantation (Dollar *et al.*, 1982; James, 2014). It is driven by the progesterone of corpus luteum after ovulation (Carp, 2020). The available single histological samples of orangutans and gibbons have shown restricted interstitial invasion and decidualization remains an uncertain process (Carter and Pijnenborg, 2011). In other primates (new-world monkeys), the decidualization only occurs after implantation (Siriwardena and Boroviak, 2022). Chimpanzee and human embryos are entirely buried into decidua basalis, whereas the embryos of cynomolgus and rhesus macaques are only partially buried in the endometrium (Nakamura *et al.*, 2016). It has been reported that all trophoblast cell types among non-human primates and humans are similar morphologically and functionally (Enders, 1995, 2000; Carter *et al.*, 2015) but a species difference of central importance is in the EVT invasion depth, which is smaller in primates other than chimpanzees (Schmidt *et al.*, 2015; Nakamura *et al.*, 2021) (Table 1). Also, different kinetic gene expressions before and after implantation have been shown between humans and monkeys (Nakamura *et al.*, 2016). Humans and great apes have a single placental disc, while macaques and marmosets have a bi-discoidal placenta (Myers, 1972). The primitive syncytium forming lacunae filled with maternal blood (Enders, 1989), later becoming large intervillous spaces, is observed in humans and non-human primates (Schlafke and Enders, 1975). Studying implantation in different primate species is helpful to identify conserved processes applicable to all primates including humans and conversely, the differences in implantation will dissect human-specific processes. Studies on the mechanisms controlling trophoblast invasion depth in primates of different implantation modes may elucidate early trophoblast invasion and the molecular mechanisms underlying pathophysiological changes in human placental development (Siriwardena and Boroviak, 2022). Future cross-species analysis of the recently generated *in vivo* single-cell transcriptome datasets of post-implantation trophoblast in humans, rhesus macaque, and marmoset will facilitate the discovery of new regulators of EVT differentiation (Ma *et al.*, 2019; Bergmann *et al.*, 2022; Chen *et al.*, 2022).

In Table 2, we summarize most of the studies of primates and rodents as *in vivo* models of human implantation and early placentation emphasizing their advantages and disadvantages. Large-scale experiments with non-human primates are difficult because of higher expenses, longer life cycles and gestation time, specific requirements for their housing, low fertility, and inability to detect early pregnancy, as well as ethical considerations (Lee and DeMayo, 2004) (Table 2). Given the embargo on invasive procedures in apes, it seems that the best models available are macaques, baboons, and marmosets (Carter *et al.*, 2015).

From rodents, the most widespread models to study human implantation are mice and rats (Fig. 2A) because of their low cost, easy breeding, short maturation, available genetic information, and the possibility of genetic modifications (Table 2). Although rodent models can be highly informative, the translation and applicability of the findings to humans are under question because of some important differences. Unlike spontaneous decidualization in humans and some non-human primates in mice and other rodents, the presence of an embryo triggers a decidual response (Nakamura *et al.*, 2021, Table 1). Mouse pregnancy is dependent on the corpus luteum for the production of progesterone through the whole gestation, while in humans at the beginning of pregnancy (first trimester) it is the corpus luteum, but after luteolysis the placenta is the main hormone producer (Malassiné *et al.*, 2003). Mice and rats have discoidal hemochorial placenta of the labyrinth type (Malassiné *et al.*, 2003; Burke *et al.*, 2010; Elmore *et al.*, 2022) and the embryo is never entirely embedded

Table 1. Main differences in implantation between humans, primates, and rodents.

	Human	Primates (great apes, old and new world monkeys)	Rodents (mice, rats, guinea pigs, rabbits)	References
Decidualization	Spontaneous cyclic process independent on the embryo	Spontaneous cyclic process independent on the embryo	Dependent on an embryonic stimulus	Dollar *et al.*, 1982 Carter and Pijnenborg, 2011 James, 2014 Carp, 2020 Nakamura *et al.*, 2021
Time and type of implantation	9 days post coitum, Interstitial	9 days post coitum, Interstitial (great apes), centric (cynomolgus, rhesus macaques)	4 days post coitum, interstitial (guinea pigs), centric (rabbits), eccentric (mice, rats)	Lee and DeMayo, 2004 Nakamura *et al.*, 2016 Siriwardena and Boroviak, 2022
Attachment	Polar trophoblast (ICM-adjacent) adheres to and breaches the endometrial epithelium	Polar trophoblast (ICM-adjacent) adheres to and breaches the endometrial epithelium	Mural trophoblast (non-ICM-adjacent) adheres to the endometrial epithelium inducing apoptosis or entosis	Enders *et al.*, 1983 Enders, 1993, 2000 Enders and Lopata, 1999 Carter, 2007
Trophoblast subpopulations	ST, CT and EVT	ST, CT and EVT	Two syncytial and a single mononuclear layer of unknown function, spongiotrophoblasts	Enders, 1989, 1995, 2000 Enders and Blankenship, 1999 Carter, 2007 Carter *et al.*, 2015 Elmore *et al.*, 2022
Large intervillous spaces	Yes	Yes	No, yes (guinea pigs)	Schlafke and Enders, 1975 Enders, 1989
EVT invasion depth	Decidua basalis and the inner 1/3 of the myometrium	Decidua basalis mainly, decidua basalis and the inner 1/3 of the myometrium (chimpanzee)	Decidua basalis mainly, deeper in rats, decidua basalis and the inner 1/3 of the myometrium (guinea pigs)	Enders, 2000 Caluwaerts *et al.*, 2005; Vercruysse *et al.*, 2006 Nakamura *et al.*, 2021
Type of placenta	Hemochorial, villous, discoidal	Hemochorial, villous, discoidal or bi-discoidal (macaques and marmosets)	Hemochorial, labyrinth, discoidal	Myers, 1972 Carter, 2007 Burke *et al.*, 2010 Elmore *et al.*, 2022

EVT, extravillous trophoblast; ICM, inner cellular mass; ST, syncytiotrophoblast; CT, cytotrophoblast.

Table 2. Advantages and disadvantages of the primates and rodents studied as models for human implantation.

	Advantages	Disadvantages	Studies
Primates (great apes, old and new world monkeys)	Decidualization, same or similar type and time of implantation, the same type of placenta	Expensive experiments long life cycle and gestation time Specific housing Low fertility Ethical considerations	Myers, 1972 Enders, 1993, 1995, 2000 Carter and Pijnenborg, 2011; Carter *et al.*, 2015 Schmidt *et al.*, 2015 Nakamura *et al.*, 2016, 2021
Rodents (mice, rats, guinea pigs, rabbits)	Low-cost experiments Easy breeding, short maturation, available genetic information Possibility of genetic modifications	Different decidualization, substantial mechanistic differences in implantation Different trophoblast subpopulations	Schlafke and Enders, 1975 Enders and Schlafke, 1969 Enders, 1989 Caluwaerts *et al.*, 2005 Simmons and Cross, 2005 Cross, 2005 Carter, 2007 Vercruysse *et al.*, 2006 Burke *et al.*, 2010 Ramathal *et al.*, 2010 Aplin and Ruane, 2017 Elmore *et al.*, 2022

(eccentric implantation). Thus, histotrophic nutrition is additionally ensured by the formation of a second yolk sac (Ramathal *et al.*, 2010; Elmore *et al.*, 2022). Moreover, the mouse placenta has three (two syncytial and a single mononuclear layer of unknown function) (Enders and Lopata, 1999). While in the chorionic villi of the human placenta, there is a huge amount of proliferating CT, the labyrinth zone of the mouse placenta does not have an exact such layer. Nevertheless, the labyrinth zone is differentiated from the intensively proliferative polar TE of the ectoplacental cone, a structure specific for mice and rats (Enders and Lopata, 1999; Elmore *et al.*, 2022). Spongiotrophoblasts are the putative mouse counterparts of human CT and mouse trophoblast giant cells and glycogen cells (analogous to human EVT) are their derivatives but never reach the huge invasive capacity of human EVT (Cross, 2005; Simmons and Cross, 2005; Soncin *et al.*, 2018). An EVT invasion depth in mice is restricted to the decidua basalis (Schmidt *et al.*, 2015; Nakamura *et al.*, 2021) while rats show deeper trophoblast invasion, involving both endovascular and

Table 3. *Ex vivo* and *in vitro* models used to study human implantation.

Type	Endometrium: to study decidualization and endometrial receptivity	Reference	Trophoblast: to study CT proliferation, differentiation into EVT or ST, EVT migration and invasion, syncytialization/ fusion, immune interactions (tolerogenic mechanisms), apoptosis.	Reference
Tissue explants	➢ Segments of late-luteal-phase (4–7 days after the LH peak) **Pros:** Closest to the *in vivo/in situ* features, n Production and regulation of the endometrial-derived cytokines, and other bioactive substances **Cons:** Short-term cultures; The orientation of the endometrium is not preserved; Difficult standardization, timing of the biopsies, and specimen handling; *De novo* isolation for each experiment	• Kliman *et al.*, 1990 • Dudley *et al.*, 1992 • Landgren *et al.*, 1996 • Teklenburg and Macklon, 2009 • Bersinger *et al.*, 2010	➢ Segments of the villous part of 5–10 gw human placenta **Pros:** Closest to the *in vivo/in situ* features to study the early stages of placentation (proliferation and differentiation of EVT from the cytotrophoblastic columns in a preserved villous structure **Cons:** Short-term cultures; difficult standardization; restricted access to specimens; d*e novo* isolation for each experiment	• Genbacev *et al.*, 1992 • Vicovac *et al.*, 1995 • Caniggia *et al.*, 2000 • Popovici *et al.*, 2006 • Miller *et al.*, 2005 • Baczyk *et al.*, 2013 • Horii *et al.*, 2016
Primary cells	➢ **Epithelial and stromal endometrial cells**: **Pros:** Closest to the *in vivo/in situ* features; express progesterone-regulated markers of endometrial receptivity **Cons:** Time- and source-consuming isolations; considerable differences in protocols for isolation; variable yields, purity and differentiation; limited viability; constant risk for unwanted spontaneous transformations, difficult standardization and propagation; fresh cell isolates are needed for each experiment	Satyaswaroop *et al.*, 1979 • Bentin-Ley *et al.*, 1995 • Chan *et al.*, 2004 • Teklenburg and Macklon, 2009 • Masuda *et al.*, 2016 • Horii *et al.*, 2016	➢ **Cytotrophoblasts (CT)**: **Pros:** Closest to the *in vivo/in situ* features; dynamic phenotype; Specific upregulation of surface HLA-C and HLA-G upon differentiation; provide combination of protein and non-protein-coding markers for validation of a particular first- trimester trophoblast subpopulation **Cons:** Time- and source-consuming isolations; considerable difference in protocols; Often mixed population; Limited life span, rapidly cease proliferation *in vitro*; Lack of generally accepted minimal criteria for a line to be determined as a pure primary, 1st trimester, human trophoblast cell line; *De novo* isolation for each experiment	• Kliman *et al.*, 1990 • Fisher *et al.*, 1989 • King *et al.*, 2000 • Genbacev *et al.*, 2011 • Lee *et al.*, 2016 • Abbas *et al.*, 2020 • Horii *et al.*, 2020 • Eikmans *et al.*, 2022 • Greenbaum *et al.*, 2023

(continued)

Table 3. (continued)

Type	Endometrium	Reference	Trophoblast	Reference
	Pros: Immortal and easy to propagate **Cons:** Tumor cell features, constant possibility for unwanted spontaneous transformation			
Cancer cells	➢ **HEC-1A/B – luminal epithelium.** **Pros:** Express cytokeratin 13 and 18, and retain estrogen, progesterone and androgen receptors, and CD55 found in implantation. Used as a model for non-receptive endometrium **Cons:** Considerable difference in expression estrogen metabolism. Poor apical adhesiveness due to epithelial polarization	• Mo *et al.*, 2006 • Hannan *et al.*, 2010 • Hevir-Kene and Rižner, 2015	➢ **BeWo – CT** able to differentiate **into EVT or ST.** **Pros:** Express HLA-C and HLA-G mRNA, produce hCG; Adhesiveness to endometrial epithelium. Suitable for syncytialization **Cons:** Near-triploid; HLA-G is not expressed	• Pattillo *et al.*, 1968 • Kovats *et al.*, 1990 • Al-Nasiry *et al.*, 2006 • Bhat and Anderson, 2007 • Hannan *et al.*, 2010 • James *et al.*, 2016 • Rothbauer *et al.*, 2017 • Ban *et al.*, 2020 • Weber *et al.*, 2021 • Li *et al.*, 2023
	➢ **ECC-1 – luminal epithelium.** **Pros:** Well-differentiated, steroid-responsive; MUC secretion. Attachment surface with capacity to acquire a receptive phenotype **Cons:** **N.B.!** The original line has been lost. Currently available ECC-1 cells are shown to be an Ishikawa 3-H-12 derivative, MCF-7 breast cancer cells, or a mixture of both	• Satyaswaroop and Tabibzadeh, 1991 • Mo *et al.*, 2006 • Evans *et al.*, 2020 • Ban *et al.*, 2020	➢ **JEG-3 - CT** able to differentiate **into EVT or ST**. **Pros:** Express HLA-C, HLA-G and secrete HLA-G; adhesiveness to endometrial epithelium. Non- classical cell fusion model for syncytialization **Cons:** Near tetraploid which correlates with HLA-G expression; Limited intercellular membrane fusion	• Kohler and Bridson, 1971 • Kovats *et al.*, 1990 • Apps *et al.*, 2009 • Apps *et al.*, 2011 • Hannan *et al.*, 2006 • Zou *et al.*, 2015 • Poloski *et al.*, 2016 • McConkey *et al.*, 2016 • James *et al.*, 2016 • Rothbauer *et al.*, 2017 • Weber *et al.*, 2021 • Eikmans *et al.*, 2022
	➢ **Ishikawa cells – glandular + luminal epithelium.** **Pros:** Widely considered as good model for a receptive endometrium, normal endometrial function, and endocrine signaling **Cons:** Stable up to 45passages; **N.B.!** Sub clone 3-H-12 has been distributed since 1996 - not deposited in any cell bank	• Nishida,2002 • Castelbaum *et al.*, 1997 • Hannan *et al.*, 2010 • Vergaro *et al.*, 2019	➢ **JAR-CT** able to differentiate **into EVT or ST**. **Pros:** Adhesiveness to endometrial epithelium. Non- classical cell fusion model for syncytialization **Cons:** Express no or minimal amounts of HLA-C and HLA-G and did not secrete sHLA-G; Near- triploid; Limited intercellular membrane fusion	• Kovats *et al.*, 1990 • Heneweer *et al.*, 2005 • Al-Nasiry *et al.*, 2006 • Apps *et al.*, 2009 • Apps *et al.*, 2011 • Hannan *et al.*, 2010 • James *et al.*, 2016 • Rothbauer *et al.*, 2017 • Weber *et al.*, 2021
	➢ **RL95-2—glandular epithelium.** **Pros:** Receptive glandular epithelial cells with lack of epithelial polarization; strong tendency to accumulate and to form gland-like structures **Cons:** The line is trisomic 8 and has an 8% frequency of polyploidization	• Way *et al.*, 1983 • Hannan *et al.*, 2010 • Evans *et al.*, 2020 • Ban *et al.*, 2020 • John *et al.*, 1993	➢ **AC1M-88—EVT to model EVT adhesion and migration** **Pros:** Adhesiveness to endometrial epithelium; constitutively express HLA-G mRNA **Cons:** The trophoblast cells are from the chorion leave- the non-villous part of the chorion that is not part of the placenta	• Frank *et al.*, 2000 • King *et al.*, 2000 • Hannan *et al.*, 2010

(continued)

Table 3. (continued)

Type	Endometrium	Reference	Trophoblast	Reference
Epithelial and stromal endometrial cells **Pros:** Derived from normal early pregnancy endometrium; easy to propagate; Healthy placental cell features; signs of preserved stemness **Cons:** Immortalization includes viral transduction or plasmid transfection that may alter initial cell features; sourced from different cycle time points or women in menopause				
Immortalized cell lines	➢ **EM-E6/E7/TERT (epithelial, glandular)** **Pros:** Retain the natural characteristics of endometrial glands **Cons:** Preferential use for cancer research	• Kyo *et al.*, 2003 • Fitzgerald *et al.*, 2021	➢ **HESC (stromal)** **Pros:** Typical stroma morphology, karyotype and phenotype, similar reaction to treatment with ovarian steroids, retain the ability to undergo decidualization **Cons:** Not clear if the decidualization is sustainable	• Krikun *et al.*, 2004 • Holmberg *et al.*, 2012 • You *et al.*, 2019; You *et al.*, 2021; You *et al.*, 2023 • Ban *et al.*, 2020
Trophoblast cell lines **Pros:** Derived from normal early pregnancy primary trophoblast; easy to propagate; healthy placental cell features; signs of preserved stemness **Cons:** Immortalization includes viral transduction or plasmid transfection that may alter initial cell features; sourced from an advanced stage of trophoblasts differentiation than at implantation.				
Immortalized cell lines	➢ **HTR-8/SVneo - CT** able to differentiate *in vitro* to **EVT** **Pros:** CK7 positive, secrete hCG. non-tumorigenic into nude mice. Express stemness-associated factors; Can express HLA-A, HLA-B and HLA- class II molecules upon IFN-γ induction **Cons:** Mixed population of CK7+ trophoblasts and Vim+ mesenchymal cells; Near triploid; may not be suitable for the study of intrauterine infection	• Graham *et al.*, 1993 • King *et al.*, 2000 • Paiva *et al.*, 2009 • Apps *et al.*, 2009 • Weber *et al.*, 2013 • Weber *et al.*, 2021 • Zou *et al.*, 2015 • James *et al.*, 2016 • Abou-Kheir *et al.*, 2017 • Eikmans *et al.*, 2022 • Sheridan *et al.*, 2022	➢ **Swan71 (Sw71) - EVT** phenotype, migration and invasion models, immune interactions, and apoptosis **Pros:** Expression of trophoblast markers, cytokines and growth factors, migration, and invasion abilities close to the primary trophoblasts. Preserved stemness; hybrid CK7+/Vim+, HLA- C+/HLA-G+ phenotype like primary EVT, Secrete fetal fibronectin and low levels of hCG; also retain the ability to fuse spontaneously. can express HLA-A and HLA-B, and HLA- class II molecules upon IFN-γ induction **Cons:** Some authors reported no HLA-G or CK7 expression; near pentaploid	• Straszewski-Chavez *et al.*, 2009 • Apps *et al.*, 2009 • Fraccaroli *et al.*, 2009 • Holmberg *et al.*, 2012 • Racicot *et al.*, 2014 • Reiter *et al.*, 2017 • You *et al.*, 2019 • You *et al.*, 2021 • You *et al.*, 2023 • Guzman-Genuino *et al.*, 2019 • Ban *et al.*, 2020 • Alexandrova *et al.*, 2023 • Alexandrova *et al.*, 2022b • Pastuschek *et al.*, 2021 • Dietrich *et al.*, 2023
	➢ **HChEpC1b (EVT)—EVT function, autophagy as a protective mechanism** **Pros:** Derived from normal early pregnancy (7gw) placenta. CK7, HLA-G, and CD9 positive. Vimentin positive. Non-tumorigenic in nude mice. Near-diploid karyotype **Cons:** Difficult to determine the origin	• Omi *et al.*, 2009 • Takahashi *et al.*, 2017 • Nakashima *et al.*, 2019	➢ **SGHPL-4 (MC-4)—EVT function and apoptosis, phagocytic activity.** **Pros:** Derived from early pregnancy primary EVT; constitutively express HLA-G mRNA; secrete hCG and express CK7 when grown on collagen, fibronectin, and gelatin; amenable to stable genetic manipulation **Cons:** Rather long-lived than immortal	• Choy and Manyonda, 1998 • Cartwright *et al.*, 1999 • Cartwright *et al.*, 2002 • McCormick *et al.*, 2009 • Hannan *et al.*, 2010 • James *et al.*, 2016

(continued)

Table 3. (continued)

Type	Trophoblast cell lines			
Immortalized cell lines	➢ **TEV-1 (EVT) - EVT function and immune interactions** **Pros:** CK7, HLA-G and CD9 positive; non-tumorigenic in nude mice; near-diploid karyotype; can express HLA-A and HLA-B and HLA-class II molecules upon IFN-γ induction **Cons:** Not reported	• Feng *et al.*, 2005 • Chen and Zou, 2008 • Apps *et al.*, 2009 • Wang *et al.*, 2011 • Wong *et al.*, 2013	➢ **HIPEC 65 (EVT) – EVT function** **Pros:** Derived from normal first-trimester primary EVT. CK7, HLA-G, and CD9 positive **Cons:** A very weak expression of HLA-G and loss of CK7 expression on ongoing passages, no secretion of hCG	• Pavan *et al.*, 2003 • Handschuh *et al.*, 2007
	➢ **B6Tert-1 (VT (CT) to EVT)—CT** able to differentiate *in vitro* to EVT (**EVT function**) or ST (**cell fusion**). **Pros:** Non-tumorigenic in nude mice; produce various biomarkers of normal EVT during the early weeks of gestation; ability for differentiation towards syncytium **Cons:** EVT differentiation requires EGF supplemented, FCS-free medium	• Wang *et al.*, 2006 • Fu *et al.*, 2010 • Li *et al.*, 2021	➢ **ACH-3P (VT (CT) to EVT)—EVT differentiation and function** in terms of autocrine and paracrine regulation. Derived after fusion of normal first-trim trophoblast with choriocarcinoma cells **Pros:** Expression of trophoblast markers, invasion abilities and transcriptome close to the primary trophoblasts **Cons:** Morphology, cytogenetics, and doubling time as of the parental cancer cells. Mixed population of villous and EVT; Near tetraploid	• Hiden *et al.*, 2007 • Rothbauer *et al.*, 2017 • Ban *et al.*, 2020 • Pastuschek *et al.*, 2021 • Weber *et al.*, 2021

EVT, extravillous trophoblast; ST, syncytiotrophoblast; CT, cytotrophoblast; hCG, human chriongonadotropin; CK7, cytokeratin 7; IFN-γ, interferon gamma; EGF, epidermal growth factor; FCS, fetal calf serum.

Table 4. Assembled models to recapitulate maternal–fetal interface during human implantation and early placentation.

Pros: Closest similarity to maternal–fetal contact during human implantation and early placentation. Suitable to study the ultrastructure of trophoblast-endometrial contact, breaching of the endometrial epithelium, stromal migration/invasion
Cons: The same as of the sourced cells/tissues, hard to find human embryos for research, require high expertise and expensive resources

Phase	Endometrium	+	Trophoblast	Reference
Apposition, adhesion, attachment	Endometrial explants of late-luteal-phase (4–7 days after the LH peak)	+	First/second/third-trimester primary trophoblast cells	• Kliman *et al.*, 1990 Endometrial receptivity, trophoblast adhesion to the stroma, ultrastructure of trophoblast-endometrial interaction.
		+	IVF embryos	Landgren *et al.*, 1996 Embryo penetration of the endometrial epithelium and stroma invasion
	Primary human endometrial epithelial cells in monolayer	+	IVF embryos	• Simon *et al.*, 1998; Meseguer *et al.*, 2001; Caballero-Campo *et al.*, 2002; Dominguez *et al.*, 2010 Endometrial epithelium receptivity, trophoblast differentiation • Galán *et al.*, 2000 Apoptosis of the endometrial epithelial cells upon contact with the embryo-derived TE during apposition and adhesion • Evans *et al.*, 2020 Adhesion and differentiation of the TE upon contact to the primary endometrial epithelial cells
	Primary human endometrial epithelial cells in monolayer	+	Blastoids	• Kagawa *et al.*, 2022 Endometrial receptivity and epiblast-induced local maturation of the polar TE, endowing blastoids with the capacity to directionally attach to hormonally stimulated endometrial cells as during implantation
	HEC-1A cells	+	JAR or JEG-3 spheroids	• John *et al.*, 1993 Attachment of spheroid to uterine epithelial cell monolayer • Thie and Denker, 2002 Adhesiveness of endometrial epithelial cells
		+	Mouse embryo	• Martin *et al.*, 2000 Adhesiveness of endometrial epithelial cells
	ECC-1 cells	+	Sw71 BLS	• Holmberg *et al.*, 2012 Attachment of spheroids to endometrial epithelial cells with acquired receptive phenotype
		+	Stem cell-derived spheroids	Evans *et al.*, 2020

(continued)

Table 4. (continued)

Pros: Closest similarity to maternal–fetal contact during human implantation and early placentation. Suitable to study the ultrastructure of trophoblast-endometrial contact, breaching of the endometrial epithelium, stromal migration/invasion
Cons: The same as of the sourced cells/tissues, hard to find human embryos for research, require high expertise and expensive resources

Phase	Endometrium	+	Trophoblast	Reference
				Adhesion of the spheroids to the endometrial epithelial cell lines
Apposition, adhesion, attachment	Ishikawa cells	+	JAR or JEG-3 spheroids	• Vergaro *et al.*, 2019 Transcriptomic analysis of the interaction of the trophoblasts with receptive vs. non-receptive (HEC-1A) endometrial epithelium cell lines • Evans *et al.*, 2020 Adhesion of the spheroids to endometrial epithelial cell lines
		+	IVF embryos or stem cell-derived spheroids	• Ruane *et al.*, 2020, 2022 Human embryo breaching of the epithelium and the gene networks involved in implantation
	RL95-2 cells	+	BeWo, JEG-3 or JAR spheroids	• Thie and Denker, 2002 Thie *et al.*, 1998 Adhesiveness of the apical plasma membrane of endometrial epithelial cell lines • Hohn *et al.*, 2000 The relationship between the adhesion of the trophoblast to epithelium and its differentiation • Heneweer *et al.*, 2005 Modulation of epithelial adhesiveness and apicobasal polarity by contact with JAR spheroids • Ho *et al.*, 2012 A high-throughput *in vitro* model for fluorometric assessment of spheroid attachment to human endometrial epithelial cells
	hTERT-EEC cells	+	JAR spheroids	• Aboussahoud *et al.*, 2010 Trophoblast attachment
	Matrix-embedded epithelial and stromal cells (EMO)	+	IVF embryos	• Bentin-Ley *et al.*, 2000 Ultrastructure of human blastocyst–endometrial interactions during attachment and invasion showing a displacement of endometrial epithelial cells, and formation of a penetration cone with no endometrial or trophoblastic degeneration. Epithelial penetration was achieved primarily by cellular syncytiotrophoblast-like cells, developed simultaneously with penetration of the epithelium • Lalitkumar *et al.*, 2007 Endometrial receptivity and TE attachment • Rawlings *et al.*, 2021 Single-cell transcriptomics of differentiated and senescent subpopulations in both glands and stroma and role of the senescence in glandular epithelium and in the stroma on the implantation.
		+	JAR spheroids	• Wang *et al.*, 2012 Attachment of human trophoblast spheroids to a 3D endometrium-like culture system with overall architecture, similar to that seen in endometrial tissue
	Apical-out (AO)–EMO	+	IVF embryos or blastoids	• Shibata *et al.*, 2024 Model with exposed apical epithelium surface, dense stromal cells, and a self-formed endothelial network to recapitulate apposition, adhesion, and Syn invasion.
	OFEL, open-faced endometrial layer from EMO	+	Blastoids	• Kagawa *et al.*, 2022 Attachment on the receptive endometrial epithelium, epiblast-induced local maturation of the polar TE endows blastoid with the capacity to directionally attach to hormonally stimulated endometrial cells

(continued)

Table 4. (continued)

Phase	Endometrium	+	Trophoblast	Reference
Pros: Closest similarity to maternal–fetal contact during human implantation and early placentation. Suitable to study the ultrastructure of trophoblast-endometrial contact, breaching of the endometrial epithelium, stromal migration/invasion				
Cons: The same as of the sourced cells/tissues, hard to find human embryos for research, require high expertise and expensive resources				
Invasion, stromal endometrial receptivity	Primary human endometrial stromal cells monolayer	+	AC-1M88 cells or spheroids	• Gellersen *et al.*, 2010 The invasiveness of human endometrial stromal cells promoted by decidualization and by trophoblast-derived signals, by encapsulation of the conceptus the decidua supports trophoblast invasion. • Weimar *et al.*, 2012 DSC of women with recurrent miscarriage have higher migratory response to trophoblast • Gonzalez *et al.*, 2011 DSC supports trophoblast invasion by paracrine signals
		+	First trim villous trophoblasts	• Vicovac *et al.*, 1995 Trophoblast–stroma interaction, differentiation of trophoblasts
		+	Mouse embryos	• Hanashi *et al.*, 2003 Human decidual b1-integrin and focal adhesion kinase participate in the trophoblast invasion • Grewal *et al.*, 2010 Rho GTPase-PTK2-dependent remodeling of the endometrial stromal cell compartment may be critical for successful embryo implantation
		+	IVF embryos	• Weimar *et al.*, 2012 DSC of fertile women discriminate between high- and low-quality embryos unlike women with recurrent miscarriage • Carver *et al.*, 2003 Trophoblast attachment to endometrial stroma and further implantation (invasion and migration of EVT) of the human embryo into the endometrial stroma • Teklenburg *et al.*, 2012 Lineage development and epi- and hypoblast segregation, a lineage-specific inactivation of parental X chromosome
	Decidua parietalis explants	+	First-trimester villous trophoblast explants	• Dunk *et al.*, 2003 Trophoblast-mediated decidual blood vessel remodeling • Hazan *et al.*, 2010 Trophoblast-mediated decidual blood vessel remodeling and role of uNK cells and macrophages
	Proliferative-phase endometrial stromal cells	+		• Popovici *et al.*, 2006 Trophoblasts attachment and gene expression of the endometrial stromal cells during implantation
	HEC-1A spheroids	+	AC-1M88 cells	• Buck *et al.*, 2015, 2021 Model system for trophoblast invasion into gland-like endometrial spheroids
	Ishikawa spheroids	+		
	RL95-2 spheroids	+		
	HESC	+	Sw71 trophoblast spheroids	• Holmberg *et al.*, 2012 Trophoblast migration into endometrial stroma • You *et al.*, 2019, 2021, 2023 Trophoblast invasion and migration into endometrial stroma
	Spiral arteries' segments from myometrial biopsies	+	Primary trophoblasts or SGHPL-4 cells	• Cartwright *et al.*, 2002 To study interstitial invasion and endovascular invasion

IVF, *in vitro* fertilization; LH, luteinizing hormone; BLS, blastocyst-like structure; EMO, endometrial organoids; DSC, decidual stromal cells.

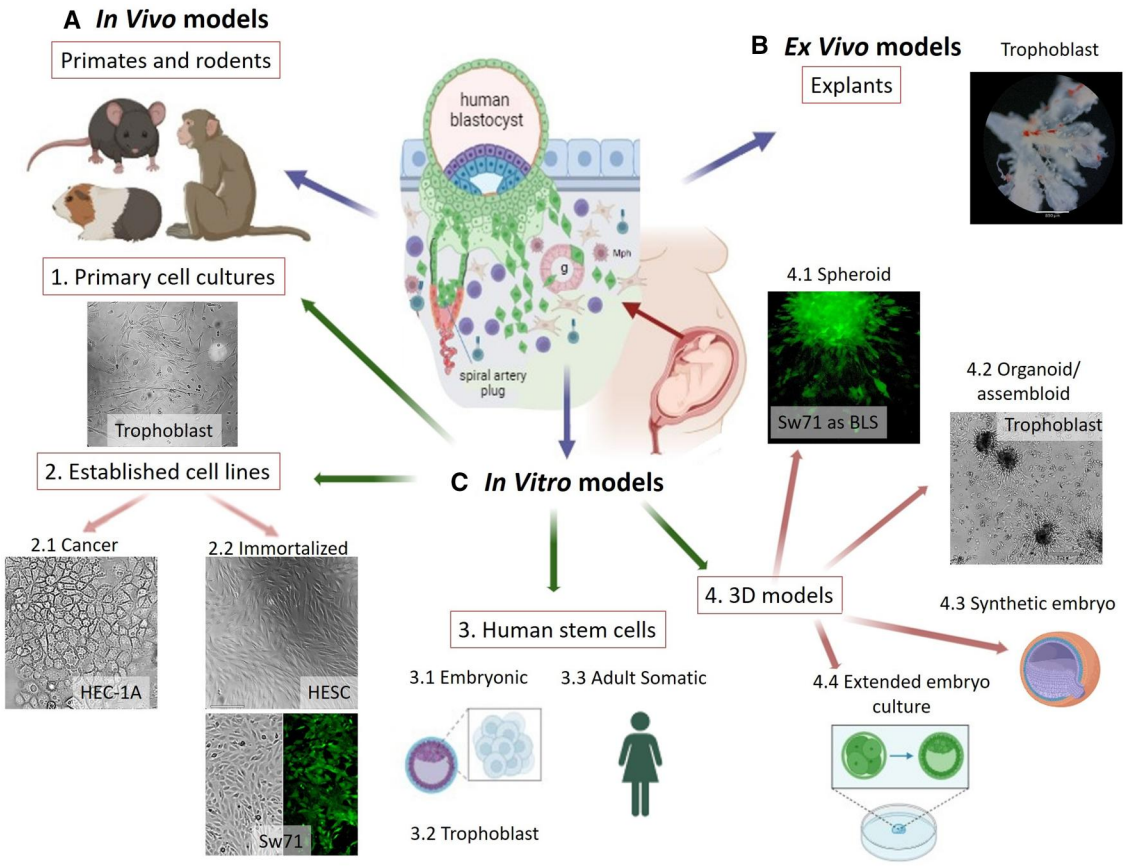


Figure 2. Modeling of human implantation. The range of *in vivo*, *ex vivo*, and conventional (2D) and advanced (3D) *in vitro* models, used to recapitulate trophoblast or/and decidua behavior in the peri-implantation period. (**A**) *In vivo* (animal) models: primates and rodents. (**B**) *Ex vivo* models: placental explants, representing the fetal part of the placenta. (**C**) *In vitro* conventional (2D) models: (1) primary cells (isolated from the maternal or fetal part of the placenta); (2) cancer (2.1) and immortalized (2.2) cell lines of maternal or fetal origin); (3) stem cells; (4) advanced 3D models: 4.1. spheroids of maternal or fetal origin, 4.2. self-organized organoids or assembloids, 4.3. synthetic embryos (blastula/gastrula) and 4.4. extended embryo culture. The scheme is created on Biorender.com. The microscope images are the authors' property and have not been published before.

interstitial invasion (Caluwaerts *et al.*, 2005; Vercruysse *et al.*, 2006). In guinea pigs, the invasion involves decidua basalis and the inner third of the myometrium like in humans, thus, it is the only rodent model for intervillous space formation (Schlafke and Enders, 1975; Enders, 1989).

Because of the short-term pregnancy and fast apposition, attachment, and invasion of the luminal epithelium, the mice and rats cannot model the mechanisms of early implantation in humans. However, they are a good model for studying decidualization because the decidual response can be elicited without trophoblastic attachment (Lee and DeMayo, 2004), highlighting the potential other factors, such as hormonal or environmental stimuli, as triggers of the decidual response. Of note, guinea pigs might serve as an efficient model for apposition and attachment, trans-epithelial penetration, and deep endovascular invasion but there are other limitations such as longer estrous and hidden implantation sites (Hennessy, 2014). Although many substantial differences between mouse and human implantation, gene ablation techniques in the mouse have proven to be a powerful tool in elucidating gene function during implantation (Table 2). For instance, the key transcriptional regulators of human TE specification and development (Cdx2, Tead4, and Gcm1) were first identified in mice (Baczyk *et al.*, 2013; Blakeley *et al.*, 2015).

In summary, although the experiments on animals are controversial due to a deficiency in cross-species extrapolation, the primates and rodents are valuable *in vivo* models for human implantation research and have already shed some light on the 'black box' of human implantation. As early human implantation stages are not accessible, non-human primate models are imperative for our understanding of embryo implantation. The comprehensive analysis of the expressed genes in mouse, monkey and human embryos is important for understanding trophoblast specification. It must be taken into account that human TE establishment differs in terms of the expression of some crucial genes during early development (Soncin *et al.*, 2018; Hemberger *et al.*, 2020; Shibata *et al.*, 2020). The formation of the human blastocyst is regulated differently as well (Niakan and Eggan, 2013; Rossant and Tam, 2018). The difference of central importance, however, is the deep interstitial, glandular, and endovascular invasion of EVT into the inner third of the human myometrium which is extremely important for the survival of the fetus. This invasion depth is accompanied by a range of human-specific factors and is responsible for the difference in the supplied blood volume. We accept the statement of Schmidt *et al.* in their review from 2015, that 'only humans have human placentas' and consequently, an animal model that exactly reflects human placentation does not exist (Schmidt *et al.*, 2015).

Ex vivo models: endometrial and trophoblast explants

The maternal–fetal crosstalk during human implantation involves the emergence of different, constantly changing

decidual and trophoblast cell phenotypes (Hannan *et al.*, 2010). These two principal compartments should be present in any model mimicking human implantation as a whole. However, some scientists rely on *in vitro* culture or full reconstruction of the 'pregnant' endometrium, while others rely on *de novo* construction of blastocyst-like models from explanted gestational tissue, stem cells, or cell lines. Since human endometrial tissue is often biopsied due to abdominal or menstrual pain or infertility, these endometrial explant cultures have been used as decidualization models for many years (Bersinger *et al.*, 2010) (Table 3). The idea for endometrium culture emerged in the late 1960s (Csermely *et al.*, 1969; Schatz *et al.*, 1985), and an endometrial explant system was described in the early 1990s by Dudley *et al.* (1992) where segments of late-luteal-phase endometrium were obtained aseptically, cut into small pieces, and cultured for a few days. These explants remained viable and responsive for about 5 days. Although useful in the study of the production of cytokines and other bioactive substances, the orientation of the endometrium in this model is not preserved and thus the investigation of the blastocyst-endometrium interactions cannot be guaranteed (Lindenberg *et al.*, 1989; Kliman *et al.*, 1990; Dudley *et al.*, 1992).

By using this model, it has been shown that the addition of progesterone initiates decidualization and prolongs the survival of the primary endometrial stromal cells of the explant (Bersinger *et al.*, 2010) (Table 3). Nevertheless, a later detailed study pointed out that human endometrial explants are a complex model due to limited viability and difficult standardization (Schäfer *et al.*, 2011; Teklenburg and Macklon, 2009).

Human trophoblast explants are valuable *ex vivo* models for the fetal part of the embryo-endometrial interaction that mimics the *in vivo* situation as closely as possible (Aghajanova, 2020). Table 3 shows the main reports using villus placenta explants as a model of CT proliferation and bi-potency differentiation. Genbacev *et al.* (1992) described a model to study the differentiation of EVT through a culture of first and second-trimester human chorionic villi explants on Matrigel and proved that the EVTs differentiation was restricted to first-trimester villous tissue. Accumulating data noted the great importance of the trophoblasts explants, containing both anchoring and floating first-trimester chorionic villi to study early stages of placentation like the reconstitution of cell columns and CT to EVT differentiation *in vitro* (Vicovac *et al.*, 1995; Caniggia *et al.*, 2000; Miller *et al.*, 2005; Popovici *et al.*, 2006; Baczyk *et al.*, 2013) (Table 3). Previous results from our laboratory showed that after 48–72 h in culture, near the villous explants abundant cytotrophoblast clusters containing HLA-C positive EVTs emerged. Explants remained vital for at least a week (Alexandrova *et al.*, 2022b) (Fig. 2B–1).

In summary, although the placental explants closely reflect peri-implantation placental development, they are short-term cultures, and difficult to standardize. Of note, the access to tissues from elective terminations of pregnancy is often limited by ethical and/or legal constraints. All the pros and cons of explants and extended culture of human embryos as *ex vivo* models of human implantation are summarized in Table 3.

In vitro models: primary cells, cell lines, stem cells, and 3D models

Most of the models currently being used do not recreate the entire implantation process but rather certain steps of implantation or implantation-related events. These include the conventional 2D mode of cultured endometrial or trophoblast cells or their 3D derivatives such as spheroids and organoids. The endometrial or trophoblast cells can be sourced either from early pregnancy gestational tissues or established cancerous, normal immortalized cells and stem cells. Table 3 lists the available endometrial and trophoblast primary and established cell lines used as models in the studies of decidualization and receptivity of human endometrium as well as of apposition, attachment, and invasion phases of implantation, respectively, alongside their pros and cons.

Primary endometrial (epithelial and stromal) and trophoblast cell cultures

Early pregnancy-derived gestational tissues allow the isolation of relatively pure primary cell cultures to model either endometrial receptivity status near implantation (Satyaswaroop *et al.*, 1979; Bentin-Ley *et al.*, 1995; Chan *et al.*, 2004; Masuda *et al.*, 2016) or early differentiation and function of ready-to-implant trophoblasts (Kliman *et al.*, 1986; Fisher *et al.*, 1989; Lee at al., 2016) (Fig. 2C–1, Table 3). Of note, primary endometrial epithelial cells fall into senescence within 2 weeks when cultured on plastic dishes (Kyo *et al.*, 2003). Moreover, primary endometrial (epithelial and stromal) cells showed reduced biological activities and diminished response towards sex hormones after several passages. Therefore, results could not be directly extrapolated to the *in vivo* situation (Li *et al.*, 2023). The same is valid for the primary trophoblasts. CT after isolation could be differentiated into EVT or ST but due to their primary nature and limited life span, rapidly cease proliferation *in vitro* (Genbacev *et al.*, 2011; Horii *et al.*, 2020). Another important aspect is that the nature and purity of isolated populations must be confirmed with a panel of phenotypic markers. Unfortunately, the lack of generally accepted minimal criteria for a line to be determined as a pure primary, first-trimester, human trophoblast cell line obscures the trophoblast research. This is determined partly by the preserved stemness of these cells and their dynamic phenotype (Lee *et al.*, 2016; West *et al.*, 2019; Abbas *et al.*, 2020; Greenbaum *et al.*, 2023). For example, the relatively specific trophoblast markers such as hCG, hPL, HLA-G, and cytokeratin-7 (CK-7) are also expressed by a range of malignant cells (Loke, 1978; Heyderman *et al.*, 1985; Cabestre *et al.*, 1999; Real *et al.*, 1999). Lack of Vimentin (Vim) is mostly considered normal for the trophoblast lineage, but it is expressed to varying extents by EVT in EMT or vice versa (MET, mesenchymal-epithelial transition) (Loke, 1988; Aboagye-Mathiesen *et al.*, 1996; Alexandrova *et al.*, 2023). While the HLA class I expression pattern is null in villous trophoblast and ST, the EVTs are HLA-G+ and HLA-C+ (Papúchová *et al.*, 2019; Salvany-Celades *et al.*, 2019; Eikmans *et al.*, 2022; Alexandrova *et al.*, 2023). Eikmans *et al.* (2022) published a detailed characterization of HLA profiles and immune cell interactions of their primary trophoblast cultures. For trophoblast isolation, they used enzymatic digestion of first-trimester placental tissue (6–9 gw) and subsequent Percoll gradient separation and trophoblast enrichment by magnetic bead retraction. These primary trophoblasts were easily maintained for several passages with upregulated trophoblast markers (GATA3, TFAP2C, chromosome-19 microRNAs). Cultured CT was differentiated into EVT in a Matrigel-containing medium expressing HLA-C and HLA-G with HLA-G1 predominance and secreting soluble HLA-G. The CT fraction abundantly secreted the cytokines TNF-α and IL-8, which levels were minimal in EVT cultures. In their hands, 3 days of co-culturing of EVT and peripheral immune cells led only to a decreased HLA-DR expression on CD4 T cells (Eikmans *et al.*, 2022). The EVT-conditioned media alone had no such effect, while other authors reported the same effect but with conditioned media from first-trimester placental explants (Svensson-Arvelund *et al.*, 2015). The EVT-conditioned medium is composed 1:1 mixture of EVT medium (DMEM/F-12 supplemented with 0.1 mM 2-mercaptoethanol, 0.5%

Penicillin-Streptomycin, 0.3% BSA, 1% ITS-X supplement, 7.5 μM A 83-01, 2.5 μM Y-27632, 100 ng/ml of NRG1, and 4% KnockOut Serum Replacement) (Okae *et al.*, 2018) with RPMI medium with supplements. In our hands, the trophoblasts, gained through enzymatic digestion and subsequent Percoll gradient separation (to reduce fibroblast contamination) (Stenqvist *et al.*, 2008), showed a tendency to form CT clusters with varying morphology and ST-like fragments abundant in the mixture. The cell passaging led CT clusters to self-organization of spheroid-like structures. The EVTs expanded from such structures showed preserved strong migration capacity and HLA-G+/HLA-C+ profile (Alexandrova *et al.*, 2023). New technologies constantly add novel markers corresponding to the dynamics of trophoblast differentiation during peri-implantation (West *et al.*, 2019; Greenbaum *et al.*, 2023).

In summary, many studies utilize primary isolated and cultured endometrial epithelial and/or stromal cells, and human trophoblasts as simple monolayer models or as a part of more complex 3D constructs or assembloids to study human implantation (discussed later). Although they closely represent the *in vivo* or *in situ* features, the isolations are time- and source-consuming with unpredictable outcomes (variable yields, purity, differentiation). Fresh cell isolates are needed for each new experiment to keep the initial characteristics that obscure their standardization. In addition, the primary cells are difficult to propagate in long-term culture.

Endometrial (epithelial and stromal) and trophoblast cell lines: cancer and immortalized cells

The limitations of primary cultures have forced researchers to turn towards the use of established cancer (Hannan *et al.*, 2010) and immortalized non-cancer human cell lines, of which multiple have been generated over the years (Horii *et al.*, 2020) (Fig. 2C-2, Table 3). Since cancer cell lines are relatively easy to obtain and maintain (Mirabelli *et al.*, 2019), it is not surprising that most endometrial epithelial cell lines originate from endometrial adenocarcinomas. For example, the HEC-1A cells widely used for endometrium research were isolated from a well-defined human endometrial adenocarcinoma (Kurarmoto *et al.*, 2002; Hannan *et al.*, 2010) (Fig. 2C-2.1, Table 3). The ECC-1 line closely represents luminal epithelial cells since expresses CK-13 and -18, and retains estrogen, progesterone, and androgen receptors, while HES cells express the adhesion molecule mucin1 (MUC1) and have embryotrophic potential (Satyaswaroop and Tabibzadeh 1991; Mo *et al.*, 2006; Ban *et al.*, 2020; Evans *et al.*, 2020). MUC1-expressing Ishikawa cells (mixed glandular and luminal epithelium characteristics) are considered a good model for endometrium receptivity (Castelbaum *et al.*, 1997; Nishida, 2002). The epithelioid RL95-2 cells, isolated from moderately differentiated adenosquamous carcinoma, are generally used as a model for receptive glandular epithelial cells, as these are highly adhesive to trophoblast cell lines (Way *et al.*, 1983; John *et al.*, 1993).

Trophoblast-derived choriocarcinoma cell lines have been used extensively as an alternative to primarily trophoblasts for elucidation of the biology and functionality, and to model the early placental formation/development (King *et al.*, 2000; Hannan *et al.*, 2010) (Table 3). By the year 2000, there was already data from 14 cell lines originating from malignant tissue and five cell lines from embryonal carcinomas which have evidence of trophoblast differentiation (King *et al.*, 2000). Of them, BeWo (1968), JAR (1971), and JEG-3 (1971) are still popular trophoblast model systems and are widely used to study *in vitro* trophoblast cell fusion, migration, and invasion (Table 3). BeWo and JEG-3 cells are capable of fusing to form ST, retaining the ability to secrete specific hormones, and are used to study syncytialization, adhesion, proliferation, and early placental endocrine function (Zhou *et al.*, 2021), whereas JAR is mostly used to model differentiation of CT to EVT (Table 3).

It is important to mention that both the choriocarcinoma cell lines and primary trophoblastic cells are already at a later developmental stage than TE/trophoblasts in implantation and thus, do not perfectly recapitulate the multipotent early trophoblasts. Choriocarcinoma cells show an abnormal chromosome number, a substantially different transcriptomic profile from EVT, and genome-wide DNA methylation patterns that are different from primary trophoblast (Abbas *et al.*, 2020; Nikitina and Lebedev, 2022). Moreover, cancer cells have HLA status differing considerably from primary tissues (Bilban *et al.*, 2010). The advantages and disadvantages of cancer cell lines are highlighted in Table 3.

The use of immortalized endometrial cell lines in implantation models is not common. Researchers prefer to use instead endometrial biopsies containing both epithelial and stromal cells for a short time to represent the receptive or non-receptive human endometrium (Fitzgerald *et al.*, 2021). However, Desai *et al.* (1994) generated spontaneously immortalized human endometrial epithelial cell line with excellent embryotrophic potential for mice embryos (Desai *et al.*, 1994). Human endometrial glandular EM-E6/E7/TERT cells were established in 2003 as useful tools for various experimental models including the implantation model. EM-E6/E7/TERT cells maintain responsiveness to sex steroids, such as estrogen and progesterone, lack a transformed phenotype, and have an unlimited life span (Kyo *et al.*, 2003). Krikun *et al.* (2004) created a new immortalized human endometrial stromal cell line (HESC) with a normal progestational response and no clonal chromosomal structural or numerical abnormalities (Fig. 2C-2.2, Table 3). Barbier *et al.* (2005) elaborated the stromal cell line SHT290 used as a model for endometrium decidualization. This cell line is similar to the parental strain regarding proliferation and karyotype as well as basal gene expression (Barbier *et al.*, 2005).

Nowadays, several immortalized trophoblast cell lines derived from healthy human placentas are in use (Table 3). These were created from first-trimester CT/EVTs by genetic manipulation, virus transfection, or fusion with choriocarcinoma cells. These include B6-TERT1 (Rong-Hao *et al.*, 1996; Wang *et al.*, 2006), HTR8/SVneo (Graham *et al.*, 1993), HPT-8 and its HBV transfected variant (Zhang *et al.*, 2011), ACH-3P (Hiden *et al.*, 2007), HChEpC1b (Omi *et al.*, 2009), Swan 71 (Straszewski-Chavez *et al.*, 2009), TEV-1 (Feng *et al.*, 2005), and HIPEC65 (Pavan *et al.*, 2003). Of them, the HIPEC65 cells that do not endogenously secrete hCG and express the luteinizing hormone/choriongonadotropin receptor were used to study the effect of hCG on the invasion process *in vitro* (Handschuh *et al.*, 2007). The HTR-8/SVneo cell line was widely used to study trophoblast cell fusion, migration, and invasion (Bačenková *et al.*, 2022) (Table 3). It was developed from primary EVT and interestingly expresses the pluripotency markers OCT4 and NANOG. Such expression of stemness-associated factors is a sign of self-renewal and repopulation activity of these cells (Weber *et al.*, 2013). However, some authors question their homogeneity (Abou-Kheir *et al.*, 2017) raising doubt about whether they are truly representative of either villous or extravillous normal trophoblast (Abbas *et al.*, 2020). Interesting data from Abbas *et al.* (2020) on first-trimester trophoblast models used in human implantation research between 2015 and 2020 showed that from 1044 studies only 76 used primary human EVT and another 629 used the cancer JEG-3, BeWo, or JAR cell lines. The other 339 studies used the immortalized HTR-8/Svneo and Swan-71 (Sw71)

pointing out their accumulating popularity. Of note, the Sw71 line was from the newest established immortalized normal human trophoblast cell lines explaining its still limited usage (only 31 of the listed studies) (Abbas *et al.*, 2020). Sw71 cells exhibit EVT characteristics, preserve stemness, and an ability to attach to the endometrium, migrate, and invade in ECM and between endometrial stromal cells (Straszewski-Chavez *et al.*, 2009; Holmberg *et al.*, 2012; Hackmon *et al.*, 2017; You *et al.*, 2019; Alexandrova *et al.*, 2022b) (Fig. 2C-2.2). This cell line is amenable to gene editing, and a gene-specific knockout clone targeting the TWIST1 gene, which is involved in the regulation of mesenchymal differentiation and cell motility was recently created (You *et al.*, 2023). This line was established more than 10 years ago and has been used in several models over the years to test different factors crucial for human implantation. For example, it was used to test EVT invasion properties (You *et al.*, 2021; Alexandrova *et al.*, 2022), the impact of maternal–fetal immune interactions on trophoblast migration and invasion as well as the reciprocal effect of Sw71 cells on the degranulation of the cytotoxic immune cells (Racicot *et al.*, 2014; Grasso *et al.*, 2018). The Sw71 line was successfully validated by primary trophoblast in the context of their hybrid phenotype (CK7+/Vim+) and HLA pattern (HLA-G+/HLA-C+) (Straszewski-Chavez *et al.*, 2009; Alexandrova *et al.*, 2023) unlike cancer trophoblast cell lines JEG-3 and JAR which do not specifically upregulate HLA-C and HLA-G on their surface (Eikmans *et al.*, 2022). On the contrary, some authors reported no HLA-G or CK7 expression by Sw71 cell line (Apps *et al.*, 2009; Pastuschek *et al.*, 2021; Dietrich *et al.*, 2023).

In summary, while carcinoma cells are easy to culture, there are many limitations including their nature of being prone to spontaneous transformation and their lack of decent tissue characteristics. Therefore, it is not surprising that parallel research with immortalized, non-cancer human endometrial and trophoblast cell lines has been done over the years. Altogether, monolayer cell cultures from human immortalized endometrium and trophoblast are an important part of the implantation research since aspects of the very early microenvironment during peri-implantation and early placentation can be investigated at the level of a single cell type. The source and characteristics of the cancer and immortalized cell lines and their potential as models of human implantation are shown in Table 3.

Stem cells (embryonic and trophoblast)-derived models

Human pluripotent embryonic (ESC) and induced (iPSC) stem cells as well as trophoblast stem cells are another possible source for the generation of human implantation models (Fig. 2C-3). The potential of ESC or iPSC to generate 3D advanced models to study embryogenesis and/or implantation is discussed thoroughly in the section below. The report of the first human ESC lines that emerged from outgrowths of peri-implantation human blastocysts dates from 1998 (Thomson *et al.*, 1998). Within the next few years, it was defined that the pluripotent ESC can differentiate into trophoblast cells when induced with bone morphogenetic protein-4 (BMP4) or in specific culture conditions (Xu *et al.*, 2002; Gerami-Naini *et al.*, 2004). Currently, the conversion of ESC and iPSC to trophoblast-like cells by BMP4 treatment, alone or in combination with small important molecules is a common method (Takahashi *et al.*, 2007; Amita *et al.*, 2013; Horii *et al.*, 2016, 2020; Wei *et al.*, 2017; Li *et al.*, 2019; Castel *et al.*, 2020; Cinkornpumin *et al.*, 2020; Dong *et al.*, 2020; Liu *et al.*, 2020; Kobayashi *et al.*, 2022). The resulting cells show bi-potency: some express ST-specific markers and secrete placental hormones, such as hCG, progesterone, and hPL, while others are capable of differentiating to invasive HLA-G+ EVT-like cells (Horii *et al.*, 2016). Whether the cells obtained from BMP4-treated iPSC correspond to the trophoblast is still under discussion, since different protocols may result in a mixture of trophoblast and mesoderm cells and further separation or purification may be needed (Horii *et al.*, 2019; Tsuchida *et al.*, 2020; Nikitina and Lebedev, 2022). Amita *et al.* (2013) introduced the so-called BAP protocol to inhibit mesoderm induction and accelerate trophoblast differentiation of hESC to terminally differentiated ST or EVT, compared to BMP4 treatment alone. The main limitation of this approach is the lack of self-renewal, short-time proliferation, and quick differentiation. Importantly, even though many TE- and trophoblast-associated genes are induced in this system, BMP4-treated PSCs do not fully resemble primary trophoblast based on marker expression (Li *et al.*, 2013; Aghajanova, 2020). Nevertheless, the use of iPSC as a cell source offers the possibility to generate patient-specific trophoblast-like cells and to perform genetic manipulations (Shpiz *et al.*, 2015; Horii *et al.*, 2016; Ahern *et al.*, 2022; Nikitina and Lebedev, 2022). The possibility of reprogramming cells of a full-term placenta to iPSC and then differentiating them into trophoblast could be a key to overcome the need for first-trimester specimens and would give models for primary trophoblasts from cells with known pregnancy outcomes (Horii *et al.*, 2020). Another approach is to obtain trophoblast cells by establishing culture conditions for the growth and expansion of human trophoblast stem cells (hTSC) (Okae *et al.*, 2018). In 2018, the first derivation of self-renewing trophoblast stem cells from human blastocyst TE and first-trimester placental isolates was implemented. These hTSCs use a combination of WNT (Wingless and Int-1) activation and transforming growth factor beta (TGFβ) inhibition to self-renew, giving rise to long-term expanding CT that can differentiate into ST and EVT and can be cryopreserved for later usage. Of note, Okae *et al.* (2018) proved that hTSC has similar transcriptomes and methylomes to the primary trophoblast cells and mimic trophoblast invasion during implantation. The exact location of trophoblast stem cells within the placenta remains unknown but they probably reside in a specific niche within the first-trimester placenta, since they could not be derived from later gestation placental tissues. Recently, Wei *et al.* (2021) obtained hTSC from primed pluripotent stem cells (hESC lines H1 and HN10) and revealed that BMP4 treatment significantly enhanced the efficiency of the process. These cells are named hTS^{PS} cells and share identical features with primary blastocyst-derived hTSC, including morphological characteristics, gene expression profiles, and the capacity for differentiation toward EVT and ST cells. Endometrial stem/progenitor cells were discovered 10 years ago (Chan *et al.*, 2004) and studied mainly in the context of endometrium regeneration, rather than modeling of human implantation.

In summary, the work with ESC is labor-intensive and highly restricted, and the generation of iPSC has varying success and reproducibility. Although embryonic and trophoblast stem cells could be a valuable source for the generation of patient-specific models suitable for genetic manipulations, they may remain of limited value due to the ethical and legal challenges against their use.

Advanced 3D cultures: spheroids, organoids, IVF embryos and embryoids, and assembloids

A single endometrial or trophoblast culture system is unlikely to address all of the biological questions related to human implantation but is the first step towards this is complex 3D models and assembloids. In the last decade, the researchers turned to 3D spheroid and organoid systems using most of the

above-mentioned cells: primary, cancerous, immortalized or stem cells (Fig. 2C-4, Table 3). In these systems, cells benefit from cell-to-cell and cell-to-ECM contacts. Moreover, the cells exist in a more biochemically relevant state with gradients of oxygen, nutrients, and metabolites. There are two ways of constructing 3D models of human implantation: (i) self-organizing cultures to model early blastocyst/TE differentiation and migration (spheroids, organoids), and (ii) controlled assembly approaches to model embryo attachment and trophoblast invasion. *De novo* construction of both the pregnant endometrial wall (endometrial organoids) and implanting blastocyst (blastocyst-like structures, BLS) is based on the use of scaffold-dependent or matrix-free cell culture techniques to facilitate self-organization of the cells in organoid-like structures. A fundamental requirement is the correlation to clinical outcomes. The more closely certain 3D model represents receptive endometrium, blastocyst, or early placenta as well as maternal–fetal interface as a whole, the more likely it can provide accurate results. With the implication of 3D cell cultures worldwide, the terminology about the types of 3D cultures gets more and more complicated. For example, while the definition of spheroids, as self-assembling cell aggregates in an environment preventing their attachment to a flat surface (Białkowska *et al.*, 2020), is common for laboratories working with healthy and cancer cell models, the definition of organoids is more unspecified. Some authors have determined organoids as 3D cultures that contain more than one type of differentiated cells and their progenitors (Mulaudzi *et al.*, 2024). Organoids might be derived from ESC or adult progenitor cells that self-organize in particular culture conditions and recapitulate the differentiation and function of the organ of origin (Clevers, 2016; Abbas *et al.*, 2020). Others have stated that organoids are genetically stable, 3D culture systems containing both progenitor (stem) and differentiated cells (Cui *et al.*, 2020). A third party described the organoids just as 3D multicellular tissue models that mimic their corresponding *in vivo* tissue (Francés-Herrero *et al.*, 2021). On the other hand, assembloids are 3D cultures created by the combination of two or more distinctly patterned organoids or an organoid plus additional cells or tissues that are used to model usually cell migration, invasion, and attachment during implantation (Levy and Paşca, 2023). In the current review, we consider the spheroids as single-cell type-based, self-assembling 3D cultures, the organoids as self-organizing 3D co-cultures of two or more cell types independent of the origin of the cell lines, and the assembloids as controlled organoids co-culture or an organoid along additional types of cell or tissue.

The idea for a 3D model of the human endometrium dates from the 1980s, and organoid-like structures from endometrial epithelial cells were first derived from primary gland fragments seeded into Matrigel (Rinehart *et al.*, 1988). The endometrial organoids (EMO) with primary endometrial epithelial cells as 3D endometrial models are promising tools for recapitulation of the receptivity of maternal tissue in human implantation (Bentin-Ley *et al.*, 1995). Despite being relatively complex to generate and maintain, EMOs originate from more easily obtainable specimens and can be manipulated in various ways. To generate and maintain human EMOs, Matrigel droplets with enzymatically dissociated primary endometrial tissue or iPSCs-derived endometrial fibroblasts were cultured in a defined medium (Turco *et al.*, 2017; Miyazaki *et al.*, 2018). The defined medium includes activators of WNT signaling (WNT ligands and R-respondin-1), growth factors (epidermal growth factor, EGF, fibroblast growth factor 10, FGF10), TGFβ inhibitors (A83-01), BMP inhibitor (Noggin), and nicotinamide. Importantly, the organoids could be generated from biopsies obtained from cycling and non-cycling (pregnant or post-menopausal) endometrium. Moreover, these can be extensively cultured (more than 6 months) and cryopreserved showing stable genotypes and phenotypes, (Turco *et al.*, 2017; Turco and Moffett, 2019). EMOs are an unprecedented opportunity to study the human endometrium in terms of normal endometrial development and menstrual cycle, and their remarkable aspects have been comprehensively discussed (Hibaoui and Feki, 2020). However, the cystic structure and reversed apico-basal polarity of conventional EMOs limit their utility in studying interactions with embryo surrogates during implantation (Bergmann *et al.*, 2021). To reconstitute normal apico-basal polarity, Shibata *et al.* created apical-out EMOs (AO-EMO) that expose the apical surface of the epithelium to be available for interaction with trophoblasts (Shibata *et al.*, 2024). In the same year, Francés-Herrero broadly discussed the importance of mimicking a favorable native microenvironment for the development of human EMO and proposed supplementations based on hydrogels from pig endometrium (Francés-Herrero *et al.*, 2021). Fraser *et al.* presented a 3D endometrial organotypic model simulating the acute inflammatory decidualization initiation phase characterized by epithelial induction of the key endometrial receptivity marker integrin αVβ3 as a new concept to investigate endometrial receptivity. Accumulating data show a continuous tendency for the generation of closer-to-the-natural settings models to serve as irreplaceable tools for studying human implantation (Fraser *et al.*, 2021).

Trophoblast spheroids (Fig. 2C-4.1) constructed from either primary immortalized trophoblasts or choriocarcinoma cell lines are commonly used as blastocyst surrogates for implantation studies. Their greatest advantage is that they do not include the use of human stem cells and they lack an ICM so they could not potentially give rise to an organism. This lifts many of the ethical constraints related to human stem cell usage (Caulfield *et al.*, 2015; Matthews and Moralí, 2020; Matthews *et al.*, 2021). Meanwhile, this kind of embryo surrogate contains trophoblast cells that, in particular settings, sufficiently represent TE development and differentiation during peri- and early implantation. Therefore, the trophoblast spheroid models have been used for many years to assess embryo attachment (Tacey *et al.*, 1988; Wang *et al.*, 2012), trophoblast migration and invasion during implantation (Holmberg *et al.*, 2012; Gao *et al.*, 2019; You *et al.*, 2019) (Table 4). Mor's group created spheroid models from the immortalized, non-cancer, seventh gestational week derived trophoblast Sw71 cell line. As mentioned above this line has an EVT nature, and preserved stemness so the models effectively resemble the human blastocyst during the peri-implantation period. Sw71 spheroids were proven to migrate between endometrial stromal cells in appropriate conditions and by numerous protrusions to digest and invade Matrigel, which resembles the behavior of the invasive EVT *in vivo* (Holmberg *et al.*, 2012; You *et al.*, 2019). Recently, we extended these data showing over 70% production efficiency, closer to hatched or implanting blastocyst morphology and behaviors or functions that describe the 3D Sw71 spheroids as destined to be implanting structures, able to survive and migrate in serum-free media (Alexandrova *et al.*, 2022b). Most importantly, we validated the Sw71 blastocyst-like model by direct comparison to primary trophoblasts regarding their hybrid (Vim+/CK7+) phenotype and HLA-G+/HLA-C+ pattern as well as by their ability to generate differentiated and functional spheroid models (Alexandrova *et al.*, 2023). After the significant compactization during their 48 h of differentiation, these BLS lacking inner cellular mass have a blastocoel-cavity,

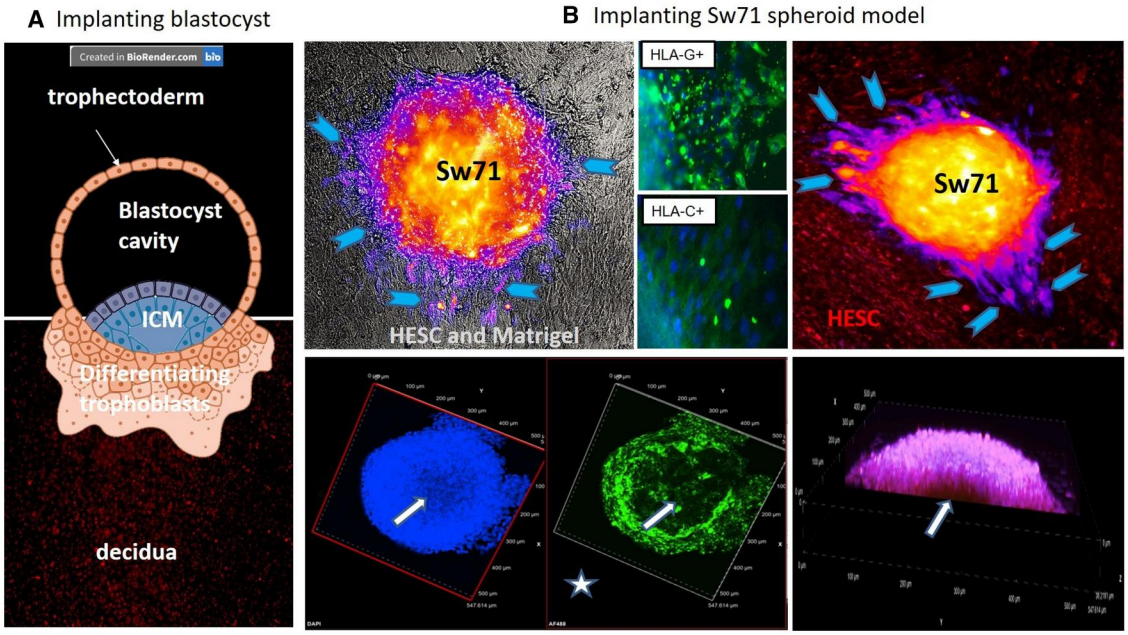


Figure 3. Suitability of the 3D Sw71 spheroid model as a blastocyst-like surrogate to study trophoblast migration and invasion during human implantation. (**A**) Overall view of an implanting human blastocyst (Day 7–9 post-fertilization). (**B**) Upper panel (light microscopy, ECHO Revolve microscope RVL-100-M, Echo, San Diego, CA, USA, magnification 10×): Left—Sw71 spheroid (yellow) placed on human endometrial stromal cells (HESC) monolayer (grey), covered with Matrigel as a substitute for ECM. Note the invading trophoblast cells from the spheroid periphery (blue arrowheads, purple zone). Right—Sw71 spheroid trophoblast cells (blue arrowheads, purple zone), migrating between the stromal endometrial cells (HESC in red). Middle—HLA-G and HLA-C expression (in green) by Sw71 spheroid's trophoblast cells. The staining is performed with antibodies against HLA-G (rabbit, polyclonal, E-AB-18031, Elabscience) and HLA-C (mouse, monoclonal, sc-166088, Santa Cruz Biotechnology), and cell nuclei are counterstained with Hoechst (in blue); B. Lower panel (confocal microscopy, Nikon AX/AX R Confocal Microscope System): Blastocoel-like cavity (white arrow) of the Sw71 spheroid. Left and right—The nuclei of Sw71 cells are visualized with Hoechst staining (blue and magenta, respectively); middle—Sw71 trophoblast cells are stained for HLA-G molecule (green).

proven by confocal imaging (Fig. 3B, lower panels). Both events compactization and cavitation are specific for early blastocyst development *in vivo*. The comparison of the Sw71 spheroids to implanting human blastocyst confirms the invasion and migration of HLA-G- and HLA-C-positive trophoblast cells from a differentiated Sw71 spheroid, placed on endometrial stromal monolayer with or no ECM (Fig. 3B, upper panels).

The trophoblast is a functional bridge between the embryo and the mother and an important player in the establishment of immune tolerance during implantation. The trophoblast Sw71 cells express/produce both HLA-G and HLA-C molecules (Hackmon *et al.*, 2017; Alexandrova *et al.*, 2022b) as ligands for maternal KIRs on both NK cells and T cells. Thus, the Sw71 spheroid model may be a key structure for the evaluation of the immune interactions via the KIR/HLA axis at the MFI during implantation and early placentation (Alexandrova *et al.*, 2022a). The inclusion of immune components in our model would be an asset to delineate mechanisms of immune tolerance, albeit at the expense of increased complexity. Sw71 blastocyst surrogate is a promising model for studying human implantation either in the presence or absence of partner endometrial cells.

In addition to immortalized and cancer trophoblast cell lines, an embryonic stem cell line VAL3 was differentiated into solely trophoblastic cells that were cultured in a AggreWell plate to form a human embryonic stem cell-derived trophoblastic spheroid implantation model, cultured in BAP differentiation medium (Lee *et al.*, 2015; Yue *et al.*, 2020). BAP differentiation medium is mouse embryonic fibroblast conditioned medium (MEF-CM), supplemented with 10 ng/ml BMP4, 1 μM of an ALK4/5/7 inhibitor, and 0.1 μM of an FGF2-signaling inhibitor (PD173074). BAP-induced differentiation leads to reduced OCT4 expression and upregulation of trophoblastic markers HLA-G, β-hCG, and CK7. Moreover, the BAP-treated VAL3 cells showed enhanced capacity for invasion and migration *in vitro*, consistent with their trophoblastic properties. Their mRNA resembled that of the TE of blastocyst before implantation (Yue *et al.*, 2020). Compared to the classic trophoblast spheroids, BAP spheroids are derived from ESC, enabling long-term culture with an unlimited supply, but an ethical issue needs to be considered. BAP spheroids possess a blastocoel-like cavity, resembling the human blastocyst at a more physiological extent, and selectively attach to the receptive endometrial cells (Li *et al.*, 2022).

The establishment of trophoblast organoids sourced from first-trimester placental villi (Fig. 2C-4.2) was a major advancement in the trophoblast research field. Early placenta-derived villous CT proved to differentiate to hCG-secreting ST and/or HLA-G+ invading EVT could serve as a sophisticated 3D model for trophoblast differentiation, invasion and migration studies. For more than 20 years, Moffet's team strived to grow a human placental model for the first few weeks of pregnancy (King *et al.*, 2000; Moffett and Loke, 2006; Moffett and Shreeve, 2023). In 2018, they reported the generation of a human, single donor-based long-term, genetically stable trophoblast organoids that grow as complex 3D structures with the fusion of villous trophoblasts to hCG-secreting ST or differentiate to HLA-G+ EVT cells that vigorously invade and digest Matrigel, and anatomically and functionally resemble the villous placenta *in vivo* (Turco *et al.*, 2018). By ameliorating culture conditions for these 'mini-placentas', they stated that the organoid culture system provides a translational model for early placentation that may be passaged for more than a year and also may be frozen (Turco *et al.*, 2018; Sheridan *et al.*, 2020, 2022). Concurrently, Knöfler's group reported the

establishment of long-term living human CT organoids from purified from pooled early placental tissues, expressing markers of human CT stemness, and proliferation, and with gene expression profiles highly similar to those of the primary human trophoblasts. The authors succeeded in establishing 16 different organoid cultures with 100% efficiency, that could be cryopreserved and re-cultivated (Haider *et al.*, 2018). Both groups suggested that the activation of Wnt and EGF signaling and inhibition of the TGFb pathway could be sufficient for the derivation and long-term expansion of human trophoblast organoids (Nikitina and Lebedev, 2022). These reports remain highly debated as effective ways to derive trophoblast organoids from primary cells to model both normal and pathological human placenta in Horii *et al.*, (2020), Li *et al.* (2022), Nikitina and Lebedev (2022), James *et al.* (2022). However, much work remains to be done to translate these models in the research of implantation-based complications (Nishiguchi *et al.*, 2019; Horii *et al.*, 2020; Li *et al.*, 2022). For example, these 'mini-placentas' need further optimization like reversing the polarity since they have internal ST covered by EVT (Sheridan *et al.*, 2020; James *et al.*, 2022) or the treatment should be consistent with these features (Sheridan *et al.*, 2022).

Recent studies have highlighted the potential of ESC or iPSC to generate 3D BLS called 'blastoid' or 'iBlastoid' respectively (Yu *et al.*, 2021; Kagawa *et al.*, 2022; Karvas *et al.*, 2022) (Fig. 2C-4.3). These structures have many names such as blastoids (Rivron *et al.*, 2018), iBlastoids, BLS (Nikitina and Lebedev, 2022), embryoid bodies (Li *et al.*, 2022), embryoids (Nicolas *et al.*, 2021), and stem cell-based embryo models (SCB-Ems). Synthetic human embryo models show functional, molecular and morphological similarities to the human blastocysts at Days 6–10 post-fertilization *in vivo* consisting of epiblast, hypoblast and TE layers (Ball, 2023). These structures are derived from naïve hESC (Yu *et al.*, 2021) or iPSC (Liu *et al.*, 2021) exposed to designated differentiation media ensuring sequential lineage differentiation and self-organization. Up to now, human blastoids have been successfully generated using hESC in both the naive (Yanagida *et al.*, 2021; Yu *et al.*, 2021; Kagawa *et al.*, 2022; Karvas *et al.*, 2022; Yu *et al.*, 2023) and primed-to-naive intermediate pluripotency states (Tu *et al.*, 2023) as well as from iPSCs (Fan *et al.*, 2021; Liu *et al.*, 2021; Sozen *et al.*, 2021; Mazid *et al.*, 2022; Yu *et al.*, 2022). These studies exploit single-cell transcriptome analyses to confirm the segregation of TE, hypoblast, and epiblast. By mimicking the interaction between the epiblast and TE, the crucial role of the epiblast was revealed as an inductor of the local maturation of polar TE and its attachment to the endometrial epithelium (Kagawa *et al.*, 2022). They might serve as embryo surrogates in modeling normal and impaired implantation as well as genetic and other manipulations (Morris, 2017). Blastoids cultured on three-dimensional (3D) extracellular matrix undergo early post-implantation events, including epiblast lumenogenesis, rapid expansion, and diversification of trophoblast lineages, and robust invasion of EVT by Day 14. By Day 21, the extended blastoid culture shows localized activation of the markers of the primitive streak and the emergence of embryonic germ layers. This structure is called a gastruloid (post-implantation model) (Karvas *et al.*, 2022, 2023) (Fig. 2C-4.3). So far, there have been no reports of human blastoid formation using human trophoblast cells (Okae *et al.*, 2018) probably due to the closer similarity of human trophoblasts to post-implantation cytotrophoblasts rather than TE (Okae *et al.*, 2018; Castel *et al.*, 2020; Mischler *et al.*, 2021).

Advanced assisted reproduction requires the maintenance of human gametes, fertilization, and optimal development of the zygote to the blastocyst stage *ex utero*, permitting an evaluation of embryo quality before transfer. Although ethically controversial, the extended cultures of human embryos bring remarkable advances in our understanding of the peri-implantation stages of human development (Fig. 2B-4.4). In 2016, the successful growth of human embryos up to Day 14 post-fertilization (start of gastrulation, the legal limit of human embryo culture) (Warnock, 1884) was reported and approved for replication of the *in vivo* transition from pre- to post-implantation stages (Morris, 2017). These extended embryo cultures reveal self-organization of the human embryo after attachment in the absence of maternal influence as well as the early TE and subsequent trophoblast differentiation (Deglincerti *et al.*, 2016; Shahbazi *et al.*, 2016; Popovic *et al.*, 2019; West *et al.*, 2019; Xiang *et al.*, 2020; Zhou *et al.*, 2021; Greenbaum *et al.*, 2023). By using single-cell RNA sequencing, plenty of trophoblast lineages from peri-implantation embryos in culture were assessed to gain insights into events driving early placental emergence (Blakeley *et al.*, 2015; Petropoulos *et al.*, 2016). The transcriptome dynamics in trophoblast cells occurring between D8 and D12 post-fertilization, a time that, *in vivo*, corresponds to the first 5 days after the embryo begins to implant into the uterine wall, were successfully captured (West *et al.*, 2019; Greenbaum *et al.*, 2023). Zhou *et al.* (2021) made a great parallel between data obtained from the 13th day post-fertilization human embryo culture and from the Cambridge and Carnegie collections of *in situ* developing human embryos.

The perfect *in vitro* implantation model would present the interaction between endometrial epithelial, stromal, endothelial, and immune cells and embryos (or surrogates). But this complex model is still hypothetical. In their efforts to reach the complete recapitulation of maternal–fetal contact during implantation, the researchers turned to different assembloid (layered) models. As shown in Table 4, these two-part assembloids could be less or more complex.

These assembloids combine primary or cancer endometrial (epithelial and/or stromal cells) or endometrial segments/tissue or EMO organoids with genuine human embryos or embryo surrogates (mouse embryo, trophoblast spheroid or organoid) to study embryonic adhesion and attachment, trophoblasts differentiation, and migration/invasion (Table 4) (Kliman *et al.*, 1990; Galán *et al.*, 2000; Hohn *et al.*, 2000; Heneweer *et al.*, 2003, 2005; Harun *et al.*, 2006; Uchida *et al.*, 2007; Aboussahoud *et al.*, 2010; Gonzalez *et al.*, 2011; Liu *et al.*, 2011; Ho *et al.*, 2012; Holmberg *et al.*, 2012; He *et al.*, 2019; Kinnear *et al.*, 2019).

Assembling of extended embryo cultures with maternal tissue is an optimal system allowing modeling of the *in vivo* endometrium-conceptus crosstalk in norm and pathology (Menezo *et al.*, 1995; Cartwright *et al.*, 1999, 2002; Carver *et al.*, 2003; Teklenburg and Macklon, 2009; Teklenburg *et al.*, 2012; Zhou *et al.*, 2021). Rawlings *et al.* (2021b) assembled endometrial organoids with human IVF embryos to study the impact of the senescence of decidual stromal cells on embryo invasion. Buck *et al.* (2015) used an original approach to develop a novel 3D assembloid cell culture system consisting of trophoblast choriocarcinoma monolayer (AC-1M88) co-cultured with spheroids from endometrial epithelial cell lines HEC-1A, Ishikawa, or RL95-2 cells to mimic trophoblast invasion into endometrial glands (Table 4). In this model, three differently differentiated and polarized endometrial adenocarcinoma cell lines formed gland-like structures in a reconstituted basement membrane with apicobasal polarization towards their well-developed internal lumen, for studying the impact of the epithelial junction on trophoblast-endometrium interaction and trophoblast invasion of endometrial glands (Buck *et al.*, 2015). By the same approach, an Ishikawa

cell spheroid culture served as an experimental model system to prove the effect of hormonal changes during the window of implantation (Buck *et al.*, 2021).

The invasion of the decidual vessels by endovascular trophoblasts and the role of uNK cells and macrophages in this process was followed by an *in vitro* co-culture system of first-trimester villous explants and decidua parietalis embedded in paraffin (Dunk *et al.*, 2003; Hazan *et al.*, 2010). Earlier, the uterine biopsy embedded in fibrin gel and seeded with fluorescence-tagged trophoblasts (SGHPL-4 line) was used as an assembloid model for evaluation of the interstitial and spiral artery invasion (Cartwright *et al.*, 1999, 2002).

To evaluate the attachment potential of recently created blastoids, these were placed onto an open-faced endometrial layer (OFEL) obtained from endometrium organoids (Kagawa *et al.*, 2022). These models are promising tools for the study of peri-implantation and early (post-implantation) events (Sozen *et al.*, 2021; Kagawa *et al.*, 2022; Li *et al.*, 2022). For example, they may provide important insights into the validation of the expression profile of the primary ST (Sheridan *et al.*, 2022).

A relatively complete *in vitro* model of maternal–fetal contact during human implantation was recently created by Shibata *et al.* (2024). In their complex assembloid, they co-cultured AO-EMO (containing endometrial epithelial and stromal cells), and an endothelial network with human blastoids or human embryos. Although lacking the immune cells, this feto-maternal assembloid successfully recapitulated all stages of implantation. The direct interaction between fetal and maternal cells could be observed, including the breaching of the endometrial epithelial barrier by ST as well as the subsequent invasion between stromal cells (Shibata *et al.*, 2024).

Of note, the 2021 guidelines of the International Society for Stem Cell Research were updated to ban the transfer of human research embryos, human-animal chimeric embryos, or human embryo models into an animal or human uterus. However, this restriction did not apply to blastoids or gastruloids. Recently it has been shown that blastoids can induce decidualization upon transfer into the uteri of pseudo-pregnant mice, and they showed improved differentiation into post-implantation embryo-like structures both *in vitro* and/or *in utero* (Li *et al.*, 2019; Sozen *et al.*, 2019; Oura *et al.*, 2023). In the primate study, the authors stated that the results reveal the capacity of cynomolgus monkey blastoids to implant although the pregnancies all aborted spontaneously (Li *et al.*, 2023). In their press statement, the team recognized that the work may be controversial but they noted that discussions between the scientific community and the public are needed for progress to be made in this field of research (https://www.wired.com/story/stem-cells-monkey-synthetic-embryos/). These results raise active discussion on the findings as well as the ethical aspects in recent scientific publications (Conroy, 2023) and numerous public-oriented science communications (Devlin, 2023; Hamzelou, 2023). We would like to note that although they could bring valuable findings on the implantation processes, the studies confronting the published rules and guidelines raise ethical concerns.

In summary, for accurate results with the endometrial 3D cultures, researchers should carefully control the maturation process of endometrial and trophoblast spheroids or organoids, while also being critical in discriminating true phenotypes from the observations of sporadic events in a dish. Another hurdle is the limited size and the appearance of necrotic central zones due to insufficient internal oxygen and nutrient supply. Although all these facts question reproducibility and sustainability, the advanced 3D models and especially assembloids are valuable and very promising tools for fundamental research in peri-implantation and early placentation events. Since the interaction of the immune cells with trophoblasts is a readout for successful implantation, key steps forward would be the selective incorporation of components of the immune system. The extended cultures of human embryos as well as the embryoid models are considered only the beginning of the full discovery of the peri-implantation stages in humans. However, the use of such models is still restricted by the specific culturing conditions, and the requirement for expertise, as well as the ethical and legal concerns raised. There has been an ongoing discussion on the appropriacy of the extension of these cultures beyond the legal 14 days (Hurlbut *et al.*, 2017).

Perspectives

As Rivron has noted, 'embryos teach us everything about how we are formed and how we fail'. Therefore, we must profit from the highly advanced 3D models to study and understand implantation and to employ the findings in favor of infertile couples (Rivron *et al.*, 2018; Powell, 2021). Since the terminology still lacks systematization, it would be of great value if a robust classification of all the available 3D endometrial, embryo culture systems, and assembloids is produced. The existing type of models should be defined under certain designations. After determining the best models, they should be implemented in practice, their handling should be mastered, and they should be proved reliable in parallel with natural settings (primary cells, extended embryo cultures, possible *in vivo* observations). Thus, scientists all over the world could use standardized protocols for generation and maintenance, as well as strict guidelines to work with 3D human MFI surrogates. New sources for information about human implantation are the extended human embryo cultures and the synthetic embryo models. Both models raise controversies and ethical questions. To date, none of the synthetic embryos can biologically produce a fetus. Since some artificial models could originate from iPSC, they could even escape the use of ESC but 'as they get more sophisticated, with the potential to form recognizable structures or even organs, they enter their ethical grey area' (Powell, 2021). Their similarity to human blastocysts/gastrula provides promising avenues of research and treatment for developmental biology, regenerative medicine, drug discovery, and reproductive health, but they still raise social, ethical, and legal questions that will affect future research and widespread adoption in industry and clinical settings. Moreover, such cultures are not widely accessible. A very recent comment on the artificial embryo models in *Nature* presented data for a pair of studies with the most advanced to date lab-grown human embryo models. The main message was that those most advanced synthetic human embryos spark controversy provoking many arguments about the merits of claims made, and raising ethical concerns and legal questions (Ball, 2023). New ethical dilemmas such as the moral status of the human embryo and whether we should treat synthetic embryos in the same way as natural ones or whether, because of their laboratory origin, we have no moral responsibility. As Foreman *et al.* stated recently, it would be of benefit for scientists to voluntarily and proactively strive for efficient regulation and consistent ethical guidelines for research on human embryoids in collaboration with members of the public, clinicians, patients, bioethicists, sociologists, legal experts, and funding organizations (Nicolas *et al.*, 2021; Foreman *et al.*, 2023). The embryos generated through ART but not used for

reproductive purposes are at disposal (where this is allowed by the country's ART regulation) as donated human embryos for research (spare embryos, surplus embryos). The usage of such embryos also raises moral unease about the instrumentalization of human life (Scully *et al.*, 2012), whether the donation of the embryo could be an act of possible coercion 'wrapped' in altruism, and whether is acceptable for the embryo to be 'a gift'. The statement that synthetic embryos will eliminate the need to use natural human embryos is controversial because, their creation involves the extraction of a stem cell from a natural embryo, which could reduce, but not eliminate, the use of natural embryos at this stage. On the other hand, there are great uncertainties about their comparability with natural embryos and how well they can show actual early embryonic development. If the synthetic embryos created from manipulated somatic cells develop into full-grown humans, it will confront us with the ethical dilemma of access to this technology for reproductive purposes in same-sex couples. It will lead us to the questions of genetic parentage in the background of genetic manipulation and the moral justification of asexual reproduction as a whole. The opportunity of synthetic embryos to replace donated human embryos provokes the question of 'what's next for lab-grown human embryos?' (Powell, 2021) or even 'what is an embryo'? Although the potential of the new artificial human embryo models to induce a pregnancy is yet unknown (Ball, 2023), some researchers speculate that it is time to change the definition of the human embryo and include embryo models with the potential to develop into a fetus (Rivron *et al.*, 2023) They propose the embryo to be defined as 'a group of human cells supported by elements fulfilling extraembryonic and uterine functions that, combined, have the potential to form a fetus'. We and others remain conservative, stating that it is too soon to start formalizing such distinctions (Ball, 2023). We state that genuine donated-for-research or synthetic embryos should not in any circumstances complete this cycle since it will exceed any ethical tolerance and would highly impact society in a negative way. Also, natural and model human embryos used in science should be classified under a single term like 'human research embryo' or another term, to distinguish them from human embryos that will eventually develop into humans. Surplus embryos should be replaced with BLS for research and used only in the final validation steps under strict regulation.

Concluding remarks

The human implantation process is still obscure and remains a limiting step for the success of *in vivo* and *in vitro* fertilization. Conventional animal and 2D culture models have had an important role in the first steps of revealing the uniqueness of this process, helping to define the stages, and suggesting important study targets. Tissue explants and extended human embryo cultures as well as primary, cancer, and immortalized cell line models, with all their pros and cons, have given crucial results in human implantation research. Of them, the normal immortalized cell lines promise to model the closest to the *in situ* process. The advanced 3D models might be the key to widening the knowledge of the intimate maternal–embryo contact at the very beginning of pregnancy. From the already available models, the endometrium spheroids or organoids are a powerful tool for assessing the impact of different factors on implantation/early placentation with increased physiological relevance. The relative autonomy in early trophoblast development makes trophoblast spheroids relevant models for studying their implantation potential independently of the embryoblast and/or maternal signals. Since these models comprise a single cell line (independent of the origin), they overcome all the legal and ethical constraints for work with ESC and are more accessible and affordable for laboratories all over the world. However, such models need to be validated with similar constructs from primary cells and confronted with small decidua biopsies recapitulating the MFI. For example, the Sw71 spheroids are useful and biologically relevant embryo surrogates for the evaluation of TE behavior during human implantation, including maternal–fetal immune recognition at peri-implantation. The natural or induced pluripotent stem cell-derived embryoids could be used in studies on human implantation/placentation and embryogenesis. For epiblast research, the trophoblast stem cells or stem cells generated from iPSC should be preferentially used to avoid the use of ESC. The complex assembloids including organoid-based 3D culture endometrial and trophoblast models can better recapitulate the trophoblast-endometrium interaction for the investigation of the successful (and failed) human implantation. Patient-derived endometrial organoids/assembloids are a new avenue for developing novel treatment strategies. Much work remains to be done for the optimization and standardization of these integrated and complex models. Given their diversity as well as the frequent lack of validation with primary cultures or *in vivo* observations, it is preferable that findings from studies on human implantation, using a particular model are confirmed with others and/or verified *in vivo*. We believe that the proposed consecutive approach including systematization of the available information and creation of strict classification with denomination of the 3D models with their advantages, limitations, preferred use, and methodology for modeling of a particular event, would tremendously aid the modeling of human implantation. When combined with contemporary molecular techniques such as genomic microarrays, RT-PCR, multiplex assays, and proteomics, these models could provide reliable and important data about human implantation. This would be a key for reproductive science providing an opportunity to investigate fundamental pathways of human implantation as well as to evaluate the impact of many factors, new therapies, and treatments related to women's health, IVF success, and embryo development. Last but not least, it would be ideal if the legislation and regulations on the 3D embryo models were unified and applicable worldwide to ensure the same standards and practices, equalize research quality, and provide reliable output. Synthetic embryo research presents scientists with the dilemma of how both science and ethics can go hand in hand. Warnock's report (1884) as a regulatory framework for the oversight of experiments with human embryos was questioned and new guidelines relaxing the '14-day rule' about human embryo culture took away the hard barrier for researchers (Powell, 2021). Some research groups admired such an extension envisaging possible scientific and clinical advances and determining an ethical basis for such an extension (Williams and Johnson, 2020; McCully, 2021), while others denied it (Blackshaw and Rodger, 2021; Nicolas *et al.*, 2021). Warnock herself was concerned that the extension would put at risk the whole field of embryo research (Hurlbut *et al.*, 2017). We support Warnock's opinion that it is too soon to continue past Day 14 for human embryo cultures. Moreover, extended human embryo cultures have to be used as a last resort tool for *in vivo* confirmation purposes only. Nevertheless, the careful appraisal and development of national legal and ethical frameworks are crucial for better regulation of studies using human embryo models to obtain all the potential benefits for human reproduction.

Data availability

The data underlying this article are available in the article.

Authors' roles

T.D.: conceptualization, data curation, funding acquisition, supervision, writing of the original draft, review, and editing. M.A.: data curation, visualization, formal analysis, investigation, writing of original draft. I.V.: formal analysis, investigation. Y.Y.: formal analysis, investigation. G.M.: resources, writing—review and editing. All authors revised the article critically for important intellectual content. All authors approved the final version of the article to be published.

Funding

This work was financially supported by the Bulgarian National Science Fund via VIHREN, project KP-06-DV3.

Conflict of interest

All authors declare no conflict of interest.

References

Abbas Y, Turco M, Burton GJ, Moffett A. Investigation of human trophoblast invasion in vitro. *Hum Reprod Update* 2020;**26**:501–513.

Aboagye-Mathiesen G, Laugesen J, Zdravkovic M, Ebbesen P. Isolation and characterization of human placental trophoblast subpopulations from first-trimester chorionic villi. *Clin Diagn Lab Immunol* 1996;**3**:14–22.

Abou-Kheir W, Barrak J, Hadadeh O, Daoud G. HTR-8/SVneo cell line contains a mixed population of cells. *Placenta* 2017;**50**:1–7.

Aboussahoud W, Bruce C, Elliott S, Fazeli A. Activation of Toll-like receptor 5 decreases the attachment of human trophoblast cells to endometrial cells in vitro. *Hum Reprod* 2010;**25**:2217–2228.

Aghajanova L. How good are we at modeling implantation? *J Assist Reprod Genet* 2020;**37**:17–19.

Ahern DT, Bansal P, Armillei MK, Faustino IV, Kondaveeti Y, Glatt-Deeley HR, Banda EC, Pinter SF. Monosomy X in isogenic human iPSC-derived trophoblast model impacts expression modules preserved in human placenta. *Proc Natl Acad Sci USA* 2022;**119**:e2211073119.

Alexandrova M, Manchorova D, Dimova T. Immunity at maternal-fetal interface: KIR/HLA (Allo)recognition. *Immunol Rev* 2022a;**308**:55–76.

Alexandrova M, Manchorova D, You Y, Mor G, Dimitrova V, Dimova T. Functional HLA-C expressing trophoblast spheroids as a model to study placental-maternal immune interactions during human implantation. *Sci Rep* 2022b;**12**:10224.

Alexandrova M, Manchorova D, You Y, Terzieva A, Dimitrova V, Mor G, Dimova T. Validation of the Sw71-spheroid model with primary trophoblast cells. *Am J Reprod Immunol* 2023;**90**:e13800.

Al-Nasiry S, Spitz B, Hanssens M, Luyten C, Pijnenborg R. Differential effects of inducers of syncytialization and apoptosis on BeWo and JEG-3 choriocarcinoma cells. *Hum Reprod* 2006;**21**:193–201.

Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. *Nat Immunol* 2004;**5**:266–271.

Amita M, Adachi K, Alexenko AP, Sinha S, Schust DS, Schulz LS, Roberts RM, Ezashi T. Complete and unidirectional conversion of human embryonic stem cells to trophoblast by BMP4. *Proc Natl Acad Sci USA* 2013;**110**:E1212–E1221.

Aplin JD, Ruane T. Embryo-epithelium interactions during implantation at a glance. *J Cell Sci* 2017;**130**:15–22.

Apps R, Murphy SP, Fernando R, Gardner L, Ahad T, Moffett A. Human leucocyte antigen (HLA) expression of primary trophoblast cells and placental cell lines, determined using single antigen beads to characterize allotype specificities of anti-HLA antibodies. *Immunology* 2009;**127**:26–39.

Apps R, Sharkey A, Gardner L, Male V, Trotter M, Miller N, North R, Founds S, Moffett A. Genome-wide expression profile of first trimester villous and extravillous human trophoblast cells. *Placenta* 2011;**32**:33–43.

Bačenková D, Trebuňová M, Čížková D, Hudák R, Dosedla E, Findrik-Balogová A, Živčák J. In vitro model of human trophoblast in early placentation. *Biomedicines* 2022;**10**:904.

Baczyk D, Kibschull M, Mellstrom B, Levytska K, Rivas M, Drewlo S, Lye SJ, Naranjo JR, Kingdom JCP. DREAM mediated regulation of GCM1 in the human placental trophoblast. *PLoS One* 2013;**8**:e51837.

Ball P. Most advanced synthetic human embryos yet spark controversy. *Nature* 2023;**618**:653–654.

Ban Z, Knöspel F, Schneider M. Shedding light into the black box: advances in in vitro systems for studying implantation. *Dev Biol* 2020;**463**:1–10.

Barbier CS, Becker K, Troester MA, Kaufman DG. Expression of exogenous human telomerase in cultures of endometrial stromal cells does not alter their hormone responsiveness. *Biol Reprod* 2005;**73**:106–114.

Benirschke K, Kaufmann P. *Pathology of the Human Placenta*, 4th edn. Springer: New York, USA, 2000. [Database]

Bentin-Ley U, Horn T, Sjögren A, Sørensen S, Falck Larsen J, Hamberger L. Ultrastructure of human blastocyst-endometrial interactions in vitro. *J Reprod Fertil* 2000;**120**:337–350.

Bentin-Ley U, Lindenberg S, Horn T, Larsen JF. Ultrastructure of endometrial epithelial cells in a three-dimensional cell culture system for human implantation studies. *J Assist Reprod Genet* 1995;**12**:632–638.

Bergh P, Navot D. The impact of embryonic development and endometrial maturity on the timing of implantation. *Fertil Steril* 1992;**58**:537–542.

Bergmann S, Penfold C, Slatery E, Siriwardena D, Drummer C, Clark S, Strawbridge S, Kishimoto K, Vickers A, Tewary M *et al.* Spatial embryo profiling of primate gastrulation. *Nature* 2022;**609**:136–143.

Bergmann S, Schindler M, Munger C, Penfold CA, Boroviak TE. Building a stem cell-based primate uterus. *Commun Biol* 2021;**4**:749.

Bersinger NA, Genewein EM, Müller O, Altermatt H, McKinnon B, Mueller M. Morphology of human endometrial explants and secretion of stromal marker proteins in short- and long-term cultures. *Gynecol Surg* 2010;**7**:75–80.

Bhat P, Anderson DA. Hepatitis B virus translocates across a trophoblastic barrier. *J Virol* 2007;**81**:7200–7207.

Białkowska K, Komorowski P, Bryszewska M, Miłowska K. Spheroids as a type of three-dimensional cell cultures-examples of methods of preparation and the most important application. *Int J Mol Sci* 2020;**21**:6225.

Bilban M, Tauber S, Haslinger P, Pollheimer J, Saleh L, Pehamberger H, Wagner O, Knöfler M. Trophoblast invasion: assessment of cellular models using gene expression signatures. *Placenta* 2010;**31**:989–996.

Bischof P, Irminger-Finger I. The human cytotrophoblastic cell, a mononuclear chameleon. *Int J Biochem Cell Biol* 2005;**37**:1–16.

Blackshaw BP, Rodger D. Why we should not extend the 14-day rule. *J Med Ethics* 2021;**47**:712–714.

Blakeley P, Fogarty N, del Valle I, Wamaitha S, Hu T, Elder K, Snell P, Christie L, Robson P, Niakan K. Defining the three cell lineages of the human blastocyst by single-cell RNA-seq. *Development* 2015; **142**:3613–3165.

Blaschitz A, Hutter H, Dohr G. HLA Class I protein expression in the human placenta. *Early Pregnancy (Cherry Hill)* 2001;**5**:67–69.

Boyd J. *Boyd Collection*. Cambridge, UK: University Cambridge, 1950. https://www.repository.cam.ac.uk/collections/421ae3b9-2c6d-4f34-a124-cc4678557331 (date accessed 20 February).

Buck VU, Gellersen B, Leube RE, Classen-Linke I. Interaction of human trophoblast cells with gland-like endometrial spheroids: a model system for trophoblast invasion. *Hum Reprod* 2015; **30**:906–916.

Buck VU, Kohlen MT, Sternberg AK, Rösing B, Neulen J, Leube R, Classen-Linke I. Steroid hormones and human choriogonadotropin influence the distribution of alpha6-integrin and desmoplakin 1 in gland-like endometrial epithelial spheroids. *Histochem Cell Biol* 2021;**155**:581–591.

Burke SD, Barrette V, Gravel J, Carter A, Hatta K, Zhang J, Chen Z, Leno-Durán E, Bianco J, Leonard S *et al.* Uterine NK cells, spiral artery modification and the regulation of blood pressure during mouse pregnancy. *Am J Reprod Immunol* 2010;**63**:472–481.

Burton G, Jauniaux E. The cytotrophoblastic shell and complications of pregnancy. *Placenta* 2017;**60**:134–139.

Caballero-Campo P, Domínguez F, Coloma J, Meseguer M, Remohí J, Pellicer A, Simón C. Hormonal and embryonic regulation of chemokines IL-8, MCP-1 and RANTES in the human endometrium during the window of implantation. *Mol Hum Reprod* 2002; **8**:375–384.

Cabestre FA, Lefebvre S, Moreau P, Rouas-Friess N, Dausset J, Carosella ED, Paul P. HLA-G expression: immune privilege for tumour cells? *Semin Cancer Biol* 1999;**9**:27–36.

Caluwaerts S, Vercruysse L, Luyten C, Pijnenborg R. Endovascular trophoblast invasion and associated structural changes in uterine spiral arteries of the pregnant rat. *Placenta* 2005;**26**:574–584.

Caniggia I, Mostachfi H, Winter J, Gassmann M, Lye S, Kuliszewski M, Post M. Hypoxia-inducible factor-1 mediates the biological effects of oxygen on human trophoblast differentiation through TGFβ3. *J Clin Invest* 2000;**105**:577–587.

Carp H. Progestogens in luteal support. *Horm Mol Biol Clin Investig* 2020;**42**:143–148.

Carter A. Animal models of human placentation—a review. *Placenta* 2007;**28 Suppl A**:S41–S47.

Carter AM, Enders A, Pijnenborg R. The role of invasive trophoblast in implantation and placentation of primates. *Philos Trans R Soc Lond B Biol Sci* 2015;**370**:1663.

Carter AM, Pijnenborg R. Evolution of invasive placentation with special reference to non-human primates. *Best Pract Res Clin Obstet Gynaecol* 2011;**25**:249–257.

Cartwright JE, Holden DP, Whitley GS. Hepatocyte growth factor regulates human trophoblast motility and invasion: a role for nitric oxide. *Br J Pharmacol* 1999;**128**:181–189.

Cartwright JE, Kenny LC, Dash PR, Crocker IP, Aplin JD, Baker PN, Whitley GS. Trophoblast invasion of spiral arteries: a novel in vitro model. *Placenta* 2002;**23**:232–235.

Carver J, Martin K, Spyropoulou I, Barlow D, Sargent I, Mardon H. An in-vitro model for stromal invasion during implantation of the human blastocyst. *Hum Reprod* 2003;**18**:283–290.

Castel G, Meistermann D, Bretin B, Firmin J, Blin J, Loubersac S, Bruneau A, Chevolleau S, Kilens S, Chariau C *et al.* Induction of human trophoblast stem cells from somatic cells and pluripotent stem cells. *Cell Rep* 2020;**33**:108419.

Castelbaum A, Ying L, Somkuti S, Sun J, Ilesanmi A, Lessey B. Characterization of integrin expression in a well differentiated endometrial adenocarcinoma cell line (Ishikawa). *J Clin Endocrinol Metabol* 1997;**82**:136–142.

Caulfield T, Kamenova K, Ogbogu U, Zarzeczny A, Baltz J, Benjaminy S, Cassar PA, Clark M, Isasi R, Knoppers B *et al.* Research ethics and stem cells: is it time to re-think current approaches to oversight? *EMBO Rep* 2015;**16**:2–6.

Cha J, Sun X, Dey SK. Mechanisms of implantation: Strategies for successful pregnancy. *Nat Med* 2012;**18**:1754–1767.

Chan RW, Schwab K, Gargett CE. Clonogenicity of human endometrial epithelial and stromal cells. *Biol Reprod* 2004;**70**:1738–1750.

Chard T. Frequency of implantation and early pregnancy loss in natural cycles. *Baillieres Clin Obstet Gynaecol* 1991;**5**:179–189.

Chen H, Zou L. IL-24 expression at maternal-fetal interface and its roles in trophoblast invasion. *J Huazhong Univ Sci Technol [Med Sci]* 2008;**28**:456–459.

Chen Y, Siriwardena D, Penfold C, Pavlinek A, Boroviak TE. An integrated atlas of human placental development delineates essential regulators of trophoblast stem cells. *Development* 2022; **149**:dev200171.

Choy MY, Manyonda I. The phagocytic activity of human first trimester extravillous trophoblast. *Hum Reprod* 1998;**13**:2941–2949.

Cinkornpumin JK, Kwon S, Guo Y, Hossain I, Sirois J, Russett CS, Tseng HW, Okae N, Arima T, Duchaine TF *et al.* Naive human embryonic stem cells can give rise to cells with a trophoblast-like transcriptome and methylome. *Stem Cell Rep* 2020;**15**:198–213.

Clark D. Is there any evidence for immunologically mediated or immunologically modifiable early pregnancy failure? *J Assist Reprod Genet* 2003;**20**:63–72.

Clevers H. Modeling development and disease with organoids. *Cell* 2016;**165**:1586–1597.

Conroy G. Stem-cell-derived 'embryos' implanted in monkeys. *Nature* 2023;**616**:422–423.

Cross J. How to make a placenta: mechanisms of trophoblast cell differentiation in mice-a review. *Placenta* 2005;**26 Suppl A**:S3–S9.

Csermely T, Demers LM, Hughes EC. Organ culture of human endometrium: effects of progesterone. *Obstet Gynecol* 1969;**34**:252–259.

Cui Y, Zhao H, Wu S, Li X. Human female reproductive system organoids: applications in developmental biology, disease modelling, and drug discovery. *Stem Cell Rev Rep* 2020;**16**:1173–1184.

Deglincerti A, Etoc F, Guerra MC, Martyn I, Metzger J, Ruzo A, Simunovic M, Yoney A, Brivanlou AH, Siggia E *et al.* Self-organization of human embryonic stem cells on micropatterns. *Nat Protoc* 2016;**11**:2223–2232.

Desai N, Kennard EA, Kniss D, Friedman C. Novel human endometrial cell line promotes blastocyst development. *Fertil Steril* 1994; **61**:760–766.

Devlin H. Synthetic human embryos created in groundbreaking advance. *The Guardian* 2023. https://www.theguardian.com/science/2023/jun/14/synthetic-human-embryos-created-in-groundbreaking-advance (July 2023, date last accessed).

Dietrich B, Kunihs V, Pollheimer J, Knöfler M, Haider S. 3D organoid formation and EVT differentiation of various trophoblastic cell lines. *Placenta* 2023;**133**:19–22.

Dimova T, Nagaeva O, Stenqvist A-K, Hedlund M, Kjellberg E, Strand M, Dehlin E, Mincheva-Nilsson L. Maternal Foxp3 expressing CD4+ CD25+ and CD4+ CD25 regulatory T-cell populations are enriched in human early normal pregnancy decidua: a phenotypic study of paired decidual and peripheral blood samples. *Am J Reprod Immunol* 2011;**66 Suppl 1**:44–56.

Dollar JR, Graham CE, Reyes FI. Postovulatory pre-decidual development in the baboon, chimpanzee, and human. *Am J Primatol* 1982; **3**:307–313.

Dominguez F, Gadea B, Mercader A, Esteban F, Pellicer A, Simón C. Embryologic outcome and secretome profile of implanted

blastocysts obtained after coculture in human endometrial epithelial cells versus the sequential system. *Fertil Steril* 2010;**93**:774–782.e771.

Dong C, Beltcheva M, Gontarz P, Zhang V, Popli P, Fischer LA, Khan SA, Park KM, Yoon EJ, Xing X *et al.* Derivation of trophoblast stem cells from naïve human pluripotent stem cells. *Elife* 2020;**9**:e52504.

Dudley DJ, Hatasaka HH, Branch DW, Hammond E, Mitchell MD. A human endometrial explant system: validation and potential applications. *Am J Obstet Gynecol* 1992;**167**:1774–1780.

Dunk C, Petkovic L, Baczyk D, Rossant J, Winterhager E, Lye S. A novel in vitro model of trophoblast-mediated decidual blood vessel remodeling. *Lab Invest* 2003;**83**:1821–1828.

Eikmans M, van der Keur C, Anholts JDH, Drabbels JJM, van Beelen E, de Sousa Lopes SMC, van der Hoorn ML. Primary trophoblast cultures: characterization of HLA profiles and immune cell interactions. *Front Immunol* 2022;**13**:814019.

Elmore SA, Cochran R, Bolon B, Lubeck B, Mahler B, Sabio D, Ward JM. Histology atlas of the developing mouse placenta. *Toxicol Pathol* 2022;**50**:60–117.

Emera D, Romero R, Wagner G. The evolution of menstruation: a new model for genetic assimilation: Explaining molecular origins of maternal responses to fetal invasiveness. *Bioessays* 2012;**34**:26–35.

Enders AC. Trophoblast differentiation during the transition from trophoblastic plate to lacunar stage of implantation in the rhesus monkey and human. *Am J Anat* 1989;**186**:85–98.

Enders AC. *Overview of the Morphology of Implantation in Primates. Serono Symposia, USA*. New York: Springer, 1993.

Enders AC. Transition from lacunar to villous stage of implantation in the macaque, including establishment of the trophoblastic shell. *Acta Anat (Basel)* 1995;**152**:151–169.

Enders AC. Trophoblast-uterine interactions in the first days of implantation: models for the study of implantation events in the human. *Semin Reprod Med* 2000;**18**:255–263.

Enders AC, Blankenship TN. Comparative placental structure. *Adv Drug Deliv Rev* 1999;**38**:3–15.

Enders AC, Hendrickx AG, Schlafke S. Implantation in the rhesus monkey: initial penetration of endometrium. *Am J Anat* 1983;**167**:275–298.

Enders AC, Lopata A. Implantation in the marmoset monkey: expansion of the early implantation site. *Anat Rec* 1999;**256**:279–299.

Enders AC, Schlafke S. Cytological aspects of trophoblast-uterine interaction in early implantation. *Am J Anat* 1969;**125**:1–29.

European IVF Monitoring Consortium (EIM) for ESHRE; Smeenk J, Wyns C, De Geyter C, Kupka M, Bergh C, Cuevas Saiz I, De Neubourg D, Rezabek K, Tandler-Schneider A, Rugescu I *et al.* ART in Europe, 2019: results generated from European registries by ESHRE. *Human Reproduction* 2023;**38**:2321–2338.

Evans J, Walker K, Bilandzic M, Kinnear S, Salamonsen LA. novel "embryo-endometrial" adhesion model can potentially predict "receptive" or "non-receptive" endometrium. *J Assist Reprod Genet* 2020;**37**:5–16.

Fan Y, Min Z, Alsolami S, Ma Z, Zhang E, Chen W, Zhong K, Pei W, Kang X, Zhang P *et al.* Generation of human blastocyst-like structures from pluripotent stem cells. *Cell Discov* 2021;**7**:81.

Feng HC, Choy M, Deng W, Wong HL, Lau WM, Cheung AN, Ngan HY, Tsao SW. Establishment and characterization of a human first-trimester extravillous trophoblast cell line (TEV-1). *J Soc Gynecol Investig* 2005;**12**:e21–32–e32.

Fisher SJ, Cui TY, Zhang L, Hartman L, Grahl K, Zhang GY, Tarpey J, Damsky CH. Adhesive and degradative properties of human placental cytotrophoblast cells in vitro. *J Cell Biol* 1989;**109**:891–902.

Fitzgerald HC, Schust D, Spencer TE. In vitro models of the human endometrium: evolution and application for women's health. *Biol Reprod* 2021;**104**:282–293.

Foreman A, Liddell K, Franklin S, Jackson E, Rozeik C, Niakan K. Human embryo models: the importance of national policy and governance review. *Curr Opin Genet Dev* 2023;**82**:102103.

Fraccaroli L, Alfieri J, Larocca L, Calafat M, Mor G, Perez Leiro C, Ramhorst R. A potential tolerogenic immune mechanism in a trophoblast cell line through the activation of chemokine-induced T cell death and regulatory T cell modulation. *Hum Reprod* 2009;**24**:166–175.

Francés-Herrero E, Juárez-Barber E, Campo H, López-Martínez S, de Miguel-Gómez L, Faus A, Pellicer A, Ferrero H, Cervelló I. Improved models of human endometrial organoids based on hydrogels from decellularized endometrium. *J Pers Med* 2021;**11**:504.

Frank H-G, Gunawan B, Ebeling-Stark I, Schulten H-J, Funayama H, Cremer U, Huppertz B, Gaus G, Kaufmann P, Füzesi L. Cytogenetic and DNA-fingerprint characterization of choriocarcinoma cell lines and a trophoblast/choriocarcinoma cell hybrid. *Cancer Genet Cytogenet* 2000;**116**:16–22.

Fraser R, Smith R, Lin CJ. A 3D endometrial organotypic model simulating the acute inflammatory decidualisation initiation phase with epithelial induction of the key endometrial receptivity marker, integrin αVβ3′. *Hum Reprod Open* 2021;**2021**:hoab034.

Fritz R, Jain C, Randall Armant D. Cell signaling in trophoblastuterine communication. *Int J Dev Biol* 2014;**58**:261–271.

Fu Y-Y, Gao W-L, Chen M, Chai K, Wang Y-L, Chen L-M. Prostasin regulates human placental trophoblast cell proliferation via the epidermal growth factor receptor signaling pathway. *Hum Reprod* 2010;**25**:623–632.

Galán A, O'Connor JE, Valbuena D, Herrer R, Remohí J, Pampfer S, Pellicer A, Simón C. The human blastocyst regulates endometrial epithelial apoptosis in embryonic adhesion. *Biol Reprod* 2000;**63**:430–439.

Gao J, Song T, Che D, Li C, Jiang J, Pang J, Yang Y, Li, P, Goma. The effect of bisphenol a exposure onto endothelial and decidualized stromal cells on regulation of the invasion ability of trophoblastic spheroids in in vitro co-culture model. *Biochem Biophys Res Commun* 2019;**516**:506–514.,

Gellersen B, Reimann K, Samalecos A, Aupers S, Bamberger A-M. Invasiveness of human endometrial stromal cells is promoted by decidualization and by trophoblast-derived signals. *Hum Reprod* 2010;**25**:862–873.

Genbacev O, Donne M, Kapidzic M, Gormley M, Lamb J, Gilmore J, Larocque N, Goldfien G, Zdravkovic T, McMaster M *et al.* Establishment of human trophoblast progenitor cell lines from the chorion. *Stem Cells* 2011;**29**:1427–1436.

Genbacev O, Schubach SA, Miller RK. Villous culture of first trimester human placenta-model to study extravillous trophoblast (EVT) differentiation. *Placenta* 1992;**13**:439–461.

Gerami-Naini B, Dovzhenko O, Durning M, Wegner FH, Thomson JA, Golos TG. Trophoblast differentiation in embryoid bodies derived from human embryonic stem cells. *Endocrinology* 2004;**145**:1517–1524.

Gerri C, Menchero S, Mahadevaiah CK, Turner JMA, Niakan KK. Human embryogenesis: a comparative perspective. *Annu Rev Cell Dev Biol* 2020;**36**:411–440.

Gonzalez M, Neufeld J, Reimann K, Wittmann S, Samalecos A, Wolf A, Bamberger A-M, Gellersen B. Expansion of human trophoblastic spheroids is promoted by decidualized endometrial stromal cells and enhanced by heparin-binding epidermal growth factor-like growth factor and interleukin-1β. *Mol Hum Reprod* 2011;**17**:421–433.

Graham CH, Hawley TS, Hawley RG, MacDougall JR, Kerbel RS, Khoo N, Lala PK. Establishment and characterization of first trimester human trophoblast cells with extended lifespan. *Exp Cell Res* 1993;**206**:204–211.

Grasso E, Gori S, Soczewski E, Fernández L, Gallino L, Vota D, Martínez G, Irigoyen M, Ruhlmann C, Lobo TF *et al.* Impact of the reticular stress and unfolded protein response on the inflammatory response in endometrial stromal cells. *Sci Rep* 2018;**8**:12274.

Greenbaum S, Averbukh I, Soon E, Rizzuto G, Baranski A, Greenwald NF, Kagel A, Bosse M, Jaswa EG, Khair Z *et al.* A spatially resolved timeline of the human maternal-fetal interface. *Nature* 2023; **619**:595–605.

Grewal S, Carver J, Ridley A, Mardon H. Human endometrial stromal cell rho GTPases have opposing roles in regulating focal adhesion turnover and embryo invasion in vitro. *Biol Reprod* 2010;**83**:75–82.

Guzman-Genuino R, Dimova T, You Y, Hayball AP, Mor J, Diener G. K. Trophoblasts promote induction of a regulatory phenotype in B cells that can protect against detrimental T cell-mediated inflammation. *Am J Reprod Immunol* 2019;**82**:e13187.

Hackmon R, Pinnaduwage L, Zhang J, Lye SJ, Geraghty DE, Dunk CE. Definitive class I human leukocyte antigen expression in gestational placentation: HLA-F, HLA-E, HLA-C, and HLA-G in extravillous trophoblast invasion on placentation, pregnancy, and parturition. *Am J Reprod Immunol* 2017;**77**:e12643.

Haider S, Meinhardt G, Saleh L, Kunihs V, Gamperl M, Kaindl U, Ellinger A, Burkard TR, Fiala C, Pollheimer J *et al.* Self-renewing trophoblast organoids recapitulate the developmental program of the early human placenta. *Stem Cell Rep* 2018;**11**:537–551.

Hamilton WJ, Boyd JD. Development of the human placenta in the first three months of gestation. *J Anat* 1960;**94**:297–328.

Hamzelou J. 'This is simply mind-blowing': monkeys implanted with synthetic embryos made from stem cells. *MIT Technol Rev* 2023. https://www.technologyreview.com/2023/04/06/1071112/synthetic-embryos-have-been-implanted-into-monkey-wombs/ (July 2023, date last accessed).

Hanashi H, Shiokawa S, Akimoto Y, Sakai K, Sakai K, Suzuki N, Kabir-Salmani M, Nagamatsu S, Iwashita M, Nakamura Y. Physiologic role of decidual beta 1 integrin and focal adhesion kinase in embryonic implantation. *Endocr J* 2003;**50**:189–198.

Handschuh K, Guibourdenche J, Tsatsaris V, Guesnon M, Laurendeau I, Evain-Brion D, Fournier T. Human chorionic gonadotropin produced by the invasive trophoblast but not the villous trophoblast promotes cell invasion and is down-regulated by peroxisome proliferator-activated receptor-gamma. *Endocrinology* 2007;**148**:5011–5019.

Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, Prus D, Cohen-Daniel L, Arnon T, Manaster I *et al.* Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. *Nat Med* 2006;**12**:1065–1074.

Hannan N, Jones R, White C, Salamonsen L. Chemokines, CX3CL1, CCL14, and CCL4, promote human trophoblast migration at the feto-maternal interface. *Biol Reprod* 2006;**74**:896–904.

Hannan N, Paiva P, Dimitriadis E, Salamonsen L. Models for study of human embryo implantation: choice of cell lines? *Biol Reprod* 2010;**82**:235–245.

Harris LK, Benagiano M, Brosens DMM, Benagiano I. G. Placental bed research: II. Functional and immunological investigations of the placental bed. *Am J Obstet Gynecol* 2019;**221**:457–469.

Harun R, Ruban L, Matin M, Draper J, Jenkins NM, Liew G, Andrews P, Li T, Laird S, Moore H. Cytotrophoblast stem cell lines derived from human embryonic stem cells and their capacity to mimic invasive implantation events. *Hum Reprod* 2006;**21**:1349–1358.

Hazan AD, Smith S, Jones RL, Whittle W, Lye SJ, Dunk CE. Vascular-leukocyte interactions: mechanisms of humandecidual spiral artery remodeling in vitro. *Am J Pathol* 2010;**177**:1017–1030.

He D, Zeng H, Chen J, Xiao L, Zhao Y, Liu N. H19 regulates trophoblastic spheroid adhesion by competitively binding to let-7. *Reproduction* 2019;**157**:423–430.

Hemberger M, Hanna C, Dean W. Mechanisms of early placental development in mouse and humans. *Nat Rev Genet* 2020;**21**:27–43.

Heneweer C, Adelmann H, Kruse L, Denker HW, Thie M. Human uterine epithelial RL95-2 cells reorganize their cytoplasmic architecture with respect to Rho protein and F-actin in response to trophoblast binding. *Cells Tissues Organs* 2003;**175**:1–8.

Heneweer C, Schmidt M, Denker HW, Thie M. Molecular mechanisms in uterine epithelium during trophoblast binding: the role of small GTPase RhoA in human uterine Ishikawa cells. *J Exp Clin Assist Reprod* 2005;**2**:4.

Hennessy M. Filial attachment and its disruption: insights from the guinea pig. *Dev Psychobiol* 2014;**56**:1747–1754.

Hertig AT, Rock J, Adams EC. A description of 34 human ova within the first 17 days of development. *Am J Anat* 1956;**98**:435–493.

Hevir-Kene N, Rižner T. The endometrial cancer cell lines Ishikawa and HEC-1A, and the control cell line HIEEC, differ in expression of estrogen biosynthetic and metabolic genes, and in androstenedione and estrone-sulfate metabolism. *Chem Biol Interact* 2015; **234**:309–319.

Heyderman E, Chapman DV, Richardson TC, Calvert I, Rosen SW. Human chorionic gonadotrophin and human placental lactogen in extragonodal tumors. *Cancer* 1985;**56**:2674–2682.

Hibaoui Y, Feki A. Organoid models of human endometrial development and disease. *Front Cell Dev Biol* 2020;**8**:84.

Hiby SE, Apps R, Sharkey AM, Farrell LE, Gardner L, Mulder A, Claas FH, Walker JJ, Redman CW, Morgan L *et al.* Maternal activating KIRs protect against human reproductive failure mediated by fetal HLA-C2. *J Clin Invest* 2010;**120**:4102–4110.

Hiden U, Wadsack C, Prutsch N, Gauster M, Weiss U, Frank H-G, Schmitz U, Fast-Hirsch C, Hengstschläger M, Pötgens A *et al.* The first trimester human trophoblast cell line ACH-3P: A novel tool to study autocrine/paracrine regulatory loops of human trophoblast subpopulations—TNF-α stimulates MMP15 expression. *BMC Dev Biol* 2007;**7**:137.

Ho H, Singh H, Aljofan M, Nie G. A high-throughput in vitro model of human embryo attachment. *Fertil Steril* 2012;**97**:974–978.

Hohn H, Linke M, Denker HW. Adhesion of trophoblast to uterine epithelium as related to the state of trophoblast differentiation: In vitro studies using cell lines. *Mol Reprod Dev* 2000;**57**:135–145.

Holmberg J, Haddad S, Wunsche V, Yang Y, Aldo P, Gnainsky Y, Granot I, Dekel N, Mor G. An in vitro model for the study of human implantation. *Am J Reprod Immunol* 2012;**67**:169–178.

Horii M, Bui T, Touma O, Cho HY, Parast MM. An improved two-step protocol for trophoblast differentiation of human pluripotent stem cells. *Curr Protoc Stem Cell Biol* 2019;**50**:e96.

Horii M, Li Y, Wakeland AK, Pizzo DP, Nelson KK, Sabatini K, Laurent LC, Liu Y, Parast MM. Human pluripotent stem cells as a model of trophoblast differentiation in both normal development and disease. *Proc Natl Acad Sci USA* 2016;**113**:E3882–E3891.

Horii M, Touma O, Bui T, Parast MM. Modeling human trophoblast, the placental epithelium at the maternal fetal interface. *Reproduction* 2020;**160**:R1–R11.

Hurlbut JB, Hyun I, Levine A, Lovell-Badge R, Lunshof J, Matthews K, Mills P, Murdoch A, Pera M, Scott C *et al.* Revisiting the Warnock rule nat. *Nat Biotechnol* 2017;**35**:1029–1042.

James J. *Overview of Human Implantation*. Pathobiology of Human Disease: A Dynamic Encyclopedia of Disease Mechanisms. Amsterdam, The Netherlands: Academic Press, 2014.

James J, Tun W, Clark A. Quantifying trophoblast migration: in vitro approaches to address in vivo situations. *Cell Adh Migr* 2016; **10**:77–87.

James JL, Lissaman A, Nursalim YNS, Chamley LW. Modelling human placental villous development: designing cultures that reflect anatomy. *Cell Mol Life Sci* 2022;**79**:384.

Jauniaux E, Jurkovic D. Placenta accreta: pathogenesis of a 20th century iatrogenic uterine disease. *Placenta* 2012;**33**:244–251.

John N, Linke M, Denker HW. Quantitation of human choriocarcinoma spheroid attachment to uterine epithelial cell monolayers. *In Vitro Cell Dev Biol Anim* 1993;**29**:461–468.

Juch H, Blaschitz A, Dohr G, Hutter H. HLA class I expression in the human placenta. *Wien Med Wochenschr* 2012;**162**:196–200.

Kagawa H, Javali A, Khoei H, Sommer T, Sestini G, Novatchkova M, Op Reimer Y, Castel G, Bruneau A, Maenhoudt N *et al.* Human blastoids model blastocyst development and implantation. *Nature* 2022;**601**:600–605.

Karvas RM, Khan SA, Verma S, Yin Y, Kulkarni D, Dong C, Park K-M, Chew B, Sane E, Fischer LA *et al.* Stem-cell derived trophoblast organoids model human placental development and susceptibility to emerging pathogens. *Cell Stem Cell* 2022;**29**:810–825.e818.

Karvas RM, Zemke J, Ali SS, Upton E, Sane E, Fischer LA, Dong C, Park KM, Wang F, Park K *et al.* 3D-cultured blastoids model human embryogenesis from pre-implantation to early gastrulation stages. *Cell Stem Cell* 2023;**30**:1148–1165.e1147.

King A, Thomas L, Bischof P. Cell culture models of trophoblast II: trophoblast cell lines—a workshop report. *Placenta* 2000;**21 Suppl A**:S113–S119.

Kinnear S, Salamonsen LA, Francois M, Harley V, Evans J. Uterine SOX17: a key player in human endometrial receptivity and embryo implantation. *Sci Rep* 2019;**9**:15495.

Kliman HJ, Feinberg RF, Haimowitz JE. Human trophoblast-endometrial interactions in an in vitro suspension culture system. *Placenta* 1990;**11**:349–367.

Kliman HJ, Nestler JE, Sermasi E, Sanger JM, Strauss JF. Purification, characterization, and in vitro differentiation of cytotrophoblasts from human term placentae. *Endocrinology* 1986;**118**:1567–1582.

Kobayashi N, Okae H, Hiura H, Kubota N, Kobayashi EH, Shibata S, Oike A, Hori T, Kikutake C, Hamada H *et al.* The microRNA cluster C19MC confers differentiation potential into trophoblast lineages upon human pluripotent stem cells. *Nat Commun* 2022;**13**:3071.

Kohler P, Bridson W. Isolation of hormone-producing clonal lines of human choriocarcinoma. *J Clin Endocrinol Metab* 1971;**32**:683–687.

Kojima J, Ono M, Kuji N, Nishi H. Human chorionic villous differentiation and placental development. *Int J Mol Sci* 2022;**23**:8003.

Koot Y, Teklenburg G, Salker MS, Brosens JJ, Macklon NS. Molecular aspects of implantation failure. *Biochim Biophys Acta* 2012; **1822**:1943–1950.

Kovats S, Main E, Librach C, Stubblebine M, Fisher S, Demars R. Class I antigen, HLA-G, expressed in human trophoblasts. *Science* 1990; **248**:220–223.

Krikun G, Mor G, Alvero A, Guller S, Schatz F, Sapi E, Rahman M, Caze R, Qumsiyeh M, Lockwood CJ. A novel immortalized human endometrial stromal cell line with normal progestational response. *Endocrinology* 2004;**145**:2291–2296.

Kurarmoto H, Hamano M, Imai M. HEC-1 cells. *Hum Cell* 2002; **15**:81–95.

Kyo S, Nakamura M, Kiyono T, Maida Y, Kanaya T, Tanaka M, Yatabe N, Inoue M. Successful immortalization of endometrial glandular cells with normal structural and functional characteristics. *Am J Pathol* 2003;**163**:2259–2269.

Lalitkumar P, Lalitkumar S, Meng C, Stavreus-Evers A, Hambiliki F, Bentin-Ley U, Gemzell-Danielsson K. Mifepristone, but not levonorgestrel, inhibits human blastocyst attachment to an in vitro endometrial three-dimensional cell culture model. *Hum Reprod* 2007;**22**:3031–3037.

Landgren B-M, Johannisson E, Stavreus-Evers A, Hamberger L, Eriksson H. A new method to study the process of implantation of a human blastocyst in vitro. *Fertil Steril* 1996;**65**:1067–1070.

Lee CQ, Gardner L, Turco M, Zhao N, Murray MJ, Coleman N, Rossant J, Hemberger M, Moffett A. What is trophoblast? A combination of criteria define human first-trimester trophoblast. *Stem Cell Reports* 2016;**6**:257–272.

Lee K, DeMayo F. Animal models of implantation. *Reproduction* 2004; **128**:679–695.

Lee YL, Fong S-W, Chen AC, Li T, Yue C, Lee CL, Ng E, Yeung W, Lee K-F. Establishment of a novel human embryonic stem cell-derived trophoblastic spheroid implantation model. *Hum Reprod* 2015;**30**:2614–2626.

Leiser R, Kaufmann P. Placental structure: in a comparative aspect. *Exp Clin Endocrinol* 1994;**102**:122–134.

Levy R, Paşca S. Have organoids and assembloids taught us about the pathophysiology of neuropsychiatric disorders? *Biol Psychiatry* 2023;**93**:632–641.

Li J, Zhu Q, Cao J, Liu Y, Lu Y, Sun Y, Li Q, Huang Y, Shang S, Bian X et al Cynomolgus monkey embryo model captures gastrulation and early pregnancy. *Cell Stem Cell* 2023;**30**:362–377.e7.

Li R, Zhong C, Yu Y, Liu H, Sakurai M, Yu L, Min Z, Shi L, Wei Y, Takahashi Y *et al.* Generation of blastocyst-like structures from mouse embryonic and adult cell cultures. *Cell* 2019;**179**: 687–702.e618.

Li X, Kodithuwakku S, Chan RWS, Yeung WSB, Yao Y, Ng EHY, Chiu PCN, Lee CL. Three-dimensional culture models of human endometrium for studying trophoblast-endometrium interaction during implantation. *Reprod Biol Endocrinol* 2022;**20**:120.

Li Y, Li J, Yang D. ADAM7 promotes the proliferation and invasion in trophoblast cells. *Exp Mol Pathol* 2021;**121**:104659.

Li Y, Moretto-Zita M, Soncin F, Wakeland A, Wolfe L, Leon-Garcia S, Pandian R, Pizzo D, Cui L, Nazor K *et al.* BMP4-directed trophoblast differentiation of human embryonic stem cells is mediated through a ΔNp63+ cytotrophoblast stem cell state. *Development* 2013;**140**:3965–3976.

Li Z, Kurosawa O, Iwata H. Establishment of human trophoblast stem cells from human induced pluripotent stem cell-derived cystic cells under micromesh culture. *Stem Cell Res Ther* 2019; **10**:245.

Lindenberg S, Hyttel P, Sjøgren A, Greve T. A comparative study of attachment of human, bovine and mouse blastocysts to uterine epithelial monolayer. *Hum Reprod* 1989;**4**:446–456.

Liu S, Yang X, Liu Y, Wang X, Yan Q. SLeX/L-selectin mediates adhesion in vitro implantation model. *Mol Cell Biochem* 2011; **350**:185–192.

Liu X, Ouyang JF, Rossello FJ, Tan JP, Davidson KC, Valdes DS, Schröder J, Sun YBY, Chen J, Knaupp AS *et al.* Reprogramming roadmap reveals route to human induced trophoblast stem cells. *Nature* 2020;**586**:101–107.

Liu X, Tan J, Schröder J, Aberkane A, Ouyang J, Mohenska M, Lim M, Sun Y, Chen J, Sun G *et al.* Modelling human blastocysts by reprogramming fibroblasts into iBlastoids. *Nature* 2021; **591**:627–632.

Loke Y. *Immunology and Immunopathology of the Human Foetal-Maternal Interaction*. The Netherlands: Elsevier/North-Holland Biomedical Press, 1978.

Loke Y. Heterogeneity of human trophoblast populations. Immunobiology of human trophoblast. In G. P. Talwar (ed). *Contraception Research for Today and the Nineties: Progress in Birth Control Vaccines*. New York: Springer, 1988, 133–142.

Loke YW, King A. *Human Implantation: Cell Biology and Immunology*. Cambridge, UK: Cambridge University Press, 1995.

Ma H, Zhai J, Wan H, Jiang X, Wang X, Wang L, Xiang Y, He X, Zhao Z-A, Zhao B *et al.* In vitro culture of cynomolgus monkey embryos beyond early gastrulation. *Science* 2019;**366**:eaax7890.

Macklon NS, Geraedts J, Fauser BC. Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. *Hum Reprod Update* 2002;**8**:333–343.

Malassiné A, Frendo JL, Evain-Brion D. A comparison of placental development and endocrine functions between the human and mouse model. *Hum Reprod Update* 2003;**9**:531–539.

Manchorova D, Papadopoulou M, Alexandrova M, Dimitrova V, Djerov L, Zapryanova S, Dimitrova P, Vangelov I, Vermijlen D, Dimova T. Human decidual gamma/delta T cells possess unique effector and TCR repertoire profiles during pregnancy. *Cell Immunol* 2022;**382**:104634.

Martin J, Jasper M, Valbuena D, Meseguer M, Remohı J, Pellicer A, Simon C. Increased adhesiveness in cultured endometrial-derived cells is related to the absence of moesin expression. *Biol Reprod* 2000;**63**:1370–1376.

Masuda A, Katoh N, Nakabayashi K, Kato K, Sonoda K, Kitade M, Takeda S, Hata K, Tomikawa J. An improved method for isolation of epithelial and stromal cells from the human endometrium. *J Reprod Dev* 2016;**62**:213–218.

Matthews K, Iltis A, Marquez NG, Wagner DS, Robert JS, de Melo-Martín I, Bigg M, Franklin S, Holm S, Metzler I *et al.* Rethinking human embryo research policies. *Hastings Cent Rep* 2021;**51**:47–51.

Matthews K, Moralí D. National human embryo and embryoid research policies: A survey of 22 top research-intensive countries. *Regen Med* 2020;**15**:1905–1917.

Mazid MA, Ward C, Luo Z, Liu C, Li Y, Lai Y, Wu L, Li J, Jia W, Jiang Y *et al.* Rolling back human pluripotent stem cells to an eight-cell embryo-like stage. *Nature* 2022;**605**:315–324.

McConkey C, Delorme-Axford E, Nickerson C, Sik Kim K, Sadovsky Y, Boyle J, Coyne CA. three-dimensional culture system recapitulates placental syncytiotrophoblast development and microbial resistance. *Sci Adv* 2016;**2**:e1501462.

McCormick J, Whitley G, Le Bouteiller P, Cartwright J. Soluble HLA-G regulates motility and invasion of the trophoblast-derived cell line SGHPL-4. *Hum Reprod* 2009;**24**:1339–1345.

McCully S. The time has come to extend the 14-day limit. *J Med Ethics* 2021;medethics-2020;106406.

Medawar P. *Some Immunological and Endocrinological Problems Raised by the Evolution of Viviparity in Vertebrates*. New York: Academic Press, 1953.

Menezo Y, Sakkas D, Janny L. Co-culture of the early human embryo: factors affecting human blastocyst formation in vitro. *Microsc Res Tech* 1995;**32**:50–56.

Meseguer M, Aplin J, Caballero-Campo P, O'Connor J, Martín J, Remohí J, Pellicer A, Simón C. Human endometrial mucin MUC1 is up-regulated by progesterone and down-regulated in vitro by the human blastocyst. *Biol Reprod* 2001;**64**:590–601.

Miller RK, Genbacev O, Turner MA, Aplin JD, Caniggia I, Huppertz B. Human placental explants in culture: approaches and assessments. *Placenta* 2005;**26**:439–448.

Mirabelli P, Coppola L, Salvatore M. Cancer cell lines are useful model systems for medical research. *Cancers (Basel)* 2019;**11**:1098.

Mischler A, Karakis V, Mahinthakumar J, Carberry CK, San Miguel A, Rager JE, Fry RC, Rao BM. Two distinct trophectoderm lineage stem cells from human pluripotent stem cells. *J Biol Chem* 2021;**296**:100386.

Miyazaki K, Dyson MT, Coon VJS, Furukawa Y, Yilmaz BD, Maruyama T, Bulun S. Generation of progesterone-responsive endometrial stromal fibroblasts from human induced pluripotent stem cells: role of the WNT/CTNNB1 pathway. *Stem Cell Rep* 2018;**11**:1136–1155.

Mo B, Vendrov A, Palomino W, DuPont B, Apparao K, Lessey B. ECC-1 cells: a well-differentiated steroid-responsive endometrial cell line with characteristics of luminal epithelium. *Biol Reprod* 2006;**75**:387–394.

Moffett A, Loke C. Implantation, embryo-maternal interactions, immunology and modulation of the uterine environment—a workshop report. *Placenta* 2006;**27 Suppl A**:S54–5.

Moffett A, Shreeve N. Local immune recognition of trophoblast in early human pregnancy: controversies and questions. *Nat Rev Immunol* 2023;**23**:222–235.

Morris S. Human embryos cultured in vitro to 14 days. *Open Biol* 2017;**7**:170003.

Moser G, Gauster M, Orendi K, Glasner A, Theuerkauf R, Huppertz B. Endoglandular trophoblast, an alternative route of trophoblast invasion? Analysis with novel confrontation co-culture models. *Hum Reprod* 2010;**25**:1127–1136.

Mulaudzi PE, Abrahamse H, Crous A. Insights on three dimensional organoid studies for stem cell therapy in regenerative medicine. *Stem Cell Rev Rep* 2024;**20**:509–523.

Muter J, Kong C-S, Brosens J. The role of decidual subpopulations in implantation, menstruation and miscarriage. *Front Reprod Health* 2021;**3**:804921.

Myers R. The pathology of the rhesus monkey placenta. *Acta Endocrinol Suppl (Copenh)* 1972;**166**:221–257.

Nakamura T, Fujiwara K, Saitou M, Tsukiyama T. Non-human primates as a model for human development. *Stem Cell Rep* 2021;**16**:1093–1103.

Nakamura T, Okamoto I, Sasaki K, Yabuta Y, Iwatani C, Tsuchiya H, Seita Y, Nakamura S, Yamamoto T, Saitou M. A developmental coordinate of pluripotency among mice, monkeys and humans. *Nature* 2016;**537**:57–62.

Nakashima A, Higashisaka K, Kusabiraki T, Aoki A, Ushijima A, Ono Y, Tsuda S, Shima T, Yoshino O, Nagano K *et al.* Autophagy is a new protective mechanism against the cytotoxicity of platinum nanoparticles in human trophoblasts. *Sci Rep* 2019;**9**:5478.

Niakan K, Eggan K. Analysis of human embryos from zygote to blastocyst reveals distinct gene expression patterns relative to the mouse. *Dev Biol* 2013;**375**:54–64.

Nicolas P, Etoc F, Brivanlou AH. The ethics of human-embryoids model: a call for consistency. *J Mol Med (Berl)* 2021;**99**:569–579.

Nikitina TV, Lebedev I. Stem cell-based trophoblast models to unravel the genetic causes of human miscarriages. *Cells* 2022;**11**:1923.

Nishida M. The Ishikawa cells from birth to the present. *Hum Cell* 2002;**15**:104–117.

Nishiguchi A, Gilmore C, Sood A, Matsusaki M, Collett G, Tannetta D, Sargent IL, McGarvey J, Halemani ND, Hanley J *et al.* In vitro placenta barrier model using primary human trophoblasts, underlying connective tissue and vascular endothelium. *Biomaterials* 2019;**192**:140–148.

Norwitz ER, Schust DJ, Fisher SJ. Implantation and the survival of early pregnancy. *N Engl J Med* 2001;**345**:1400–1408.

Ojosnegros S, Seriola A, Godeau A, Veiga A. Embryo implantation in the laboratory: an update on current techniques. *Hum Reprod Update* 2021;**27**:501–530.

Okae H, Toh H, Sato T, Hiura H, Takahashi S, Shirane K, Kabayama Y, Suyama M, Sasaki H, Arima T. Derivation of human trophoblast stem cells. *Cell Stem Cell* 2018;**22**:50–63.e6.

Omi H, Okamoto A, Nikaido T, Urashima M, Kawaguchi R, Umehara N, Sugiura K, Saito M, Kiyono T, Tanaka T. Establishment of an immortalized human extravillous trophoblast cell line by

retroviral infection of E6/E7/hTERT and its transcriptional profile during hypoxia and reoxygenation. *Int J Mol Med* 2009;**23**:229–236.
Oura S, Hamilton J, Wu J. Recent advances in stem cell-based blastocyst models. *Curr Opin Genet Dev* 2023;**81**:102088.
Paiva P, Salamonsen L, Manuelpillai U, Dimitriadis E. Interleukin 11 inhibits human trophoblast invasion indicating a likely role in the decidual restraint of trophoblast invasion during placentation. *Biol Reprod* 2009;**80**:302–310.
Papúchová H, Meissner T, Li Q, Strominger JL, Tilburgs T. The dual role of HLA-C in tolerance and immunity at the maternal-fetal interface. *Front Immunol* 2019;**10**:2730.
Pastuschek J, Nonn O, Gutiérrez-Samudio R, Murrieta-Coxca J, Müller J, Sanft J, Huppertz B, Markert U, Groten T, Morales-Prieto D. Molecular characteristics of established trophoblast-derived cell lines. *Placenta* 2021;**108**:122–133.
Pattillo R, Gey G, Delfs E, Mattingly R. Human hormone production in vitro. *Science* 1968;**159**:1467–1469.
Pavan L, Tarrade A, Hermouet A, Delouis C, Titeux M, Vidaud M, Thérond P, Evain-Brion D, Fournier T. Human invasive trophoblasts transformed with simian virus 40 provide a new tool to study the role of PPARgamma in cell invasion process. *Carcinogenesis* 2003;**24**:1325–1336.
Petropoulos S, Edsgärd D, Reinius B, Deng Q, Panula S, Codeluppi S, Plaza Reyes A, Linnarsson S, Sandberg R, Lanner F. Single-cell RNA-Seq reveals lineage and X chromosome dynamics in human preimplantation embryos. *Cell* 2016;**165**:1012–1026.
Poloski E, Oettel A, Ehrentraut S, Luley L, Costa S, Zenclussen A, Schumacher A. JEG-3 trophoblast cells producing human chorionic gonadotropin promote conversion of human CD4+FOXP3- T cells into CD4+FOXP3+ Regulatory T cells and Foster T cell suppressive activity. *Biol Reprod* 2016;**94**:106–111.
Popovic M, Dhaenens L, Taelman J, Dheedene A, Bialecka M, De Sutter P, Chuva de Sousa Lopes SM, Menten B, Heindryckx B. Extended in vitro culture of human embryos demonstrates the complex nature of diagnosing chromosomal mosaicism from a single trophectoderm biopsy. *Hum Reprod* 2019;**34**:758–769.
Popovici R, Betzler N, Krause M, Luo M, Jauckus J, Germeyer A, Bloethner S, Schlotterer A, Kumar R, Strowitzki T *et al.* Gene expression profiling of human endometrialtrophoblast interaction in a coculture model. *Endocrinology* 2006;**147**:5662–5675.
Powell K. What's next for lab-grown human embryos? *Nature* 2021;**597**:22–24.
Proll J, Blaschitz A, Hutter H, Dohr G. First trimester human endovascular trophoblast cells express both HLA-C and HLA-G. *American J Rep Immunol* 1999;**42**:30–36.
Racicot KE, Wünsche V, Auerbach B, Aldo P, Silasi M, Mor G. Human chorionic gonadotropin enhances trophoblast-epithelial interaction in an in vitro model of human implantation. *Reprod Sci* 2014;**21**:1274–1280.
Ramathal CY, Bagchi I, Taylor RN, Bagchi MK. Endometrial decidualization: of mice and men. *Semin Reprod Med* 2010;**28**:17–26.
Rawlings T, Makwana K, Taylor D, Molè M, Fishwick K, Tryfonos M, Odendaal J, Hawkes A, Zernicka-Goetz M, Hartshorne G *et al.* Modelling the impact of decidual senescence on embryo implantation in human endometrial assembloids. *Elife* 2021a;**10**:e69603.
Rawlings T, Makwana K, Tryfonos M, Lucas E. Organoids to model the endometrium: implantation and beyond. *Reprod Fertil* 2021b;**2**:R85–R101.
Real LM, Cabrera T, Canton J, Oliva R, Ruiz-Cabello F, Garrido F. Looking for HLA-G expression in human tumours. *J Reprod Immunol* 1999;**43**:263–273.
Reiter J, Drendel H, Chakraborty S, Schellinger M, Lee M-J, Mor G. Cytogenetic features of human trophoblast cell lines SWAN-71 and 3A-subE. *Placenta* 2017;**52**:17–20.
Rinehart CA, Lyn-Cook BD, Kaufman DG. Gland formation from human endometrial epithelial cells in vitro. *In Vitro Cell Dev Biol* 1988;**24**:1037–1041.
Rivron N, Pera M, Rossant J, Martinez Arias A, Zernicka-Goetz M, Fu J, van den Brink S, Bredenoord A, Dondorp W, de Wert G *et al.* Debate ethics of embryo models from stem cells. *Nature* 2018;**564**:183–185.
Rivron NC, Martinez Arias A, Pera MF, Moris N, M'hamdi HI. An ethical framework for human embryology with embryo models. *Cell* 2023;**186**:3548–3557.
Roberts J, Escudero C. The placenta in preeclampsia. *Pregnancy Hypertens* 2012;**2**:72–83.
Robertson S, Moldenhauer L. Immunological determinants of implantation success. *Int J Dev Biol* 2014;**58**:205–217.
Rong-Hao L, Luo S, Zhuang LZ. Establishment and characterization of a cytotrophoblast cell line from normal placenta of human origin. *Hum Reprod* 1996;**11**:1328–1333.
Rossant J, Tam P. Exploring early human embryo development. *Science* 2018;**360**:1075–1076.
Rothbauer M, Patel N, Gondola H, Siwetz M, Huppertz B, Ertl P. A comparative study of five physiological key parameters between four different human trophoblast-derived cell lines. *Sci Rep* 2017;**7**:5892.
Ruane P, Buck C, Babbington R, Aboussahoud W, Berneau S, Westwood M, Kimber S, Aplin J, Brison D. The effects of hyaluronate-containing medium on human embryo attachment to endometrial epithelial cells in vitro. *Hum Reprod Open* 2020;**2020**:hoz033.
Ruane P, Garner T, Parsons L, Babbington PA, Wangsaputra I, Kimber SJ, Stevens A, Westwood M, Brison DR, Aplin JD. Trophectoderm differentiation to invasive syncytiotrophoblast is promoted by endometrial epithelial cells during human embryo implantation. *Hum Reprod* 2022;**37**:777–792.
Ruiz-Alonso M, Blesa D, Díaz-Gimeno P, Gómez E, Fernández-Sánchez M, Carranza F, Carrera J, Vilella F, Pellicer A, Simón C. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. *Fertil Steril* 2013;**100**:818–824.
Salvany-Celades M, van der Zwan A, Benner M, Setrajcic-Dragos V, Bougleux Gomes H, Iyer V, Norwitz E, Strominger J, Tilburgs T. Three types of functional regulatory T cells control T cell responses at the human maternal-fetal interface. *Cell Rep* 2019;**27**:2537–2547.e5.
Satyaswaroop P, Tabibzadeh SS. Extracellular matrix and the patterns of differentiation of human endometrial carcinomas in vitro and in vivo. *Cancer Res* 1991;**51**:5661–5666.
Satyaswaroop PG, Bressler RS, de la Pena MM, Gurpide E. Isolation and culture of human endometrial glands. *J Clin Endocrinol Metab* 1979;**48**:639–641.
Schäfer WR, Fischer L, Roth K, Jüllig AK, Stuckenschneider JE, Schwartz P, Weimer M, Orlowska-Volk M, Hanjalic-Beck A, Kranz I *et al.* Critical evaluation of human endometrial explants as an ex vivo model system: a molecular approach. *Mol Hum Reprod* 2011;**17**:255–265.
Schatz F, Markiewicz L, Barg P, Gurpide E. In vitro effects of ovarian steroids on prostaglandin F2 alpha output by human endometrium and endometrial epithelial cells. *J Clin Endocrinol Metab* 1985;**61**:361–367.
Schlafke S, Enders AC. Cellular basis of interaction between trophoblast and uterus at implantation. *Biol Reprod* 1975;**12**:41–65.
Schmidt A, Morales-Prieto D, Pastuschek J, Frohlich K, Markert UR. Only humans have human placentas: molecular differences between mice and humans. *J Reprod Immunol* 2015;**108**:65–71.

Scully JL, Haimes E, Mitzkat A, Porz R, Rehmann-Sutter C. Donating embryos to stem cell research. *J Bioeth Inq* 2012;**9**:19–28.

Shahbazi MN, Jedrusik A, Vuoristo S, Recher G, Hupalowska A, Bolton V, Fogarty NNM, Campbell A, Devito L, Ilic D *et al.* Self-organization of the human embryo in the absence of maternal tissues. *Nat Cell Biol* 2016;**18**:700–708.

Sheridan M, Zhou J, Franz AWE, Schust D. Modeling the human placenta to investigate viral infections during pregnancy. *FrontVirol* 2022;**2**:831754.

Sheridan MA, Fernando R, Gardner L, Hollinshead MS, Burton GJ, Moffett A, Turco MY. Establishment and differentiation of long-term trophoblast organoid cultures from the human placenta. *Nat Protoc* 2020;**15**:3441–3463.

Shibata S, Endo S, Nagai LAE, Kobayashi E, Oike A, Kobayashi N, Kitamura A, Hori T, Nashimoto Y, Nakato R *et al.* Modeling embryo-endometrial interface recapitulating human embryo implantation. *Sci Adv* 2024;**10**:eadi4819.

Shibata S, Kobayashi E, Kobayashi N, Oike A, Okae H, Arima T. Unique features and emerging in vitro models of human placental development. *Reprod Med Biol* 2020;**19**:301–313.

Shpiz A, Kalma Y, Frumkin T, Telias M, Carmon A, Amit A, Ben-Yosef D. Human embryonic stem cells carrying an unbalanced translocation demonstrate impaired differentiation into trophoblasts: an in vitro model of human implantation failure. *Mol Hum Reprod* 2015;**21**:271–280.

Simmons DG, Cross J. Determinants of trophoblast lineage and cell subtype specification in the mouse placenta. *Dev Biol* 2005; **284**:12–24.

Simon A, Hurwitz A, Zentner B, Bdolah Y, Laufer N. Transfer of frozen-thawed embryos in artificially prepared cycles with and without prior gonadotrophin-releasing hormone agonist suppression: a prospective randomized study. *Hum Reprod* 1998; **13**:2712–2717.

Siriwardena D, Boroviak T. Evolutionary divergence of embryo implantation in primates. *Philos Trans R Soc Lond B Biol Sci* 2022; **377**:20210256.

Soncin F, Khater M, To C, Pizzo D, Farah O, Wakeland A, Rajan KAN, Nelson KK, Chang C-W, Moretto-Zita M *et al.* Comparative analysis of mouse and human placentae across gestation reveals species-specific regulators of placental development. *Develoment* 2018;**145**:dev156273.

Sozen B, Cox A, De Jonghe J, Bao M, Hollfelder F, Glover D, Zernicka-Goetz M. Self-organization of mouse stem cells into an extended potential blastoid. *Dev Cell* 2019;**51**:698–712.e698.

Sozen B, Jorgensen V, Weatherbee B, Chen S, Zhu M, Zernicka-Goetz M. Reconstructing aspects of human embryogenesis with pluripotent stem cells. *Nat Commun* 2021;**12**:5550.

Stenqvist A-C, Chen T, Hedlund M, Dimova T, Nagaeva O, Kjellberg L, Innala E, Mincheva-Nilsson L. An efficient optimized method for isolation of villous trophoblast cells from human early pregnancy placenta suitable for functional and molecular studies. *Am J Reprod Immunol* 2008;**60**:33–42.

Straszewski-Chavez SL, Abrahams VM, Alvero AB, Aldo PB, Ma Y, Guller S, Romero R, Mor G. The isolation and characterization of a novel telomerase immortalized first trimester trophoblast cell line, Swan 71. *Placenta* 2009;**30**:939–948.

Svensson-Arvelund J, Mehta R, Lindau R, Mirrasekhian E, Rodriguez-Martinez H, Berg G, Lash G, Jenmalm M, Ernerudh J. The human fetal placenta promotes tolerance against the semiallogeneic fetus by inducing regulatory T cells and homeostatic M2 macrophages. *J Immunol* 2015;**194**:1534–1544.

Tacey E, White R, Di Sant'agnese A, Keng P, Sutherland R, Miller R. Human choriocarcinoma (JAr) cells grown as multicellular spheroids. *Placenta* 1988;**9**:583–598.

Takahashi H, Ohkuchi A, Kuwata T, Usui R, Baba Y, Suzuki H, Chaw Kyi T, Matsubara S, Saito S, Takizawa T. Endogenous and exogenous miR-520c-3p modulates CD44-mediated extravillous trophoblast invasion. *Placenta* 2017;**50**:25–31.

Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007;**131**:861–872.

Tal R, Taylor H. *Endocrinology of Pregnancy*. South Dartmouth, MA: MDText.com, Inc, 2000.

Teklenburg G, Macklon N. In vitro models for the study of early human embryo-endometrium interactions. *Reprod Sci* 2009; **16**:811–818.

Teklenburg G, Weimar C, Fauser BC, Macklon N, Geijsen N, Heijnen CJ, Sousa Lopes SMC, Kuijk EW. Cell lineage specific distribution of H3K27 trimethylation accumulation in an in vitro model for human implantation. *PLoS One* 2012;**7**:e32701.

Terzieva A, Dimitrova V, Djerov L, Dimitrova P, Zapryanova S, Hristova I, Vangelov I, Dimova T. Early pregnancy human decidua is enriched with activated, fully differentiated and pro-inflammatory gamma/delta T cells with diverse TCR repertoires. *Int J Mol Sci* 2019;**20**:687.

Thie M, Denker H-W. In vitro studies on endometrial adhesiveness for trophoblast: cellular dynamics in uterine epithelial cells. *Cells Tissues Organs* 2002;**172**:237–252.

Thie M, Röspel R, Dettmann W, Benoit M, Ludwig M, Gaub H, Denker H. Interactions between trophoblast and uterine epithelium: monitoring of adhesive forces. *Hum Reprod* 1998;**13**:3211–3219.

Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science* 1998;**282**:1145–1147.

Tsuchida N, Kojima J, Fukuda A, Oda M, Kawasaki T, Ito H, Kuji N, Isaka K, Nishi H, Umezawa A *et al.* Transcriptomic features of trophoblast lineage cells derived from human induced pluripotent stem cells treated with BMP 4. *Placenta* 2020;**89**:20–32.

Tu Z, Bi Y, Zhu X, Liu W, Hu J, Wu L, Mao T, Zhou J, Wang H, Wang H *et al.* Modeling human pregastrulation development by 3D culture of blastoids generated from primed-to-naïve transitioning intermediates. *Protein Cell* 2023;**14**:337–349.

Turco MY, Gardner L, Hughes J, Cindrova-Davies T, Gomez MJ, Farrell L, Hollinshead M, Marsh SGE, Brosens JJ, Critchley HO *et al.* Long-term, hormone-responsive organoid cultures of human endometrium in a chemically defined medium. *Nat Cell Biol* 2017; **19**:568–577.

Turco MY, Gardner L, Kay RG, Hamilton RS, Prater M, Hollinshead MS, McWhinnie A, Esposito L, Fernando R, Skelton H *et al.* Trophoblast organoids as a model for maternal-fetal interactions during human placentation. *Nature* 2018;**564**:263–267.

Turco MY, Moffett A. Development of the human placenta. *Development* 2019;**146**:dev163428.

Uchida H, Maruyama T, Ohta K, Ono M, Arase T, Kagami M, Oda H, Kajitani T, Asada H, Yoshimura Y. Histone deacetylase inhibitor-induced glycodelin enhances the initial step of implantation. *Hum Reprod* 2007;**22**:2615–2622.

Vercruysse L, Caluwaerts S, Luyten C, Pijnenborg R. Interstitial trophoblast invasion in the decidua and mesometrial triangle during the last third of pregnancy in the rat. *Placenta* 2006;**27**:22–33.

Vergaro P, Tiscornia G, Rodríguez A, Santaló J, Vassena R. Transcriptomic analysis of the interaction of choriocarcinoma spheroids with receptive vs. non-receptive endometrial epithelium cell lines: an in vitro model for human implantation. *J Assist Reprod Genet* 2019;**36**:857–873.

Vicovac L, Jones CJ, Aplin J. Trophoblast differentiation during formation of anchoring villi in a model of the early human placenta in vitro. *Placenta* 1995;**16**:41–56.

Wagner GP, Kin K, Muglia L, Pavli M. Evolution of mammalian pregnancy and the origin of the decidual stromal cell. *Int J Dev Biol* 2014;**58**:117–126.

Wang H, Pilla F, Anderson S, Martínez-Escribano S, Herrer I, Moreno-Moya JM, Musti S, Bocca S, Oehninger S, Horcajadas JA *et al.* A novel model of human implantation: 3D endometrium-like culture system to study attachment of human trophoblast (Jar) cell spheroids. *Mol Hum Reprod* 2012;**18**:33–43.

Wang X, Chen C, Wang L, Chen D, Guang W, French J. Conception, early pregnancy loss, and time to clinical pregnancy: a population-based prospective study. *Fertil Steril* 2003;**79**:577–584.

Wang Y, Luo X-P, Lim C, Wong W, Zhong G. Regulatory effect of peptidoglycan on the expression of toll-like receptor 2 mRNA and proteins in trophoblast cell line TEV-1 cells. *ISRN Obstet Gynecol* 2011;**2011**:692858.

Wang YL, Qiu W, Feng HC, Li YX, Zhuang LZ, Wang Z, Liu Y, Zhou JQ, Zhang DH, Tsao GS. Immortalization of normal human cytotrophoblast cells by reconstitution of telomeric reverse transcriptase activity. *Mol Hum Reprod* 2006;**12**:451–460.

Warnock M. *Report of the Committee of Inquiry into Human Fertilisation and Embryology. In Chairman: Dame Mary Warnock (ed) DBE*. Great Britain: Committee of Inquiry into Human Fertilisation and Embryology, 1884.

Way D, Grosso DS, Davis J, Surwit E, Christian CD. Characterization of a new human endometrial carcinoma (RL95-2) established in cell culture. *In Vitro* 1983;**19**:147–158.

Weber M, Knoefler I, Schleussner E, Markert UR, Fitzgerald JS. HTR8/SVneo cells display trophoblast progenitor cell-like characteristics indicative of self-renewal, repopulation activity, and expression of "stemness-" associated transcription factors. *Biomed Res Int* 2013;**2013**:243649–243610.

Weber M, Weise A, Vasheghani F, Gohner C, Fitzgerald J, Liehr T, Markert U. Cytogenomics of six human trophoblastic cell lines. *Placenta* 2021;**103**:72–75.

Wei Y, Wang T, Ma L, Zhang Y, Zhao Y, Lye K, Xiao L, Chen C, Wang Z, Ma Y *et al.* Efficient derivation of human trophoblast stem cells from primed pluripotent stem cells. *Sci Adv* 2021;**7**:eabf4416.

Wei Y, Zhou X, Huang W, Long P, Xiao L, Zhang T, Zhong M, Pan G, Ma Y, Yu Y. Generation of trophoblast-like cells from the amnion in vitro: a novel cellular model for trophoblast development. *Placenta* 2017;**51**:28–37.

Weimar C, Kavelaars A, Brosens J, Gellersen B, de Vreeden-Elbertse J, Heijnen C, Macklon N. Endometrial stromal cells of women with recurrent miscarriage fail to discriminate between high- and low quality human embryos. *PLoS One* 2012;**7**:e41424.

West RC, Ming H, Logsdon DM, Sun J, Rajput SK, Kile RA, Schoolcraft WB, Roberts RM, Krisher RL, Jiang Z *et al.* Dynamics of trophoblast differentiation in periimplantation-stage human embryos. *Proc Natl Acad Sci USA* 2019;**116**:22635–22644.

Williams K, Johnson MH. Adapting the 14-day rule for embryo research to encompass evolving technologies. *Reprod Biomed Soc Online* 2020;**10**:1–9.

Wong B, Lam K, Lee C-L, Wong V, Lam M, Chu I, Yeung W, Chiu P. Adrenomedullin enhances invasion of human extravillous cytotrophoblast-derived cell lines by regulation of urokinase plasminogen activator expression and S-nitrosylation. *Biol Reprod* 2013;**88**:34–11.

Xiang L, Yin Y, Zheng Y, Ma Y, Li Y, Zhao Z, Guo J, Ai Z, Niu Y, Duan K *et al.* A developmental landscape of 3D-cultured human pregastrulation embryos. *Nature* 2020;**577**:537–542.

Xu L, Li Y, Sang Y, Li DJ, Du M. Crosstalk between trophoblasts and decidual immune cells: the cornerstone of maternal-fetal immunotolerance. *Front Immunol* 2021;**12**:642392.

Xu R-H, Chen X, Li DS, Li R, Addicks GC, Glennon C, Zwaka TP, Thomson JA. BMP4 initiates human embryonic stem cell differentiation to trophoblast. *Nat Biotechnol* 2002;**20**:1261–1264.

Yamada S, Samtani R, Lee ES, Lockett E, Uwabe C, Shiota K, Anderson SA, Lo CW. Developmental atlas of the early first trimester human embryo. *Dev Dyn* 2010;**239**:1585–1595.

Yanagida A, Spindlow D, Nichols J, Dattani A, Smith A, Guo G. Naive stem cell blastocyst model captures human embryo lineage segregation. *Cell Stem Cell* 2021;**28**:1016–1022.e1014.

Yang Y, Wang W, Weng J, Li H, Ma Y, Liu L, Ma W. Advances in the study of HLA class Ib in maternal-fetal immune tolerance. *Front Immunol* 2022;**13**:976289.

You Y, Grasso E, Alvero A, Condon J, Dimova T, Hu A, Ding J, Alexandrova M, Manchorova D, Dimitrova V *et al.* Twist1-IRF9 interaction is necessary for IFN-stimulated gene anti-zika viral infection. *J Immunol* 2023;**210**:1899–1912.

You Y, Stelzl P, Joseph DN, Aldo PB, Maxwell AJ, Dekel N, Liao A, Whirledge S, Mor G. TNF-α regulated endometrial stroma secretome promotes trophoblast invasion. *Front Immunol* 2021;**12**:737401.

You Y, Stelzl P, Zhang Y, Porter J, Liu H, Liao AH, Aldo PB, Mor G. Novel 3D in vitro models to evaluate trophoblast migration and invasion. *Am J Reprod Immunol* 2019;**81**:e13076.

Yu L, Logsdon D, Pinzon-Arteaga CA, Duan J, Ezashi T, Wei Y, Ribeiro Orsi AE, Oura S, Liu L, Wang L *et al.* Large-scale production of human blastoids amenable to modeling blastocyst development and maternal-fetal cross talk. *Cell Stem Cell* 2023;**30**:1246–1261.e1249.

Yu L, Wei Y, Duan J, Schmitz DA, Sakurai M, Wang L, Wang K, Zhao S, Hon GC, Wu J. Blastocyst-like structures generated from human pluripotent stem cells. *Nature* 2021;**596**:E5.

Yu X, Liang S, Chen M, Yu H, Li R, Qu Y, Kong X, Guo R, Zheng R, Izsvák Z *et al.* Recapitulating early human development with 8C-like cells. *Cell Rep* 2022;**39**:110994. 110994X.

Yue C, Chen ACH, Tian S, Fong SW, Lee KC, Zhang J, Ng EHY, Lee KF, Yeung WSB, Lee YL *et al.* Human embryonic stem cell-derived blastocyst-like spheroids resemble human trophectoderm during early implantation process. *Fertil Steril* 2020;**114**:653–664.e6.

Zhang L, Zhang W, Shao C, Zhang J, Men K, Shao Z, Yan Y, Xu D. Establishment and characterization of a spontaneously immortalized trophoblast cell line (HPT-8) and its hepatitis B virus-expressing clone. *Hum Reprod* 2011;**26**:2146–2156.

Zhang S, Lin H, Kong S, Wang S, Wang H, Wang H, Armant DR. Physiological and molecular determinants of embryo implantation. *Mol Aspects Med* 2013;**34**:939–980.

Zhou J, West RC, Ehlers EL, Ezashi T, Schulz LC, Roberts RM, Yuan Y, Schust D. Modeling human peri-implantation placental development and function. *Biol Reprod* 2021;**105**:40–51.

Zinaman MJ, Clegg ED, Brown CC, O'Connor J, Selevan SG. Estimates of human fertility and pregnancy loss. *Fertil Steril* 1996;**65**:503–509.

Zou Y, Yu X, Lu J, Jiang Z, Zuo Q, Fan M, Huang S, Sun L. Decorin-mediated inhibition of human trophoblast cells proliferation, migration, and invasion and promotion of apoptosis in vitro. *Biomed Res Int* 2015;**2015**:201629.

Human Reproduction Update, 2024, 00, 1–31
https://doi.org/10.1093/humupd/dmae033
Review