Designing an agent-based model of placental development during gestation

A Technical Report submitted to the Department of Biomedical Engineering

Presented to the Faculty of the School of Engineering and Applied Science
University of Virginia • Charlottesville, Virginia

In Partial Fulfillment of the Requirements for the Degree
Bachelor of Science, School of Engineering

Shri Ramanujam

Spring, 2023

On my honor as a University Student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments

Advisor

Sepideh Dolatshahi, Department of Biomedical Engineering

Designing an agent-based model of placental development during gestation

Shri Ramanujam[a]. Remziye Erdogan[b], Sepideh Dolatshahi[c,1]

[a] Undergraduate Student, Biomedical Engineering, University of Virginia
[b] PhD Student, Biomedical Engineering, University of Virginia
[c] Assistant Professor, Biomedical Engineering, University of Virginia
[1] Correspondence: sdolatshahi@virginia.edu; PO Box 800759 Charlottesville, Virginia 22908; 434-243-7549

Abstract

During pregnancy, the placenta develops in the uterus alongside the fetus to provide it with oxygen, nutrients, and immunity. Proper development of the placenta is crucial to neonatal health, specifically because transplacental antibody transfer from pregnant person to fetus confers it with early life immune protection. This process has been leveraged to provide neonates with pathogen-specific immunity via vaccination of the pregnant person. However, little is known regarding the mechanisms of transplacental antibody transfer and how it is dynamically regulated throughout gestation, or how it is affected by the pregnant person's health (i.e. chronic stress, diabetes, etc). A recent quantitative mechanistic model, designed to uncover determinants of this process, is able to calculate the concentration of antibody in the neonate given the time of vaccination of the pregnant person, but it is limited by its inability to account for the placenta's spatial heterogeneity over the course of its development. Here, we use another modeling system to fill in the gap — agent-based models (ABMs) use a set of autonomous, decision-making individuals called agents that interact, act, and react to each other and the environment under the governance of a set of rules. This first version of the model depicts angiogenesis of fetal vasculature at the placental interface, and we used quantitative immunohistochemical images of term patient placenta samples to fit the model to human data. After parameterization, there was no significant difference (p-val = 0.0640) as measured by the number of blood vessels per mm^2. This model will uncover how the changes in placental shape and constitution over time affect transplacental antibody transfer, allowing for integration with the quantitative mechanistic model to provide a more complete and accurate model for predicting patient-specific approaches to pregnancy treatments and vaccines.

Keywords: placenta, antibody, antibody transfer, maternal, vaccination, computational, agent-based model, immunity, neonatal

Introduction

Neonates, on account of developing in the semi-allogeneic, sterile environment of the womb, are at high risk of infection upon birth, when they are exposed to the microorganism-rich world (1). About 40% of the 3 million neonatal deaths worldwide are caused by infections, and while neonatal vaccines have proven useful for older babies and children, they are least effective in the first month of life (2-4). Antibodies transferred from the pregnant person to the fetus via the placenta during pregnancy provides the neonate with passive immunity to pathogens for about 6 months *ex utero* (5), and this process has been leveraged administering vaccinations to the pregnant person (6-7). However, little is known regarding the mechanisms of transplacental antibody transfer, the dynamic regulation of it throughout placental development, and the effects of the pregnant person's health factors.

The placenta cannot be studied longitudinally in humans because it would be invasive and cause harm to the fetus and the pregnant person, so researchers are confined to one static time point (when the placenta is expelled from the body). It is also unideal to study via animal models because it is not conserved across species. This does not allow for a comprehensive look into the development. Thus, computational methods are being employed to uncover the dynamics of placental development. Recently, a quantitative mechanistic model has been designed to find out what the determinants of transplacental antibody transfer are, and how the process may be used to inform patient-specific pregnancy treatment and immunization approaches (8).

There is evidence that prenatal vaccines are less effective than they could be in transferring antibodies optimally to all populations. For example, the pertussis vaccine's effectiveness against hospitalization was only 73% in preterms as opposed to the 95% in full-terms (9). For another, several studies found that the Hib and TDap vaccines in early pregnancy resulted in insufficient antibodies in term neonates to protect against infection (10-11). Accordingly, a model that predicts a personalized approach to pregnancy vaccinations is very beneficial by promoting neonatal immunity.

A major limitation of the current quantitative mechanistic model, however, is that it does not take into account spatial heterogeneity over time, which is crucial in this biological context: the placenta is changing in shape and constitution as it undergoes development throughout gestation (12). To fill in this gap of knowledge, we are using a different modeling platform that can simulate structural changes. Agent-based models (ABMs) consist of a system of autonomous, decision-making individuals called agents assess their situations, make decisions, and execute behaviors in response to interactions with each other and the environment on the basis of a set of given rules (13). In this biological model, agents represent individual cells, the environment consists of chemical concentration gradients, and the agent behaviors are governed by rules derived from literature (13-14).

The current version of the ABM is of fetal angiogenesis at the placental interface. Eventually, it will be expanded to include more agents and environmental players, and made into a three-dimensional model to make it as closely representative of placental development, and consequently antibody transfer, as possible. Then, using the ABM in integration with the mechanistic model, we will be able to more completely and accurately develop patient-specific pregnancy treatment and vaccination plans that maximize neonatal immunity (Fig 1).

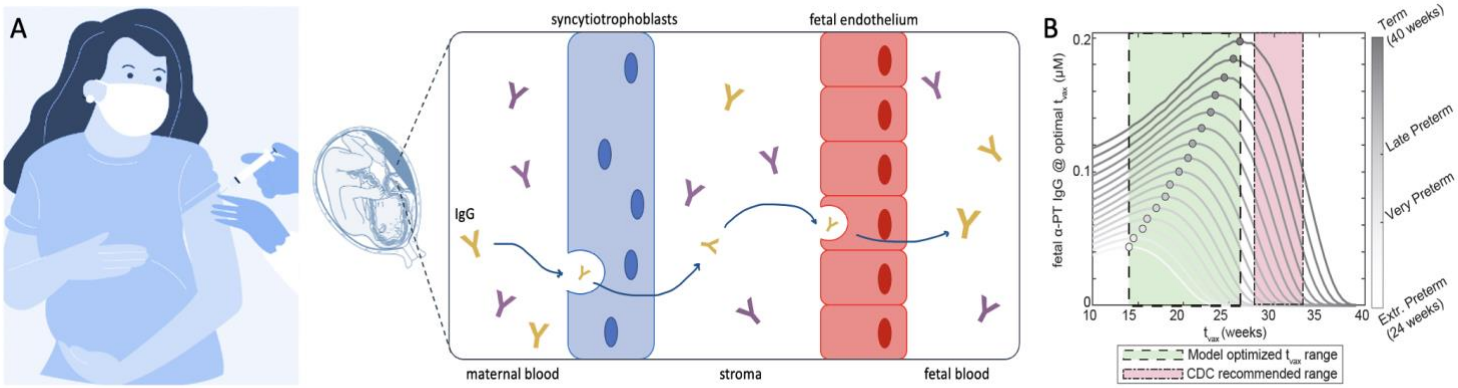

Figure 1. A) Antibody transfer from pregnant person to fetus via the placenta during gestation. B) ODE model output depicting the amount of antibody (IgG) present in the fetus depending on the time of vaccine administration to the pregnant person and the fetus's gestational age.

Results

Model Development

The placenta can be simplified down to five major components: the maternal circulation, the trophoblast cell layer, the stroma, the fetal endothelial cell layer, and the fetal bloodstream (Fig 1A). We started by modeling the fetal endothelium because ABMs of angiogenesis in other biological contexts already exist, such as the Angiogenic Growth in Cornea Micropocket Assay model (15). This model was used as a reference for basic characteristics of the endothelial cells (ECs) and the general logic of their proliferation. Two things were modified to optimize it for the placental context: the initial seeding formation of the ECs and the directionality of the VEGF gradient (Fig 2). The initial seeding in the Cornea model sets up a bed of vessels all the way down the left side of the world. However, the placenta is better simulated as starting from a smaller sprout point, as it would from an embryo. Thus, the setup process was adjusted to randomly seed endothelial cells between the coordinates of 20 and -20, instead of 100 and -100 on the y-axis. Because the actual behavior of the ECs, like growing up a concentration of growth factor, should not be be modified, the way the ECs were stimulated to proliferate radially out from the sprout was to reverse the concentration gradient from the Cornea model and shift it such that the lowest concentration would be at the center of the circle, which would coordinate with the center of the sprout (0,0).

Once the overall behavior of the ECs generally matched what we expected in the placenta, trophoblasts (TBs), specifically cytotrophoblasts (CTBs) were added into the mix. These were seeded in a rectangle around the sprout, with a height of 60 patches and a width of 20 patches (Fig 3B). Once the behaviors of the CTBs are optimized, this seeding will also be improved upon to more realistically mimic CTB layer formation. The behaviors encoded for CTBs were proliferation, and differentiation into syncytiotrophoblasts (STBs) (16-17). The intent for proliferation was as follows: if an EC is within a given radius from a CTB, that CTB would migrate some distance outwards, and proliferate until the layer was continuous again and surrounding the vessel. However, due to limitations in NetLogo, this behavior has yet to be implemented properly. Several techniques were tried, and one iteration is depicted in Figure 3C. When it became clear that CTB proliferation would not be optimized within our time constraints, we moved on to CTB to STB differentiation. CTBs, when they receive a certain signal as yet to be ascertained through literature,

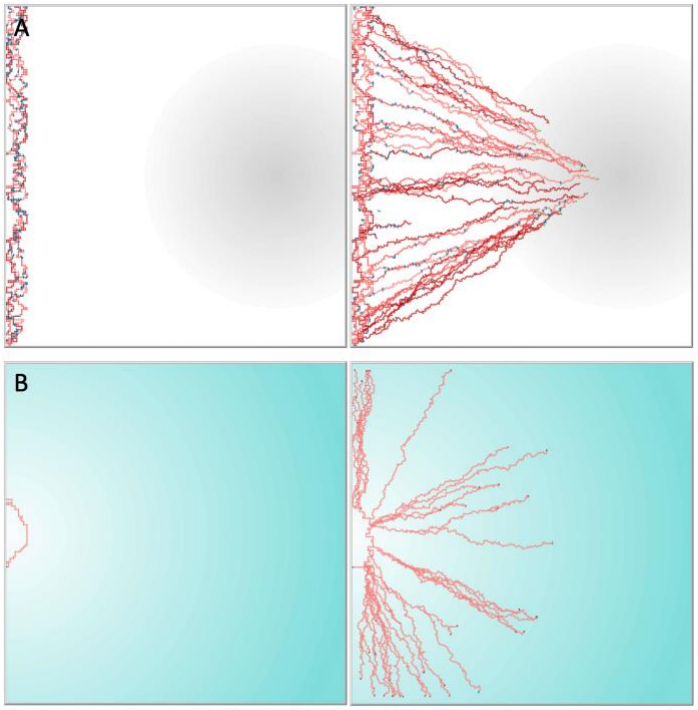

Figure 2. A) Cornea model. There is a bed of ECs against the left side of the world from which the angiogenesis begins, growing towards the highest concentration of VEGF. The concentration gradient is depicted in gray, with the lowest concentration at the edges of the circle and the highest in the center. B) Placenta model. The ECs sprout radially outward from a localized starting point to emulate the structure of a placental cotyledon because the VEGF gradient, depicted in blue, is greatest at the edges of the world and least at the sprout point.

fuse to form a multinucleated, continuous cell layer called the syncytium, which is in immediate contact with the maternal bloodstream (17-18). STBs cannot proliferate, but by the end of pregnancy, there are said to be nine times as many of them as CTBs (19), so at some point, CTB proliferation is outdone by CTB to STB differentiation, but in such a balance that there are enough CTBs to differentiate. This was modeled by giving the CTBs a probability of becoming an STB at every tick (Fig 3D). These processes will be optimized moving forward.

Parameterization of Angiogenesis via Vectra Image Analysis

The images of term-patient placenta samples were stained for several different markers, so ImageJ was used to process eight images to isolate the EC dye. The brightness and contrast of each image was adjusted manually until the ECs were the most vibrant subject, and then each blood vessel, denoted by a ring of ECs, was counted excluding

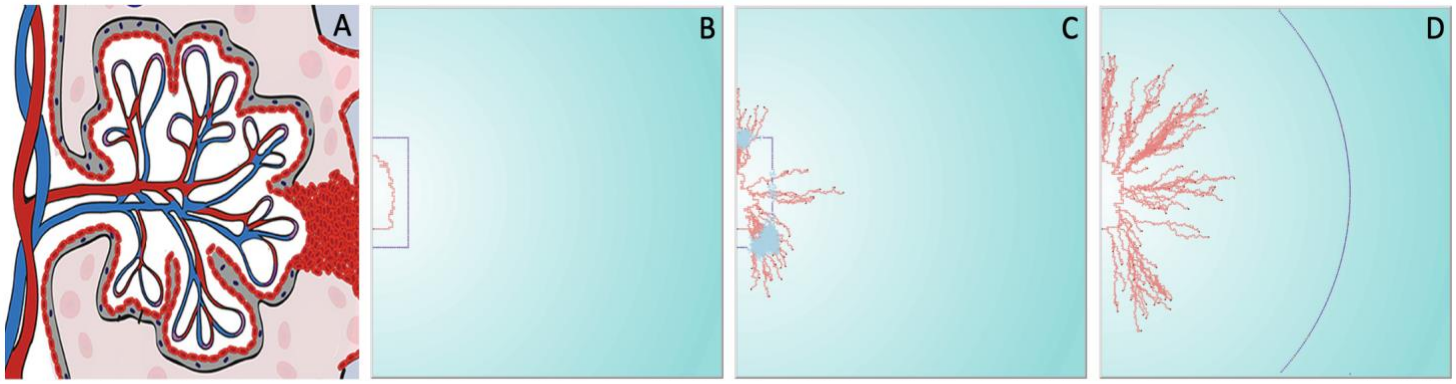

Figure 3. A) Diagram of placental cotyledon. This is the expected structure of fetal vasculature, CTBs (red cells), and STBs (gray cells). B) Initialization of CTBs (purple) around EC sprout. C) An iteration of CTB proliferation that does not function as expected. D) The CTBs were initialized far out from the ECs so that their lack of proliferation does not obstruct angiogenesis. They are differentiating into STBs (orange, not visible).

ruptured vessels or those cut off by the field of view (Figure 4 A-C). Two other images were also counted, but these were imaged only for ECs so no major adjustments were necessary. The average blood vessel count per square millimeter was 426.59 ± 116.1 (error is one standard deviation).

This was the value used to fit the model to human data. NetLogo has an *in silico* experimentation program that is sometimes used for parameterization because variables can be modified systematically and the results from each run, in this case the number of tip cells, outputted for comparison. The ABM has five parameters that can be varied to calibrate EC behavior: three from the equation (Eq. 1) used to calculate normal EC to tip cell conversion probability, a variable used to calculate the VEGF concentration gradient, and the random movement of tip cells. However, a major limitation of NetLogo is the length of time it takes to run an experiment with several varying parameters, where it has to run each combination of them at least once but preferably more — just 24 sets of parameters took almost four hours to run. In light of this, we conducted several informal sensitivity analyses to determine which variable would have the greatest effect when varied in broad strokes. Var1 was found to have the greatest effect when varied around the initial value, as derived from the Cornea model.

$$tip\ signal = ((var1 - concentration\ of\ patch) * var2) - (\#\ of\ tip\ cells\ /\ var3) \quad \textbf{[1]}$$

The number of blood vessels were plotted against time, starting at 10 weeks and ending at 40 weeks (Fig 4D), for 10 runs of the simulation where each week equaled one tick. The 40-week values of these runs were then averaged (535.40 ± 104.00 vessels per square millimeter) and compared against average Vectra blood vessel count (Fig 4E). A Wilcoxon rank sum test was conducted, and the simulations were deemed insignificantly different from the Vectra human data, with a p-value of 0.0640 at a 0.05 level of significance.

Discussion

The intention of this project was to design an agent-based model that recapitulated placental development through gestation to more accurately predict transplacental antibody transfer. By incorporating rules from several sources including an angiogenic ABM of a different biological context, we were able to capture the expected behavior of the fetal vasculature as measured by the number of blood vessels per square millimeter. We have thus established the first version of the first ABM of placental development, and are primed to continue expanding it to create more complete versions. The logical next step would be to continue optimizing the CTBs and STBs, but first, parameterization of angiogenesis needs to be conducted more robustly than was possible under the time constraints and with BehaviorSpace.

While the model displays insignificant difference to human data after the parameterization we conducted, this outcome is dubious due to how small the scope was. Ideally, parameterization is conducted iteratively, and with at least 1000 parameter sets, as opposed to a meager 10-25 with just one varying parameter. Methods such as the steepest descent algorithm would be used, and a cost function would be fitted to find which parameter set(s) generate the lowest error when compared to the calibration standard data. In this study, the calibration data consisted of ten term placenta samples due to difficulties procuring patient participants so quickly, but it would be preferable to have more samples, and from different gestational time points as well, because we are fitting a blood-vessels-over-time curve to the data. Fitting forty weeks of development data to just the term time-point does not allow for a comprehensive comparison of human data to the simulation.

NetLogo is not without its pros, but this project was obstructed several times over by NetLogo's limitations, not just during parameterization. It is very easy to begin using, but executing even slightly more complex tasks is difficult due to the simplicity of NetLogo's commands. Another limitation worth noting is that NetLogo becomes overwhelmed with agents quite quickly and will stall, thus rendering parameterization experiments very tedious and sometimes impossible. Though this is possibly a side-effect of having poorly fitted parameters, it was noticed in several published NetLogo ABMs as well. While this software is a good introduction into agent-based modeling, especially because it has thorough documentation, it might be worth it to investigate other ABM platforms to use instead that have more flexibility and capacity for nuance (20).

Future directions for this project are boundless, considering the cutting-edge nature of this research. First, all the other agents and environmental factors of the placenta must be added, such as immune cells, antibodies, and other chemical concentration gradients. Of course, the model must continue to be parameterized with the addition of each new component, and validated with new data sets as well. It will be difficult to find data for at least one, but most likely both, these processes, considering how under-researched this field is. Once the 2-D model has been validated, transferring it over to a 3-D ABM space would provide that much more insight to the same mechanisms, in an even closer representation of the placenta. These advanced versions of the model can be integrated with the existing quantitative mechanistic model (8) to provide it with the dynamic spatiotemporal aspect it lacks, thus creating a more complete and accurate system to predict patient-specific approaches to immunization such that neonates are born with maximized immunity.

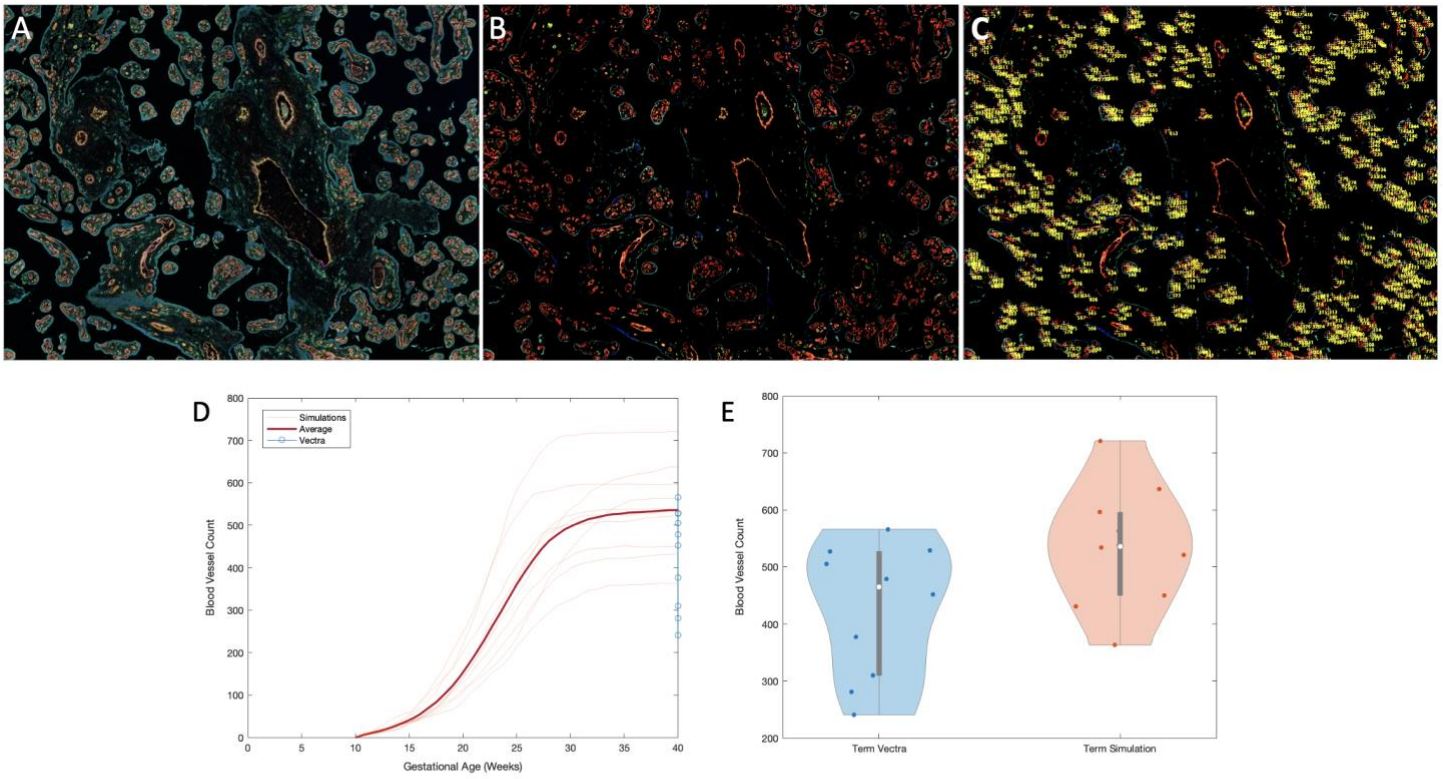

Figure 4. A) Original Vectra image of term-patient placenta sample with seven stains. B) Image contrasted and filtered with ImageJ to make ECs, stained for FcγrIIb in orange, stand out from the other cells types. C) Blood vessels per square millimeter counted with ImageJ to be used in model calibration against the model output of tip cells, a proxy count for number of vessels. D) Blood vessel count versus gestational age from 10 runs of the ABM after parameter optimization using NetLogo's BehaviorSpace to modify one parameter in the equation (1) for normal EC to tip cell conversion probability. E) Violin plots of the Vectra data versus the simulation data at term. A Wilcoxon rank sum test was conducted to give a p-value of 0.0640, indicating no significant difference between the groups, which means the model captures the expected behavior at the 0.05 significance level.

Materials and Methods

ABM Design

We created an ABM of fetal angiogenesis during placental development through pregnancy (Fig S1). To develop the model, we used a pre-existing model of angiogenesis in a different biological context (15). The agents in this model were primarily endothelial cells, but cytotrophoblasts and syncytiotrophoblasts were also in the process of being incorporated. The model's environment consisted of a concentration gradient of growth factor VEGF. The model was built in NetLogo, an agent-based modeling platform.

The ABM was a square grid of 200 patches, each patch representing 10μM in width, the average diameter of an endothelial cell (21). The initialization of the model occurs by seeding a random amount of endothelial cells in a random configuration until they form a bud from which the blood vessels could sprout. Simulations were run for 40 weeks, each cell determined its location and environment in the simulation space before taking actions determined by a probability- and logic-based decision tree (Fig 5).

Agent Actions

The simulated behaviors of the endothelial cell agents included differentiation and proliferation. At each time-step, each endothelial cell has a probability of differentiating from a "stalk" EC to a "tip" EC, or one that leads a new vessel branch. Tip cells are thus the ones undergoing proliferation, which is simulated by the tip cell moving forward in a random direction and a new agent being placed in the vacated space behind the tip cell. The rules for ECs were derived from the previously mentioned angiogenesis model (15), and modified to fit this biological context. It was presumed that several variables and behaviors would be similar, if not necessarily exactly the same. One key behavior that was changed for placental angiogenesis is the direction of vessel growth; the blood vessels of the fetal vasculature are thought to grow radially outward from a node that eventually leads down the umbilical cord and towards the fetus (22). This was done by changing the directionality and orientation of the VEGF gradient the cells grow toward, such that it was greatest around the edges of the world and least near the sprout point.

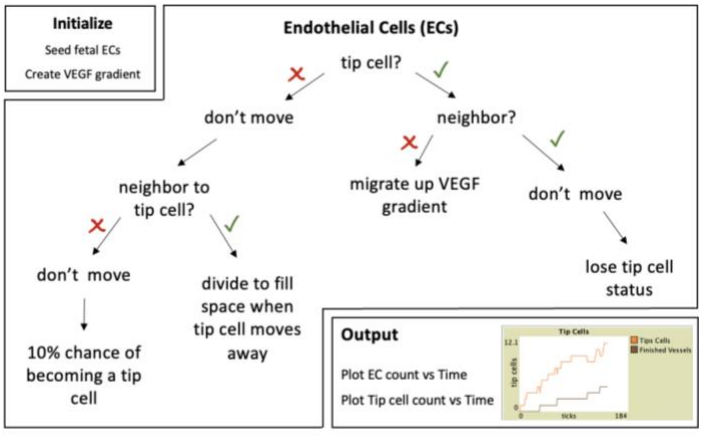

Figure 5. ABM rules, logic flow, and agent actions. Currently includes model initialization, fetal angiogenesis, and model output.

Trophoblasts were being incorporated into the model as well (Fig 6) — all of this agent type started as cytotrophoblasts. They would proliferate, and under specific environmental conditions, as yet to be determined by literature, they would differentiate into syncytiotrophoblasts. The spatial distribution was supposed to be such that the trophoblast layer enveloped the blood vessels (Fig 3A), meaning their proliferation was dependent on interactions, or spatial proximity, with the endothelial cells. The trophoblasts were not included in this version of the model due to unforeseen complications in simulating these behaviors (see Results subsection Model Development for more details).

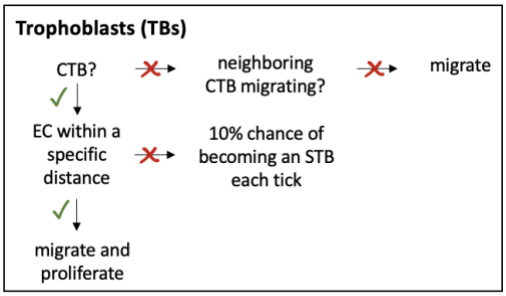

Figure 6. Rules, logic flow, and actions for TBs.

ABM Parameterization

To parameterize the model, we ran simulations and systematically adjusted the unknown model parameters using NetLogo's experimentation program, BehaviorSpace. The number of tip cells in the model at 40 weeks, as a measurement of blood vessel count, was compared to blood vessel counts from immunohistochemical images of term patient placenta samples (Vectra image analysis subsection) until the data were consistent. Because the ABM was based off another angiogenesis model, just in a different biological context, it was assumed that the parameters in this biological context would be comparable. Thus, the range for each parameter was centered around the value it was given in the cornea model. Due to limitations with BehaviorSpace, we selected one parameter, based on a set of sensitivity tests to see which one affected the ECs the most, to vary in these experiments.

Vectra Image Analysis

As part of model parameterization, we collected term patient placenta samples and used Vectra immunohistochemical staining to image them. Each image was stained for several markers, so to isolate endothelial cells, the images were processed and filtered using ImageJ until the EC stain was more vibrantly apparent than the rest. Then, we counted blood vessels, excluding those cut off by the field of view. These images are cross-sectional views, while the ABM provides a sort of bird's eye view, so a direct comparison was not possible. Instead, the number of blood vessels was counted per image to determine the number of blood vessels per square millimeter. Then, we outputted the number of tip cells from the ABM at the 40-week mark and used that as a proxy for blood vessel count. The counts of blood vessels per square millimeter were compared between the Vectra images and the ABM.

End Matter

Author Contributions and Notes

S.R, R.E, and S.D designed research, S.R performed research, S.R developed and executed model, R.E provided resources (some literature, Vectra images, etc), S.R analyzed data with the guidance of R.E and S.D, S.R wrote the paper with feedback from R.E and S.D.
The authors declare no conflict of interest.

Acknowledgments

Thank you to Shayn Peirce-Cottler for providing me with the background information I needed to dive into agent-based modeling, and endless appreciation to Remziye and Sepideh for their invaluable guidance and support through the duration of this project.

References

1. Basha, S., Surendran, N., & Pichichero, M. (2014). Immune Responses in Neonates. *Expert Review of Clinical Immunology*, *10*(9), 1171–1184. https://doi.org/10.1586/1744666X.2014.942288
2. Liu, L., Johnson, H. L., Cousens, S., Perin, J., Scott, S., Lawn, J. E., Rudan, I., Campbell, H., Cibulskis, R., Li, M., Mathers, C., Black, R. E., & Child Health Epidemiology Reference Group of WHO and UNICEF. (2012). Global, regional, and national causes of child mortality: An updated systematic analysis for 2010 with time trends since 2000. *Lancet (London, England)*, *379*(9832), 2151–2161. https://doi.org/10.1016/S0140-6736(12)60560-1
3. Wang, H., Liddell, C. A., Coates, M. M., Mooney, M. D., Levitz, C. E., Schumacher, A. E., Apfel, H., Iannarone, M., Phillips, B., Lofgren, K. T., Sandar, L., Dorrington, R. E., Rakovac, I., Jacobs, T. A., Liang, X., Zhou, M., Zhu, J., Yang, G., Wang, Y., … Murray, C. J. L. (2014). Global, regional, and national levels of neonatal, infant, and under-5 mortality during 1990–2013: A systematic analysis for the Global Burden of Disease Study 2013. *Lancet*, *384*(9947), 957–979. https://doi.org/10.1016/S0140-6736(14)60497-9
4. Wardlaw, T., You, D., Hug, L., Amouzou, A., & Newby, H. (2014). UNICEF Report: Enormous progress in child survival but greater focus on newborns urgently needed. *Reproductive Health*, *11*. https://doi.org/10.1186/1742-4755-11-82
5. Waaijenborg, S., Hahné, S. J. M., Mollema, L., Smits, G. P., Berbers, G. A. M., van der Klis, F. R. M., de Melker, H. E., & Wallinga, J. (2013). Waning of Maternal Antibodies Against Measles, Mumps, Rubella, and Varicella in Communities With Contrasting Vaccination Coverage. *The Journal of Infectious Diseases*, *208*(1), 10–16. https://doi.org/10.1093/infdis/jit143
6. Etti, M., Calvert, A., Galiza, E., Lim, S., Khalil, A., Le Doare, K., & Heath, P. T. (2022). Maternal vaccination: A review of current evidence and recommendations. *American Journal of Obstetrics and Gynecology*, *226*(4), 459–474. https://doi.org/10.1016/j.ajog.2021.10.041
7. Niewiesk, S. (2014). Maternal antibodies: Clinical significance, mechanism of interference with immune responses, and possible vaccination strategies. *Frontiers in Immunology*, *5*, 446. https://doi.org/10.3389/fimmu.2014.00446
8. Erdogan, R., Dolatshahi, S. (2023). Quantitative mechanistic model reveals key determinants of placental IgG transfer and informs prenatal immunization strategies. *bioRxiv* https://doi.org/10.1101/2023.04.18.537285
9. van der Maas, N. A. T., Sanders, E. A. M., Versteegh, F. G. A., Baauw, A., Westerhof, A., & de Melker, H. E. (2019). Pertussis hospitalizations among term and preterm infants: Clinical course and vaccine effectiveness. *BMC Infectious*

Diseases, *19*(1), 919. https://doi.org/10.1186/s12879-019-4563-5

10. Healy, C. M., Rench, M. A., & Baker, C. J. (2013). Importance of timing of maternal combined tetanus, diphtheria, and acellular pertussis (Tdap) immunization and protection of young infants. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *56*(4), 539–544. https://doi.org/10.1093/cid/cis923
11. Englund, J. A. (2007). The Influence of Maternal Immunization on Infant Immune Responses. *Journal of Comparative Pathology*, *137*, S16–S19. https://doi.org/10.1016/j.jcpa.2007.04.006
12. *Human Placenta Project | NICHD - Eunice Kennedy Shriver National Institute of Child Health and Human Development*. (2023, April 12). https://www.nichd.nih.gov/research/supported/human-placenta-project/default
13. Bonabeau, E. (2002). Agent-based modeling: Methods and techniques for simulating human systems. *Proceedings of the National Academy of Sciences*, *99*(suppl_3), 7280–7287. https://doi.org/10.1073/pnas.082080899
14. Westman, A. M., Peirce, S. M., Christ, G. J., & Blemker, S. S. (2021). Agent-based model provides insight into the mechanisms behind failed regeneration following volumetric muscle loss injury. *PLOS Computational Biology*, *17*(5), e1008937. https://doi.org/10.1371/journal.pcbi.1008937
15. Peirce-Cottler, S. (2019). *Angiogenic Growth in Cornea Micropocket Assay* (V0.1) [Computer software]
16. *Cytotrophoblast—An overview | ScienceDirect Topics*. (n.d.). Retrieved May 12, 2023, from https://www.sciencedirect.com/topics/medicine-and-dentistry/cytotrophoblast
17. Wang, Y., & Zhao, S. (2010). Cell Types of the Placenta. In *Vascular Biology of the Placenta*. Morgan & Claypool Life Sciences. https://www.ncbi.nlm.nih.gov/books/NBK53245/
18. Kaipe, H., Raffetseder, J., Ernerudh, J., Solders, M., & Tiblad, E. (2020). MAIT Cells at the Fetal-Maternal Interface During Pregnancy. *Frontiers in Immunology*, *11*. https://www.frontiersin.org/articles/10.3389/fimmu.2020.01788
19. Brown, L. M., Lacey, H. A., Baker, P. N., & Crocker, I. P. (2005). E-cadherin in the assessment of aberrant placental cytotrophoblast turnover in pregnancies complicated by pre-eclampsia. *Histochemistry and Cell Biology*, *124*(6), 499–506. https://doi.org/10.1007/s00418-005-0051-7
20. Abar, S., Theodoropoulos, G. K., Lemarinier, P., & O'Hare, G. M. P. (2017). Agent Based Modelling and Simulation tools: A review of the state-of-art software. *Computer Science Review*, *24*, 13–33. https://doi.org/10.1016/j.cosrev.2017.03.001
21. Félétou, M. (2011). Introduction. In *The Endothelium: Part 1: Multiple Functions of the Endothelial Cells—Focus on Endothelium-Derived Vasoactive Mediators*. Morgan & Claypool Life Sciences. https://www.ncbi.nlm.nih.gov/books/NBK57145/
22. *Development of Blood Vessels and Fetal Circulation | Anatomy and Physiology II*. (n.d.). Retrieved May 12, 2023, from https://courses.lumenlearning.com/suny-ap2/chapter/development-of-blood-vessels-and-fetal-circulation/

Appendix

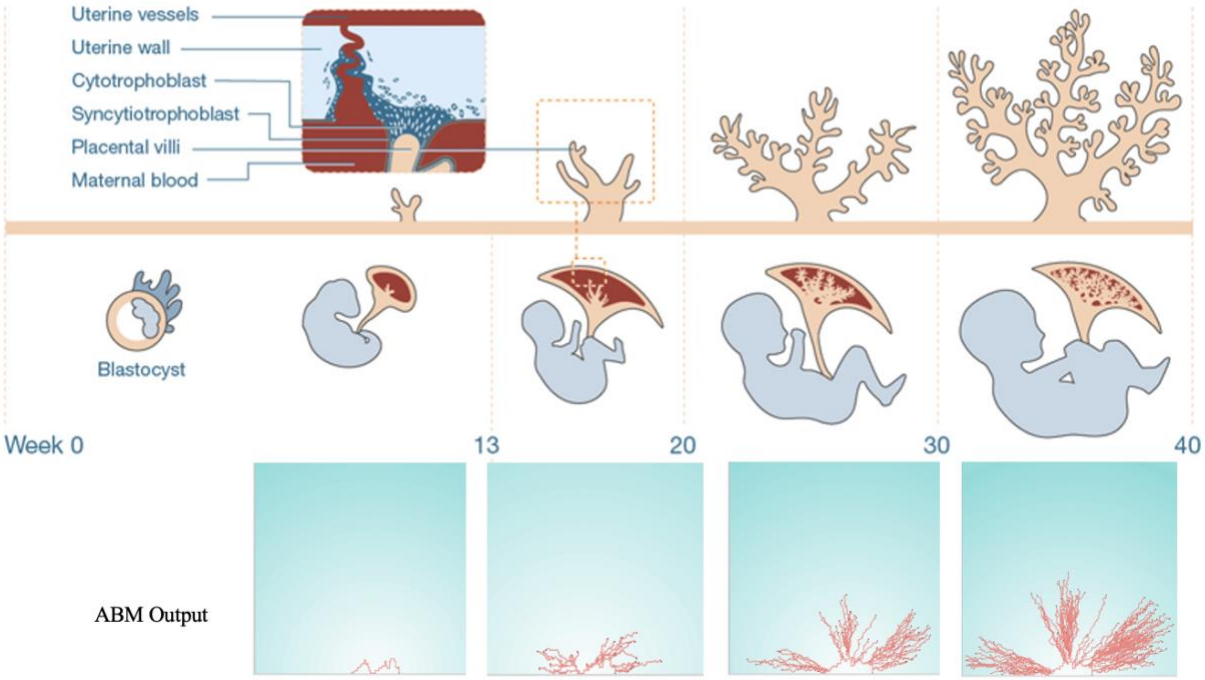

Figure S1. Model simulates placental development during gestation. (top image from *Weinburg et al., Human Placenta Project: How Does the Placenta Form?*)