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Modeling placental development and disease using human pluripotent stem cells

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Abstract

Our current knowledge of the cellular and molecular mechanisms of placental epithelial cells, trophoblast, primarily came from the use of mouse trophoblast stem cells and tumor-derived or immortalized human trophoblast cell lines. This was mainly due to the difficulties in maintaining primary trophoblast in culture and establishing human trophoblast stem cell (hTSC) lines. However, in-depth characterization of these cellular models and *in vivo* human trophoblast have revealed significant discrepancies. For the past two decades, multiple groups have shown that human pluripotent stem cells (hPSCs) can be differentiated into trophoblast, and thus could be used as a model for normal and disease trophoblast differentiation. During this time, trophoblast differentiation protocols have evolved, enabling researchers to study cellular characteristics at trophectoderm (TE), trophoblast stem cells (TSC), syncytiotrophoblast (STB), and extravillous trophoblast (EVT) stages. Recently, several groups reported methods to derive hTSC from pre-implantation blastocyst or early gestation placenta, and trophoblast organoids from early gestation placenta, drastically changing the landscape of trophoblast research. These culture conditions have been rapidly applied to generate hPSC-derived TSC and trophoblast organoids. As a result of these technological advancements, the field's capacity to better understand trophoblast differentiation and their involvement in pregnancy related disease has greatly expanded. Here, we present *in vitro* models of human trophoblast differentiation, describing both primary and hPSC-derived TSC, maintained as monolayers and 3-dimensional trophoblast organoids, as a tool to study early placental development and disease in multiple settings.

Keywords

Human pluripotent stem cells; Trophoblast stem cells; Trophoblast organoid; Disease modeling

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Author contributions

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

1. Introduction

The placenta is a transient but essential organ that plays an important role in fetal growth and development during pregnancy. The main functional epithelial cell type that makes up the placenta is called the trophoblast. Trophoblasts are originally derived from trophectoderm (TE), and begin as multipotent trophoblast stem cells called cytotrophoblast (CTB). CTB stem cells subsequently give rise to two main cell types: (1) syncytiotrophoblast (STB), which are formed by cell-cell fusion and play an important role for gas/nutrient exchange and hormone secretion; (2) extravillous trophoblast (EVT), which invade into the maternal uterine wall and remodel spiral arteries to establish blood flow at the maternal-fetal interface [1–3]. Abnormal trophoblast differentiation is known to be associated with numerous pregnancy complications, including recurrent miscarriage, preeclampsia, and fetal growth restriction [4–6]. Therefore, understanding trophoblast differentiation is critical for elucidating placental development and function across gestation, as well as placenta-based pregnancy complications.

Over the past 60 years, technological advancements have led to the development of multiple tools to study the cellular and molecular mechanisms of human trophoblast. The first *in vitro* model of human trophoblast was a cell line established from isolated choriocarcinoma (trophoblast cancer) tissue [7–9]. Subsequently, a Percoll gradient-based purification protocol for isolation of primary trophoblast from placental tissues was established [10]; however, once the cells were isolated, they were unable to be maintained in stable culture. Therefore, immortalized primary trophoblast cell lines were developed by genetically manipulating cells to proliferate, enabling long term culture [11–14]. Forty years after the initial establishment of a trophoblast cell line model, the next significant milestone was the derivation of mouse trophoblast stem cells (mTSC), which became crucial to our current understanding of trophoblast differentiation [15]. However, over time, in-depth comparisons of mouse and human trophoblast differentiation have revealed significant differences at the molecular and cellular level [16,17]. This highlights the importance of establishing human trophoblast stem cells (hTSC), which did not come to fruition until 2018, exactly 20 years after the derivation of mTSC [18].

During the 20 years between the derivation of mTSC and hTSC, alternative methods were sought to study human trophoblast. In 2002, the first study of human trophoblast differentiation using human embryonic stem cells (hESC) treated with bone morphogenic protein 4 (BMP4) was reported [19]. Since then, this method has evolved, and both human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC), have been differentiated into trophoblast [20–37]. The first reports detailing BMP4 mediated trophoblast differentiation were done in hPSC cultured in the primed- (post-implantation epiblast like) state, which is the traditional state of hESC and reprogrammed iPSC. Recent technological advancements in the human stem cell field have allowed for the method to be applied to hPSCs in an earlier naïve- (pre-implantation epiblast like) pluripotent state [38,39]. These advancements provided an in-depth understanding of developmental processes of trophoblast origin, and led to significant improvements in trophoblast differentiation protocol and its utility.

In this review, we will discuss the evolution of the *in vitro* hPSC-based trophoblast model, its application to the study of both normal and abnormal trophoblast differentiation, the different pluripotency states of the model's cell source, the importance of genetics and epigenetics to the model, and the most recent efforts in applications of 3D culture to enhance modeling of cells with *bona fide* trophoblast identity.

2. Human pluripotent stem cells as a tool to model trophoblast differentiation

Over the last two decades, multiple groups have demonstrated that human pluripotent stem cells (hPSC) can be differentiated into trophoblast by treating cells with BMP4. BMP4 principally activates a portion of the TGF-β signaling pathway by binding to receptors which are transmembrane serine/threonine kinases, leading to transcription of BMP-specific target genes through both SMAD-dependent and -independent signaling cascades. Specifically, studies have shown that exposure to BMP4 during the first 24 h is indispensable for trophoblast differentiation from hPSCs [27]. BMP4 signaling is thought to establish an epigenetic state, through chromatin remodeling, that is permissive for trophoblast development [31,40], though more research is needed to establish the exact mechanism(s). The role of BMP4 signaling in early embryonic development and trophoblast emergence, including the controversy surrounding this subject, was recently reviewed in depth [41].

As mentioned above, the initial report by Xu et al. showing that hESC could be differentiated into trophoblast using BMP4 and that this treatment produced hCG positive, multinucleated STB-like cells was published in 2002 [19]. Thereafter, multiple groups have replicated, and characterized these hPSC-derived trophoblast [20–37]. Methods using BMP4 alone were shown to have issues with co-derivation of mesoderm alongside trophoblast, with the former lineage being favored [42–44]. Therefore, significant effort was employed to improve this method resulting in two main trophoblast differentiation protocols. The first, the "BAP" protocol, uses BMP4, A83–01 (TGF-β inhibitor), and PD173074 (FGF receptor inhibitor) [27]. This protocol was developed based on the facts that (1) BMP4 in the presence of FGF2 leads to mesoderm formation [44–46], and (2) addition of a TGF-β inhibitor maximizes BMP4 signaling [44,47]. Therefore, addition of all three factors resulted in a pure population of trophoblast [44–47]. Our group has developed a second protocol using BMP4 and the WNT inhibitor IWP2, which reasons that mesoderm induction by the BMP4 is WNT-dependent [32], and therefore using a WNT inhibitor will push the BMP4-treated hPSC toward a pure trophoblast identity [32,37]. The latter protocol (BMP4+IWP2) further aimed to differentiate cells in a stepwise fashion, first into $p63^{+}/CDX2^{+}$ double positive CTB stem-like cells, which has recently been reported to have a TE-like signature [38], and subsequently into either STB or EVT lineage, using different levels of oxygen tension [37].

The next breakthrough in hTSC modeling was brought about by the formulation of hTSC medium by Okae et al., who described the derivation of self-renewing, bipotent hTSCs from blastocysts and early placenta [18]. Shortly after Okae et al. described the derivation

of hTSCs, several groups reported the differentiation of naïve hPSCs to TSCs, through passaging of these cells several times in hTSC medium [39,48–50]. These groups reported that hTSCs could be derived from naïve hPSCs but that this capacity was either lost, or significantly reduced, in primed hPSCs. The hypothesis underpinning these findings was based on the fact that, (1) in mouse, once the first lineage specification to TE and inner cell mass (ICM) is made, it is irreversible [39,51], and (2) naïve hPSC are developmentally similar to pre-implantation epiblast, while primed hPSC are traditionally derived and cultured at a state more similar to post-implantation epiblast. Therefore, because primed hPSC have passed the initial TE lineage segregation stage, they should not have the potential to generate TE [38,39]. However, recently, several groups have reported derivation of TSC from primed hPSC [38, 52–55], or by reprogramming directly from a somatic cell type [56–58], without the cells undergoing conversion to a naïve state. It has been argued that primed hPSC give rise to amnion, and not trophoblast, when treated with BMP4 [39,50], but two recent reports have addressed this concern directly and have shown that primed hPSC-derived TSC transcriptionally and functionally mirror primary (placenta-derived) trophoblast and not amnion [38,59]. Overall, these results suggest that, unlike in mouse, human pluripotent stem cells retain greater plasticity than previously thought.

Importantly, among these protocols, two methods attempted to mimic *in vivo* embryo development, and established stepwise protocols starting from TE derivation, continuing to a TSC stage, and then further differentiating into EVT or STB by lineage specific differentiation [38, 50]. Io et al. reported induction of TE from naïve-hPSC using a cocktail of A83–01, PD0325901 (MEK inhibitor), JAK inhibitor I, and BMP4 [50]. Although the authors state that BMP4 is optional for this induction, BMP4 enhances TE induction by at least 10-fold compared to the condition without BMP4, based on proportion of ENPEP/TACSTD2 double-positive cells [50]. Our group has subsequently characterized primed-hPSC derived CTB stem-like cells at the end of the first-step of trophoblast induction (day 4 of the BMP4 plus IWP2 treatment), and confirmed that these cells also contain the hallmarks of TE, however do show lower expression of the placenta specific Chromosome 19 micro-RNA cluster (C19MC) compared to naïve-PSC derived TSC or primary (first trimester placenta-derived) hTSCs [38,50,55]. Both protocols further mature the cells into TSC, then subsequently perform lineage-specific differentiation, and have shown that hPSC-derived trophoblast possess similar characteristics to primary hTSC [18,38, 50]. It is still not known if the differences between primed- and naïve-derived TSC are important for their ability to model normal and diseased trophoblast development. In the following sections, we will further discuss modeling normal and diseased placental development using hPSCs, and their applications.

3. Current use of human pluripotent stem cells to model placenta associated disease

(1) Current *in vitro* studies for pregnancy complications

Since the derivation of iPSC from somatic cells [60], multiple researchers have established iPSC-based “disease-in-a-dish” models, most commonly from blood cells or skin fibroblasts from patients with hereditary diseases [61]. Because iPSCs share the genetic background

of the patient, these cells can recapitulate the abnormal cellular phenotype associated with disease pathogenesis [61].

To-date, there are four reports associated with abnormal trophoblast differentiation using iPSC disease modeling [33,62–64]. Trisomy 21 (T21)-affected hPSC was the first genetic model to be used to validate the potential of these cells in modeling placental disease, showing that the hPSC-derived trophoblasts have the same differentiation defect seen in primary (placenta-derived) trophoblast isolated from T21-affected placentae [33]. This provided the first proof-of-concept that trophoblast-based placental diseases can be modeled *in vitro* using iPSCs. Thereafter, iPSC disease modeling has been applied to recurrent familial complete hydatidiform mole (CHM) (with NLRP7 mutations) and preeclampsia (PE) [62–64]. NLRP7 mutant patient-derived iPSC were established from maternal skin biopsies, and demonstrated expedited trophoblast differentiation compared to a control line upon BAP treatment (BMP4, A83–01, and PD173074), similar to hyperplasia of trophoblast tissue observed in CHM [62].

Although the genetic factors are not well understood, two reports have shown successful modeling of early-onset PE using patient-derived iPSC [63,64]. In both studies, PE- and non-PE affected iPSC were derived from umbilical cord cells, and subsequently differentiated into trophoblast using a BMP4-based protocol; trophoblast derived from these iPSC showed an abnormal response to oxygen in both studies, hinting at an underlying susceptibility of these cells to environmental stressors [63, 64]. Although the detailed disease mechanism and specific disease-causing genes remain to be identified, both groups were able to demonstrate the practical application of modeling a complex placenta-based disease using iPSC. Like many other complex diseases, PE is known to involve an interplay between genes and environment, which iPSC-based models have been shown to be able to model in other organs [65–67]. Exactly which epigenetic modifications (DNA methylation, chromatin modification, etc.) could be responsible for PE pathogenesis, and how they can be modeled using iPSCs, however, warrant further study.

(2) Naïve and primed iPSC derived trophoblast for disease modeling

As discussed above, the most recent reports on hPSC-based trophoblast modeling have focused on the starting cell source rather than the pathophysiologic origins of placental disease. However, to date, hPSC-based modeling of pregnancy disorders has only been done starting from primed state, and not naïve state, hPSC. Therefore, it is important to discuss the current knowledge of naïve and primed pluripotency and its application to abnormal trophoblast differentiation.

The first step in trophoblast disease modeling using pluripotent stem cells is reprogramming somatic cells to a pluripotent state. Although it is widely held that pluripotency exists along a continuum [68], there are two main *in vitro* substates: naïve and primed, which represent pre- and post-implantation epiblast, respectively. Human iPSCs were first generated in the more developmentally advanced, primed substate [60], but have since been generated in the naïve substate using the same reprogramming factors (OCT4, KLF4, c-MYC, and SOX2) but with a naïve stem cell medium [69]. Criteria for evaluating the naïve human pluripotent state by comparing it to the inner cell mass of the blastocyst have been proposed [70]. The

main characteristics of the naïve substate, which distinguish it from the primed substate, include distal enhancer activity of OCT4, expression of numerous naïve-specific transcripts and transposable elements, genome-wide DNA hypomethylation, and (in female lines), two active X chromosomes [70]. The molecular differences between these two pluripotent substates make choosing a starting point for the differentiation into human trophoblast stem cells potentially an important one.

Although several groups have shown that both naïve and primed hPSC-derived TSC have the ability to differentiate into both STB and EVT, which, at least transcriptionally, appear similar to *in vivo* isolated STB and EVT, a rigorously controlled experiment highlighting the differences between trophoblast produced by the two substates has not been done. One of the most striking differences between naïve and primed hPSCs is the relative global hypomethylation of the naïve substate. Cinkornpumin et al., compared the methylomes of naïve hPSC-derived TSCs to both primed hPSCs and placental derived hTSCs [49]. They reported a high correlation between naïve hPSC-derived TSCs and placental hTSCs. They also showed that naïve hPSC-derived TSCs gained placenta-specific methylation and increased expression of placental genes that were heavily methylated and downregulated in primed hPSCs. However, the authors noted that some imprinted regions showed aberrant hypomethylation in their naïve hPSC-derived TSCs [49]. This suggests that loss of methylation in naïve hPSCs might be important for the acquisition of a placenta-specific methylome and increased C19MC expression, but at the same time, may also lead to dysregulation of imprinted regions, which are known to be important for placental development. A similar methylome-wide investigation of primed hPSC-derived TSCs would determine whether global hypomethylation is in fact needed to acquire a placenta-specific methylome. Furthermore, a comparison of naïve-vs. primed iPSC-derived TSC in context of placenta-linked disorders has yet to be reported. Research focusing on understanding the potential of each substate to recapitulate placental disease phenotypes will be important for future modeling studies.

4. Future applications of human pluripotent stem cells to placenta-associated disease modeling

(1) Application to genetic abnormalities

The advantage of an *in vitro* cellular model is its utility in identifying the effects of genetic mutations on trophoblast differentiation. Unlike primary trophoblast, hPSCs are easy to manipulate genetically, and therefore can be used to study genetic aberrations. In human pregnancy, genetic factors (including aneuploidies) are associated with multiple complications, such as preeclampsia (PE), maternal immune dysregulation, and fetal developmental programming [71–75]. In particular, PE has been one of the most well-studied pregnancy complications using genome-wide association studies (GWAS). This was driven by the knowledge that some PE patients show a family history of PE, although the genetic inheritance pattern has less than 50% penetrance [72–74]. GWAS have identified risk loci for PE, such as single nucleotide polymorphisms in Human leukocyte antigen-G (*HLA-G*), Storkhead Box 1 (*STOX1*), Corin, Serine Peptidase (*CORIN*), and Fms-like tyrosine kinase 1 (*FLT1*) [76–78]. In particular, *HLA-G* genotyping studies have

identified mutations and polymorphisms at this locus in patients with PE [79–81], although population-based variability warrants further functional studies [81,82]. Therefore, future studies applying gene editing technologies in hPSCs to understanding the detailed cellular mechanism of PE would be highly informative. The combination of population-based, genetic, and hPSC-based studies can be applied to more fully understand the role of specific genes in placental development and disease.

(2) Application to imprinting abnormalities

Parent-of-origin/genomic imprinting is crucial for placental development and fetal growth, and is regulated by DNA methylation, non-coding RNAs, histone modifications, and chromatin interactions [83]. The use of iPSC technology is becoming increasingly attractive for researchers to study rare imprinting disorders. To date, multiple studies have shown that primed state iPSC retain both cell-of-origin-specific and donor-specific DNA methylation patterns [84–88]. Further, there have been reports of successful derivation of iPSC, with proper recapitulation of disease phenotype, from patients with known imprinting disorders, such as Angelman syndrome, Prader-Willi syndrome, and Beckwith-Wiedemann syndrome. These reports have shown that aberrant imprints present in somatic cells are retained after reprogramming, recapitulating the expected transcriptional and epigenetic features of the disease [89–92].

As stated above, there has so far been only one report using naïve hPSC-derived TSCs to study placental-specific methylation and imprints in detail [49]. This group reported similar DNA methylation levels at most imprinting control regions compared to primary hTSC, but found three placental imprinted genes, *PEG3, ZFAT and PROSER2-AS1*, which failed to activate upon TSC derivation [49]. However, it is still unclear if the large-scale erasure of DNA methylation that occurs in naïve hPSCs affects the ability of these cells to model disease phenotype. Recently, Liu et al. demonstrated that fibroblast can be directly reprogrammed to TSC-like cells (induced TSC or iTSC), by switching cells to Okae's hTSC media on day 8 post-transduction with standard iPSC reprogramming factors [56]. As with reprogramming somatic cells to the primed substate, this emerging method also holds the potential of retention of placental imprints, as the cells do not go through a naïve substate, prior to generation of iTSC. Going forward, an in-depth side-by-side comparison of naïve- and primed iPSC-derived TSCs and iTSCs, derived from the same starting material, will be highly useful for researchers making decisions with regard to the best method of placental disease modeling.

Finally, although DNA methylation is often stably maintained at the imprinting control region and at CpG islands in primed iPSC lines over prolonged culture [89,93], it is important to note that *in vitro* culture of hPSC has been associated with loss of imprinting, with some loci at higher risk than others, using current popular culture conditions [94]. Further studies to optimize media composition and culture environment will be valuable to improve imprinting stability in such *in vitro* models.

5. 3D modeling: making organoids using hPSC derived trophoblast

Advances in stem cell culture and extracellular matrix technologies have contributed to the development of several different types of self-organizing three-dimensional (3D) organoid cultures [95,96]. These organoids recapitulate at least some aspects of anatomical structure and physiological function of *in vivo* organs, and have been applied to studies in developmental biology, physiology, disease modeling, drug screening, and regenerative medicine.

Soon after the development of media for derivation of primary hTSC [18], two groups reported generation of self-renewing trophoblast organoids, using CTB from early first-trimester placenta, embedded in Matrigel [97–99]. Both groups have shown that their organoids highly express villous CTB markers (TP63, GATA3), contain a hypomethylated *ELF5* promoter, and have low EVT marker (HLA-G) expression, with both groups showing an "inside-out" organoid structure, containing an outer CTB and inner STB layer [97,98]. In addition, both groups have shown EVT differentiation capacity of these organoids, although their protocols differ slightly; Turco et al. used media similar to Okae's EVT differentiation media [98], while Haidar et al. removed WNT activators (R-spondin and CHIR99021) from their media to initiate differentiation into EVT precursors, then added the WNT activator back for further differentiation [97]. Most recently, Sheridan et al. attempted making trophoblast organoids from primary (first trimester placenta-derived) hTSC [100]. However, they reported that these cells failed to form organoids, and lacked the inner STB layer [100]. Further characterization of their primary hTSC indicated that these cells most closely resembled column niche progenitors [100]. In addition, they also reported that, while primary hTSC in 2D cultures showed upregulated expression of HLA class I molecules, hTSC in 3D culture showed downregulation of these genes, suggesting that 2D culture conditions do not always recapitulate or maintain *in vivo* characteristics [100]. While further in-depth characterization of primary hTSCs is needed to better understand its identity, incorporation of 3D culture methods may in fact be an important next step in placental disease modeling.

Using culture conditions similar to the Haider and Turco reports, two groups have reported the successful generation of organoids using naïve-hPSC derived TSC [50,101]. The organoids derived from naïve-hPSC derived TSC showed similar characteristics to primary organoids, which have CTB and STB marker expression [50,101], with the outer layer expressing CTB, and the inner layer expressing STB markers [101]. In addition, similar to primary organoids, these organoids can also differentiate into EVT [101]. Karvas et al. have further examined X chromosome inactivation (XCI) in these organoids and found that random XCI with clonal expansion recapitulated the clonal XCI patterns observed in human placental development [101]. Furthermore, they used their organoid model to study SARS-CoV-2 and Zika virus infection during placental development [101]. Their attempt to mimic viral infection was extremely valuable, as it established a renewable cell model to study emerging new pathogens and will enable researchers to understand the effect pathogens have on trophoblast and placenta. However, it remains unclear whether this "inside-out" organoid structure, which does not recapitulate the *in vivo* structure of chorionic villi, can truly mimic viral entry and associated damage to the villous structure. Additionally, this

"inside-out" architecture is suboptimal for researchers studying STB function, such as cell aging and turnover, as these cells *in vivo* shed directly into maternal circulation [102]. An alternate method, which partially overcomes this limitation, binds mononuclear trophoblast to beads in a rotating wall vessel bioreactor, which mimics physiological shear stress [103]. This model allows formation of functional STB, overlying the beads, without using forskolin or 8-bromo-cAMP [103]. However, this technology requires an initial preparation of beads with stromal cells, which are subsequently replaced by the trophoblast, and has yet to be adapted for use with primary (placenta-derived) trophoblast, in contrast to the self-assembly of primary trophoblast into organoid structures. Nevertheless, mimicking the physiological environment of an outer layer of STB is important for studying their function. Although further research and improvement of these 3D models is warranted, organoid models, combined with other new bioengineering technologies, will serve as important tools to study villous trophoblast development and disease in the future.

6. Conclusion and future prospects

Until recently, understanding the pathophysiology of placenta-associated pregnancy complications was extremely difficult, due to lack of a proper and renewable model system to study trophoblast, the epithelial component of the placenta. This review has highlighted efforts to establish and use more advanced model systems for trophoblast, the relationship between these efforts and advances in hPSC-based technologies, including the state of pluripotency and its capacity to differentiate into trophoblast, the application of trophoblast models for in-depth studies in genetic and epigenetic basis of placental disease, as well as advances in 3D (organoid) model systems (Fig. 1). The commonality in each of these advances was the development of media and culture techniques for derivation of primary hTSC and trophoblast organoids [18,97–99], which was rapidly applied to hPSC-derived trophoblast [38,48–50,52–58,101]. Although, hPSC-derived trophoblast models still have some limitations, such as primed hPSC-derived TSC's low C19MC expression [38,50,55], and iTSC and naïve hPSC-derived TSC's undetermined placental disease modeling potential [104], the efforts made in the past 20 years have significantly increased our ability to model trophoblast. Finally, researchers' current preference for cell monolayers, which has been shown to poorly represent their physiological equivalents *in vivo*, is slowly shifting to 3D culture, which will better mimic the complexities of the *in vivo* tissue environment and provide researchers a more optimal way to study cellular phenotypes of abnormal trophoblast. In the future, we expect more advances in 3D technologies and the development of more optimal assays for characterization of cellular phenotype in this context. Understanding the strengths and weaknesses of each model system will allow researchers to select the model best suited for a particular application, and we hope this review will help facilitate such decisions. Although, more work is needed to further improve these trophoblast models, we want to emphasize that a collective effort to develop robust protocols for the derivation of TSCs from hPSC will allow the field to overcome many of the current ethical and technical limitations of primary cells, and thus can be used as an alternative cell modeling system by the wider research community. In conclusion, while modeling pregnancy complications using hPSC-derived trophoblast is still in its infancy, the

ability to derive TSC from placentas at delivery, with known pregnancy outcomes, will allow for development of new interventions and treatments of placental disease.

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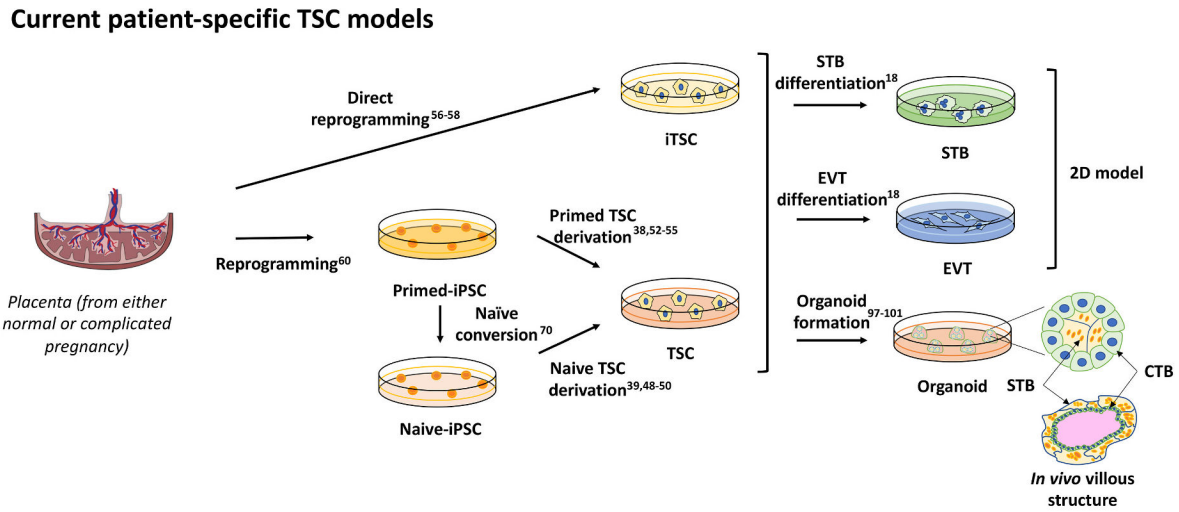

Fig. 1.

Current methods for generation of patient-specific TSC.

Schematic showing the current methods for derivation of patient-specific TSC, starting with placental cells and reprogramming, either directly to TSC ("induced TSC" or iTSC) or first into iPSC, prior to conversion to TSC from either a naïve or primed iPSC state. Emerging organoid model will further enable us to study trophoblast with phenotypes more akin to the cells *in vivo*, though the current organoid models are limited by their inside-out morphology compared to chorionic villous structures *in vivo*. In-depth comparison among these models is the next step to determine which starting material is optimal for modeling specific diseases of the placenta. References are cited at each step.