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Biophysical Properties Of Trophoblast Placental Plasma Membrane

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Abstract: The lack of experimental research on a woman's placenta due to ethical restrictions has led to little to no research on the biophysical changes at the molecular level that a woman undergoes during pregnancy. This research aims to understand biophysical changes in placental trophoblast cells that facilitate diffusion between maternal and fetal blood during the first and third trimesters. It is challenging to comprehend the function of the placental trophoblast lipid membrane in regulating molecular transport between the mother and placental trophoblast cells due to inadequate in vitro models. For this reason, we utilized recent findings on the lipid compositions of the placental trophoblast plasma membrane for the first and third trimesters to pioneer molecular-level simulation of the human placenta trophoblast membrane in the first and third trimesters. Results provided an understanding of lipids behavior between trimesters and offered insight for screening molecular transport across the placenta. Given the lipid compositions and molecular dynamics tools, the two-trimester membranes were examined and compared using a coarse-grained Martini system. Each lipid trimester composition was simulated with periodic boundary conditions in a 51 nm x 51 nm x 15 nm box. The upper and lower leaflet conditions were compared for trends within each trimester. Key observations between trimesters include a reduction in membrane thickness from the first to the third trimester, a decrease in order parameter from the first to the third trimester, an increase in area per lipid from the first trimester to the third trimester, and the difference the in the number of Chol reduction in membrane thickness from the first to the third trimester, a decrease in order parameter from the first to the third trimester, an increase in area per lipid the from the first trimester to third trimester, and the difference in the number of cholesterols between leaflets narrowed from the first to third trimester with the

number of cholesterol flip flops increasing from the first to the third trimester. Observations like the reduction in thickness reflect previously presented experimental data by researchers. In summary, using molecular dynamics for placental trophoblast membranes enables us to gain insight into the placental trophoblast lipid membrane behavior from the first to the third trimester.

BIOPHYSICAL PROPERTIES OF TROPHOBLAST PLACENTAL PLASMA MEMBRANE

by

Nelly Raissa Setchie Tchato

B.S., Carnegie Mellon University, 2020.

Thesis
Submitted in partial fulfillment of the requirements for the degree of
Master of Science in Bioengineering.

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As we progress, our learned skills bring us success in all aspects of our life. Thank everyone, for creating such a great environment.

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1. INTRODUCTION

1.1. Placenta

The placenta, a disc-shaped organ that develops in a woman's uterus during pregnancy, is a physical link between the mother and the fetus [1-2]. Its functions are to deliver essential nutrients and respiratory gases to the fetus, remove waste products, and produce gestational growth hormones that regulate the fetus's and the placenta's development. As the fetus grows and develops, the placenta also grows to provide a large surface area for mother-fetal exchange. The central functional unit of the placenta is the chorionic villus. These are vascular projections of fetal tissue surrounded by the outermost membrane around the embryo (Chorion shown in Figure 1 below) [2-3]. Within the chorionic villus, fetal blood is separated by three layers of components with different cell layers (cytotrophoblasts /syncytiotrophoblasts, mesenchymal cells, and fetal vascular cells) from the maternal blood supply in the intervillous space [3].

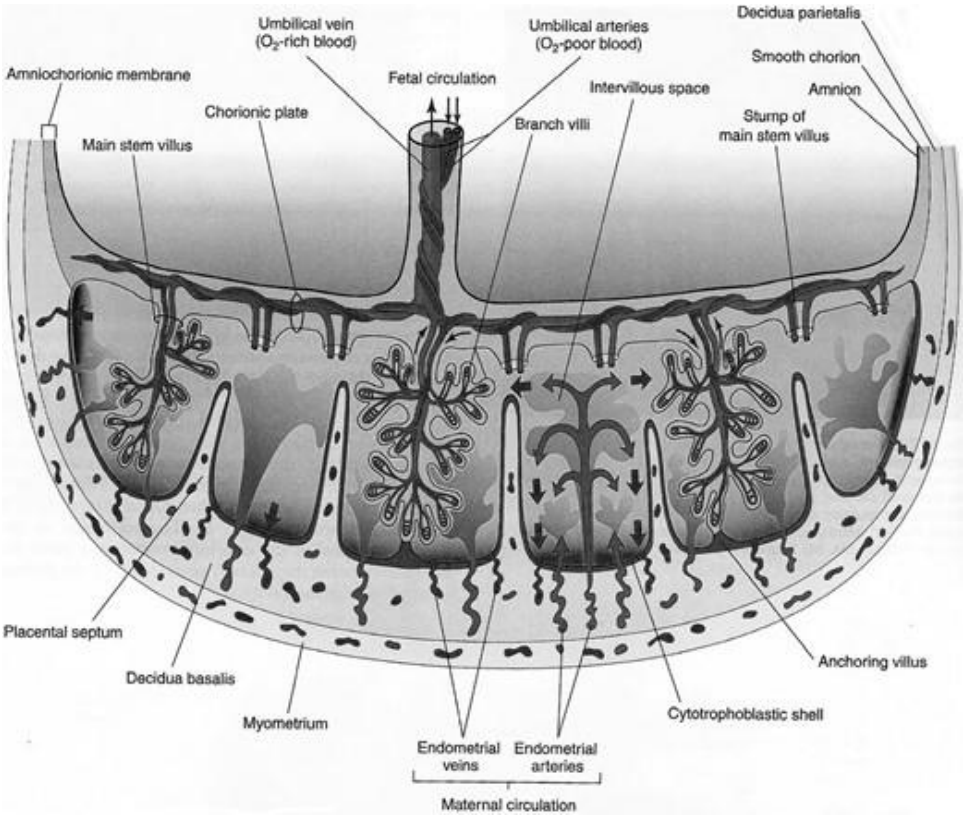


***Figure 1**: Transverse section of a full-term placenta [31]*

Upon fetus implantation into the mother's uterus, the trophoblast cells proliferate and differentiate as villous (non-migratory) and extravillous. The villous cytotrophoblast cells merge and form multinucleated syncytiotrophoblast, thus creating the outer epithelial layer of the chorionic villi. The fetal/maternal exchange occurs at the ends of these chorionic villi. As for the extravillous trophoblast cells, they migrate into the decidua and remodel the uterine arteries facilitating blood flow to the placenta through dilated compliant vessels, unresponsive to maternal vasomotor control [3].

This research is focused on the villous trophoblast cells, which form the outer layer of the blastocyst. Trophoblasts develop during the first stage of pregnancy. The trophoblast plasma membrane's physical state and molecular interactions influence the transport

activities, and membrane-associated properties orchestrate the complex biomolecular interactions between the fetus and mother. Because of the complex trophoblast interactions, trophoblast membrane lipid bilayers get modified during pregnancy to meet the changing metabolic needs of the growing fetus. The trophoblast plasma membrane's physical state and molecular interactions influence the transport activities and membrane-associated properties.

1.2. Placental Disorder and The Importance of Plasma Membrane

Disorders associated with placental development include pre-eclampsia, fetal growth restriction, recurrent miscarriage, and stillbirth. In addition, an abnormal increase in phospholipids or cholesterol in the lipid bilayer of the placental trophoblast could impair transport systems necessary for preventing pathological pregnancies. The layer which impacts the transport system is the syncytiotrophoblast cells covering the villi. Physical factors influencing transfer across the placenta include placental surface area, placental thickness, maternal and fetal blood pH, placental metabolism, uteroplacental blood flow, and placental drug transporters [4-6].

Scientific study has also indicated that changes in the phospholipid and cholesterol content within the trophoblast membrane throughout gestational progress are essential for placental function and nutrient transport [6]. During pregnancy, there is an overall increase in triglycerides, total cholesterol, and low-density lipoprotein. The ratio of cholesterol to phospholipid is a significant determinant of membrane fluidity. Changes in the balance can decrease fatty acyl chain mobility and disrupt transport across the trophoblast membrane.

These alterations can affect membrane fluidity, order, and lipid interactions, resulting in pregnancy complications related to placental permeability and the transport of essential fatty acids and solutes across the phospholipid bilayer [22].

Because the placenta is an evolutionary diverse yet highly specific organ amongst distinct species, it is challenging to find representative animal models that can reflect the characteristics of the human placenta for pregnancy. Also, ethical and logistical obstacles have stood in the way of investigating human pregnancy. Christina et al. have recently determined the lipid compositions of placental trophoblast for the first trimester and the third trimester [6]. This knowledge imparted us to pioneer a molecular-level description of the human placenta trophoblast membrane in the first (1T) and third (3T) trimesters. In addition, it provided a way to understand lipids behavior between the trimesters. In this project, we investigated the lipid composition and the physical properties of the trophoblast plasma membrane. We looked at both the extracellular leaflet (EL) and cytoplasmic leaflet (CL) layers of first and third-trimester trophoblast cells using molecular dynamics at the coarse-grained (CG) resolution.

1.3. Lipid Membranes

Lipids (fats) are water-insoluble organic compounds, referred to as “amphipathic,” containing hydrophobic (water-fearing) and hydrophilic (water-loving) moieties. Lipids placed in an aqueous environment will spontaneously form a bilayer, thus creating a lipid bilayer or lipid membrane. The cell’s plasma membrane is a protective barrier surrounding its internal organelles. It keeps the balance between the outside and inside aqueous

environment and transports substrate (e.g., nutrients) in and out of the cell. Found on/in the plasma membrane, transmembrane proteins, glycolipids, and cholesterol perform various cellular functions [26].

Eukaryotic structural membranes lipids are composed of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidic acid (PA), sphingomyelin (DPSM), cholesterol (CHOL) and other types of lipids. As the pregnancy evolves from 1T to 3T, it was observed from experimental research that the plasma lipid membrane composition changed [4]. However, the biophysical impact of the compositional changes remains unknown. The biophysical changes the team investigated include membrane thickness, density, area per lipid (APL), microdomain formation, cholesterol flip-flop, and order parameters.

1.4. Molecular Dynamics and Coarse-Grained Simulation

To understand properties associated with the assembly of molecules, structure, and atomistic interaction, scientists moved toward molecular dynamics (MD) simulations. MD broke the barrier that prevented scientists from understanding the physical properties of biological systems at an atomic level. This approach or strategy was encouraged by the fast advancement of computer science and technologies throughout the century. MD are computational simulations that study complex biomolecules. MD experiments are done 'in silico,' allowing researchers to bypass ethical restrictions by coupling with increasing computer power, established models, software, and theoretical studies. The power of this approach plays a critical role in predicting the behavior and trajectories of molecules under designed conditions. The simulations are based on Newton's second law, Newton's equation

of motion. Newton's law states that the resulting force (F) of an object is guided by the object's mass (m) times its acceleration (a) eq (1). which means that if one knows the forces atoms (with known mass) within a system exert on each other, then one can determine atoms' acceleration. The force of the atoms can be derived from their potential energy (U), which is comprised of bonded (bonds stretching, angles bending, and atoms rotating) and non-bonded (Lennard-Jones and Coulombs-potential) energy.

In summary, MD creates trajectories for all atoms with the assistance of the force field. First, initial position and velocities are given to the atoms, then their potential energies (bonded and non-bonded) energies are calculated, and the forces exerted on them along every dimension are also calculated. Finally, given forces, the atoms' acceleration can be decided based on Newton's second law of motion. Thus, the atoms' positions and velocities can be generated in the next time step.

Equations:

1) $F = ma$
2) $U = U_{bonded} + U_{non\text{-}bonded}$
3) $U_{bonded} = U_{bonds} + U_{bend\text{-}angles} + U_{torsion\text{-}angles}$
4) $U_{non\text{-}bonded} = U_{Lennard\text{-}Jones} + U_{Coulomb\text{-}potential}$

Running MD simulations at an atomistic level (all-atom MD (AAMD)) for complex biological systems, though they produce fine details, can be time-consuming, costly, and require big data storage depending on the size of the system. These disadvantages of AAMD led scientists to develop coarse-grained MD (CGMD), where small groups of atoms are treated as one bead, thus extending our simulation timestep from 2 fs to 20 fs.

The timescale increase has enabled scientists to extract more information from the system and draw more realistic conclusions. The force field used in this research is the MARTINI force field, a well-established coarse-grained model that balances computational efficiency and chemical resolution.

2. METHODS

2.1 COARSE-GRAINED MODELING

As briefly stated above, a martini is a program that maps many atoms to one (as shown in Figure 2 below). For example, three to four neighboring non-hydrogen atoms are mapped to one CG bead to preserve some properties of the chemical structures [11-12]. The trophoblast membrane constitutes multiple classes: PC (*DPPC, POPC, DOPC, PIPC, PQPC, PAP*C); PE (*POPE, DOPE, PIPE, PAPE, DIPE*); PI (*POPI, DOPI, PIPI, PQPI, PAPI*); PS (*POPS, PGPS, PEPS, PAPS*); SM (*DPSM*); and CHOL (cholesterol). These lipids were CG mapped to the MARTINI2.2 force fields using the script *martinize.py [13]*.

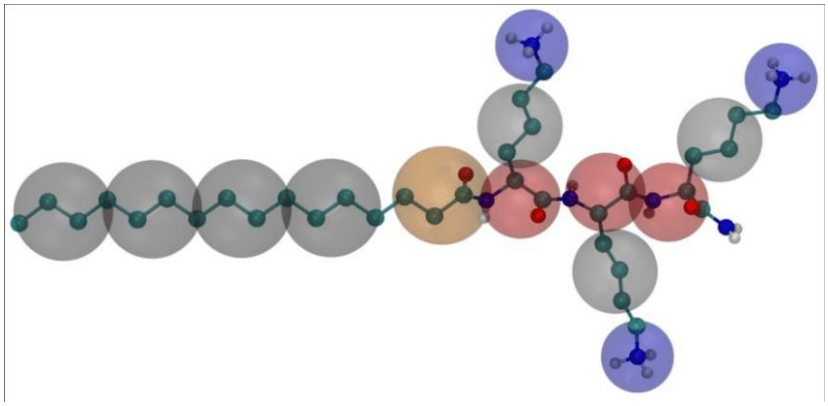


Figure 2: *All-atom to coarse grain mapping (Leung et al.) []*

2.1. Building The Lipid Membrane System

A 51 x 51 nm2 membrane patch system of the human trophoblast plasma membrane was

created in CG presentation using a locally modified insane.py script from the Martini website. As stated above, the lipid composition was obtained from a previous study, but not cholesterol. The cholesterol composition was acquired from another study and added to the system. Table 1 below shows the lipid composition difference between the first and third trimesters. PC and PE lipid composition decreased from the first to the third trimester, while PI, PS, and PSM increased. CHOL stayed constant. Every system was surrounded by Martini water and 0.15 M NaCl.

Table 1: *Lipid composition difference from 1st to 3rd trimester*

Lipid composition difference from 1st to 3rd trimester		
	EL (exoplasmic)	CL (cytoplasmic)
PC	-8.86%	-4.72%
PE	0.00%	-8.99%
PI	3.93%	3.80%
PS	0.61%	10.11%
PSM	4.63%	0.16%
CHOL	-0.30%	-0.22%

2.2. Coarse-Grained Simulations

The coarse-grained simulation transpired using GROMACS molecular dynamics package version 2018. The system underwent four steps: (1) CG energy minimization was performed using the steepest decent algorithm with a 20-fs time step until the maximum force on any bead was calculated to be below 10 kJmol-1 nm-1. (2) Isothermal-isochoric (NVT) equilibration followed for 50 ns at 310.15k using a velocity-rescale thermostat with a coupling constant Tt = 1 ps. (3) Isothermal-isobaric (NPT) equilibration continued for 100 ns with semi-isotropic pressure coupling at 1 bar using Parrinello-Rahman barostat with a Tp of 12 ps (4) Finally, the production run ran for 5 microseconds at 20-fs time step.

The non-bonded van der Waals and the electrostatic interaction cut-offs were set to 1.1 nm. The potential-shift-Verlet algorithm was applied to shift the van der Waals interactions beyond the cut-off. Coulombic interactions were calculated using the reaction-field algorithm. Periodic boundary conditions were involved in all three dimensions, and simulations were run in quadruplicate for consistency.

2.3. Biophysical Properties Analysis

Structural properties, such as area per lipid, order, mixing parameters, density, and thickness profiles, as well as cholesterol number and flip-flop, have been computed and analyzed to gain an understanding of the functional changes of the trophoblast membrane throughout pregnancy [27-28].

2.3.1. Density and Thickness Analysis

Density, which is mass per volume, indicates the packing factor of the lipids and how many ones can find per volume. Density and Thickness are good indicators of permeability through fluid membranes. The effect of the difference in thickness is observed when a membrane transits from a liquid-disordered state to a liquid-ordered and gel-like state. Thickness is obtained by calculating the length of adjacent lipid tails (or head-to-head). Increasing the length of the phospholipid acyl tails increases the hydrophobic thickness of the membrane. [27]

2.3.2. Area per Lipid and Order Parameter Analysis

Area per lipid is similar to density, but the difference is that it looks at how much area one lipid takes. Area per lipid is calculated by obtaining the lateral surface area of the lipid bilayer and then dividing it by the number of lipids in each leaflet.[28]

The lipid acyl chain's order parameter (SCD) was calculated from NMR. SCD calculates the orientation of the C–H bond vector concerning the bilayer regularly averaged over all the lipids and all the sampling time. Each lipid acyl chain was calculated with one order parameter generated, averaging over lipids of the same type in EL and CL, respectively. SCD provides information on the membrane's overall order and specific details of the conformations that the atoms within the lipid tails adopt. [29,30] Again, SCD provides information on how straight and kinked the lipid tails are.

2.3.3. Lipid Mixing and Voronoi Plot Analysis

Lipid mixing is an indicator of how different lipid types interact. Contact between two lipid molecules is formed when the distance between a lipid species and the reference lipid is within a 1.1 nm cut-off. As for the Voronoi plot, it is used to define and delineate proximal regions around individual lipids by using polygonal boundaries. Voronoi plot and lipid mixing provide insight into how “like” lipids stay together and the types of microdomains formed.

2.3.4. Cholesterol Flipping Analysis

The cholesterol flip-flop rate depends on the bilayer order; it is facilitated in bilayers with higher fluidity and lower rigidity. This information will further strengthen the previous analysis stated above.

3. RESULTS AND DISCUSSION

3.1. Membrane Density and Thickness Decrease Across Trimesters

Trophoblast plasma membranes for 1T and 3T were analyzed for lipid densities. Results

showed higher density in the 1T membrane than the 3T membrane. When looking at the thickness characteristic between both trimesters, it was observed that the 1T bilayer is thicker than the 3T bilayer. However, the 3T membrane has higher uniformity in both thickness and density than the 1T membrane, as shown in *Figure 3*.

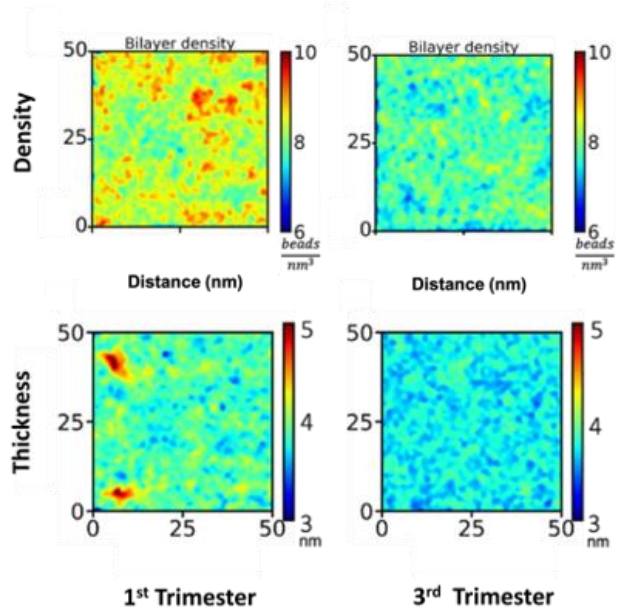


Figure 3: *Bilayer density and thickness profiles of the 1st and 3rd-trimester lipid membrane. The density plot scale ranges from 6 (blue) to 10 beads/nm^3 (red), and the thickness scale ranges from 3 (blue) to 10 beads/nm (red)*

3.2. Lipid Mixing and Voronoi Plots

We computed the lipid mixing parameter to determine whether self-association within the lipid family or co-localization with other families would be preferred Figure 4. Voronoi plots were also added to show local density to discern the lateral assembly of lipids. It was expressed that all families prefer to co-localize with PC, which has the highest proportion of mixing among each lipid family. While PC remains in the highest proportion of mixing in each of the 1T lipid families, the ratio of PC mixing in the 3T membrane is decreased

among each lipid family.

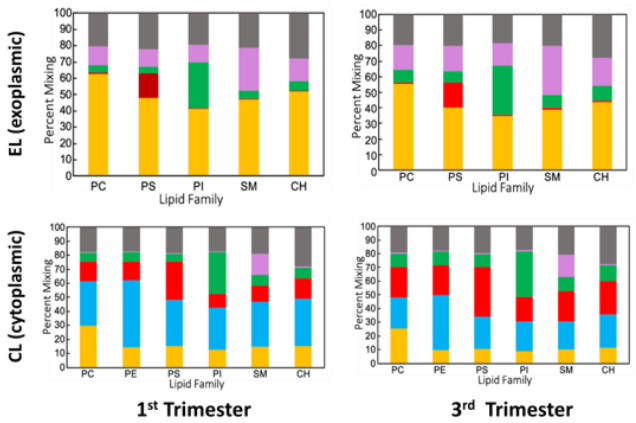


Figure 4. *Lipid mixing plots. Upper left- 1st-trimester upper leaflet, Bottom left- 1st trimester lower leaflet, upper right- 3rd-trimester upper leaflet, bottom right- 3rd trimester lower leaflet.*

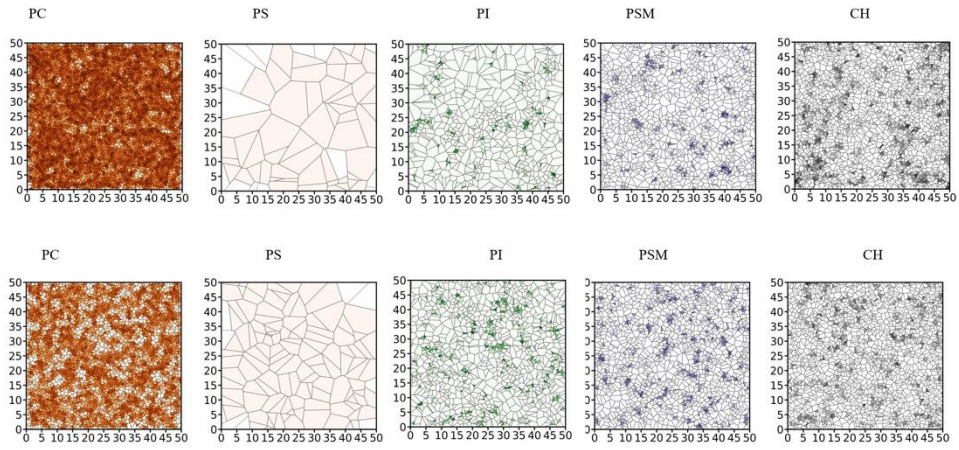
The Voronoi plots in *Figure 5* affirm this decrease across trimesters in forming PC nanodomains. As for SM and PI, their lipids comprise a significant proportion of the mixing affinity of each lipid family in the outer leaflet of the 1T membrane. Compared to the 1T outer leaflet, their proportion increases considerably in 3T. The Voronoi plots of SM and PI show increased clustering across trimesters. Because PS and SM comprise <2% lipid composition of the upper leaflet, their mixing affinity with other lipid families is negligible.

The lipid mixing parameter analysis shows that PE in the lower leaflet represents a higher proportion of lipid mixing for each lipid family, putting aside PI. PE is the most abundant lipid in the CL, corresponding to the high ratios of mixing affinity of PE with each of the 1T lipids. The mixing parameter analysis of PI shows nearly equal mixing affinity to PI and PE. PE mixing decreases from 1T to 3T, with both PI and PS mixing increasing.

Cholesterol composes about 20% of each leaflet in 1T and 3T. Cholesterol mixing in the upper and lower leaflets of 1T and 3T is roughly 20% mixing for each lipid family. Cholesterol tends to self-associate, resulting in a higher mixing affinity of approximately 28% in each leaflet for both trimesters. 3T membrane has shown excellent lipid composition distribution, correlated to a more evenly distributed mixing affinity in both leaflets. The lipid clustering in the Voronoi plots supports this conclusion.

In most situations, the frequency distributions of angles relative to the protein axis and z-axis were similar, and preferred tilt angles were nearly identical. However, some of them were different for some reason. First, when the membrane was wavy, their preferred tilt angles primarily separated from each other, which was seen in the placental results. Second, it could result from proteins tilting in different directions from CYPs.

EL



CL

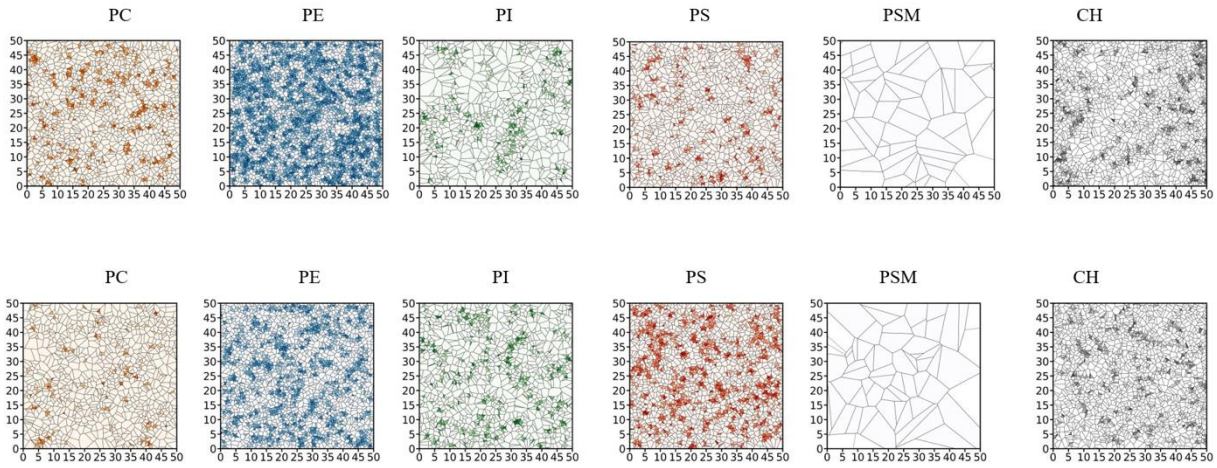


Figure 5. *Voronoi Plots of the EL and CL, 1st trimester on top and 3rd trimester at the bottom of each leaflet.*

3.3. Lipid order parameters decrease from the first trimester to the third trimester

Lipid order parameters (SCD) were generated for all acyl chain beads of the lipids to quantify the asymmetry of the trophoblast membrane. The cumulative SCD of each leaflet was calculated from the same set of carbon beads. Overall, it is observed that SCD decreases as the acyl chain beads of interest move from headgroups towards lipid tails, meaning the farther the carbon bead is located away from the headgroups, the less ordered it becomes. This trend holds for both leaflets. Consistent with the latest reports, the outer leaflet lipids have larger SCD than the lower leaflet lipids. When comparing the order parameters of the two trimesters, it is evident that the 1T membrane is more ordered than the 3T membrane. This difference is stark in the comparison of the lower leaflets. There are two main characteristics of the membranes that emerge. First, 1T membrane is more ordered than 3T. Second, the outer leaflet of the membrane (orange background) is more ordered than the inner leaflet (blue background), irrespective of the trimester. After analyzing the order parameters from the perspective of the position of the carbon beads, the

average order parameter of each acyl chain in the lipid molecule was computed. Here, it is evident that saturated and unsaturated acyl chains have remarkably different order parameters that affect the lipid shape and lipid packing in the membrane (figure 6). A box-whisker plot was generated from the average SCD values for the individual lipid acyl chains to understand this finding better.

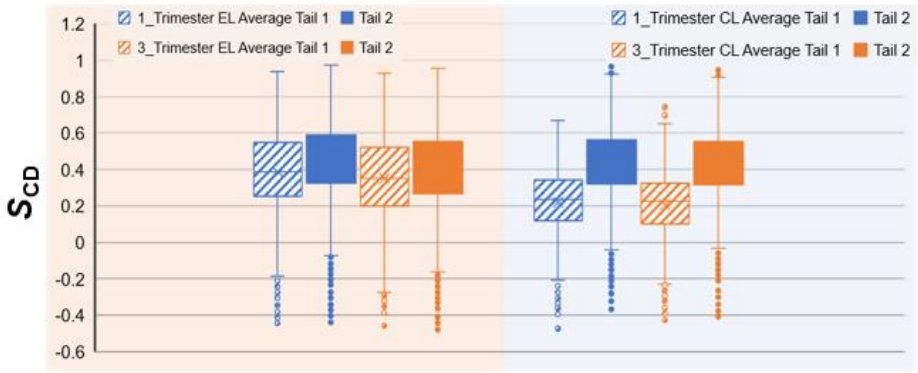


Figure 6. *The first trimester (blue) and third trimester (orange) order parameters between the EL on top and Cl at the bottom are shown as a box-whisker plot. Solid and striped boxes distinguish the two acyl chains of a lipid.*

3.4. Area per lipid increases from the first trimester to the third trimester

A bilayer's area per lipid (APL) provides insight into the sensitivity of lipids within the membrane to establish hydrophilic attraction between head groups and hydrophobic interactions between the non-polar hydrocarbon tails. The APL of the trophoblast increases from 1T to 3 T, the increase observed in the outer and inner leaflets (Figure 2). The increase in the APL could be due to a significant decrease in the abundance of PE and PC lipids from 1T to 3 T. Additionally; the average SCD parameters decrease observed in going from 1T to 3T. A lower SCD value reflects the kinks and bends in lipid tails, while a higher value reflects a straighter tail. The kinks and bends in a tail increase the lipid's area, as is reflected

in Figure 2. To recapitulate, the decrease in acyl chain SCD from 1T to 3T may explain why the area per lipid of the outer leaflet increases more than the inner leaflet from the average SCD values for the individual lipid acyl chains (Figure 7).

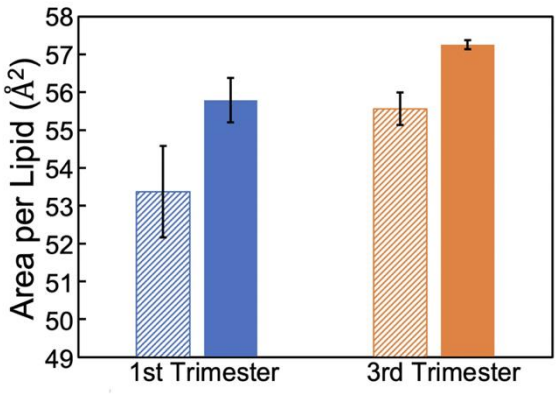


Figure 7*: Area per lipid of 1st and 3rd-trimester membranes. The striped color represents the bilayer's extracellular (EL) side, and the solid color represents the cytoplasmic side (CL).*

3.5. Cholesterol flip-flopped equally between the leaflets

An equal number of cholesterol molecules were placed in 1T and 3T membrane leaflets. Over the five microseconds simulation run, cholesterol was disproportionately distributed in favor of the outer leaflet. At equilibrium, the outer leaflet had 55.3±0.4% cholesterol compared to the inner leaflet (44.7±0.4%) in the 1T membrane. A similar behavior was observed in the 3T membrane, with 51.9±0.42% in the outer leaflet compared to 47.4±0.4% in the cytoplasmic leaflet. The rate of cholesterol flip flop was nearly equal between the upper and lower leaflet but more significant in the 3T membrane than the 1T membrane. Both observations suggest that changes in lipid composition could have a role in cholesterol distribution and flipping, considering the two-trimester membranes have similar yet different lipid compositions.

4. CONCLUSIONS

Results from this research showed that lipid composition does impact the biophysical properties of the placental trophoblast membrane when we look at the first and third trimesters. The overall change observed between the two trimesters includes a decrease in membrane lipid density, a reduction in membrane thickness, an increase in area per lipid in both the outer and inner membrane leaflets, a decrease in the lipid order parameters, an increase in the number of cholesterol flip-flop between the leaflets; and an increase in symmetric cholesterol distribution between the leaflets. The changes in these biophysical properties are bound to impact membrane-bound proteins' activity and potential drug diffusion across the membrane.

5. FUTURE WORK

This project explained the changes between the first and third trimesters, affecting the cells' biophysical properties. In addition, it gave a potential guide on how drug transport across the placenta might be affected. The results above show that the first-trimester plasma membrane is thicker and tighter than the third-trimester. From this, it can be hypothesized that transport across the placenta will be more accessible during the third trimester compared to the first trimester. To make this research have a more significant practical impact on biomedical development, our future work should investigate outside factors like nutrition and its potential effects on plasma lipid composition.

Stepping into the nutrition department as a next step to investigate is essential because it has a crucial influence on population health. Nutrition plays a significant role in health conditions in adulthood, but its impact originated in fetal life. The development of a fetus during pregnancy is critical for the programming of the future fetus' condition [14,17]. Thus, a woman's dietary fatty acid (n-3 and n-6) plays a crucial role in her cell membrane composition and her offspring's chance of survival and health after birth [15-17]. Suppose a woman's fatty acid diet can impact her cell membrane composition. In that case, it indicates that the trophoblast cells, responsible for nutrient exchange between the mother and her fetus, will also be impacted. Modifying the trophoblast lipid composition could affect the transport of nutrients across the placenta, whether by diffusion, filtration, pinocytosis, or carrier-mediated transport [20-21]. The goal is to use MD simulation to analyze the impact of diet on

the physical properties of the lipid membrane, the formation of microdomains, its impact on transmembrane protein (carrier protein) stability, and the passive diffusion of hydrophobic drugs.

Humans are heterotrophs, meaning they require organic molecules as energy sources and some specific molecules to be performed (comes and stays as is). For example, some fatty acids and amino acid members must be performed (taken in their original form), while others must be synthesized once assimilated. As previously stated, we want this research to investigate the role of dietary fat composition function on a pregnant woman. Dietary fat provides the backbone of membranes, an essential life component. If DNA is the eternal molecule of life, membranes are the infinite life structure; new membranes form from pre-existing ones. [32] The lipid membrane provides a dynamic environment where essential metabolic life chemistry occurs, and it is both adaptive and highly diverse. The main types of fatty (FA) acid chains found in the mammalian diet are categorized into saturated, monounsaturated, and polyunsaturated, with polyunsaturated further broken down into omega-3 (n-3), omega-6 (n-6) and some omega-9 (n-9). Animals can synthesize saturated and monounsaturated fatty acyl chains; however, that is not true for n-3 and n-6 polyunsaturated fatty acids (PUFAs). These two PUFAs must be obtained from the diet or synthesized by gut micro-organisms.

A fatty acyl can be identified by its head (phosphate group and a glycerol molecule) and its tail (number of carbon atoms, number of double bonds, and the position of the first double

bond relative to the methyl end (also known as omega carbons)) as shown in figure 8. The chain length and the double bonds present significantly influence the properties of fatty acids. For example, suppose 18:1 n-9 is substituted for 18:0 in the sn-2 chain (stereospecific numbering). In that case, the melting point of that phospholipid will decrease by 1 C. However, if the sn-2 chain were further modified (increase degrees of polyunsaturation) to 18:2 n-6, the result would be a slight increase in the phase transition. Due to these changes, the fatty acids that comprise the membrane lipids significantly impact membranes' fluidity and other dynamic properties.

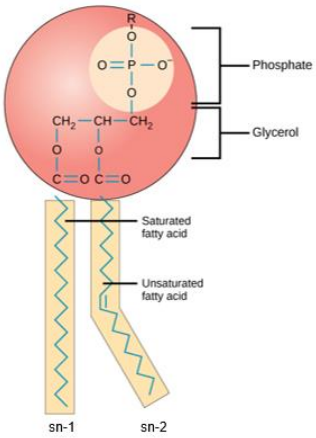


Figure 8: *Fatty acyl schematic*

Back to omega n-3 and n-6, some sources of n-3 PUFAs come from broccoli, green beans, cabbage, fish, and the brain, while n-6 PUFAs come from bread, vegetable oil, muscle, and kidney—a diet of high n-3/n-6 benefits human health [15]. Modern western diet is dominated by n-6 PUFA, with an n-3/n-6 ratio of ~0.06. This fact can be concerning since most ingested fatty acids will likely be incorporated into storage triglycerides or plasma membrane lipids. Lipid membranes are, by nature, regulators as well as conformers. The fatty acids the lipid membrane responds/conform to the most are n-3 and n-6 because higher animals cannot

synthesize these fatty acyls but must be ingested.

Previous research looked at the responsiveness of phospholipid composition of selected rat tissues (liver, colon, muscle, pancreas, thymocytes, and adipose tissue) and found that the n-3/n-6 ratio strongly influences the liver and the colon membrane.[34] Further analysis of different phospholipid classes of the plasma membrane of rat liver and their responsiveness to dietary fatty acid shows that phosphatidylcholine (PC) and sphingomyelin (SM) were the most responsive, while phosphatidylinositol (PI) was the least responsive to the different types of diet. Phosphatidylethanolamine (PE) was more responsive to the n-3 and n-6 diets. Between n-3 and n-6 diets, SM was equally responsive, while PC, PE, and PS (phosphatidylserine) were more responsive to n-3 than n-6. Because the liver and stomach have similar functions as the placental trophoblast membrane (nutrient exchange), applying this knowledge to trophoblast cells will be beneficial to understanding the impact of diet on a pregnant woman and her fetus using CGMD.

We plan to investigate two aims as we change the trophoblast plasma membrane composition based on the n-3 and n-6 diet in the first and third trimesters. Aim one is studying the plasma membrane biophysical properties, its impact on passive diffusion on hydrophobic drugs, and facilitated diffusion. Aim two looks into microdomain formation and its implications on transmembrane carrier protein conformational change.

Potential pitfalls for this research arise with computational expenses and simulation time, which might slow the investigation. The size of the system would play a significant role in

simulation time and cost, even if we achieve the simulation using a coarse grain model. This project will also require multiple job submissions (minimum 4). Thus, a possible pitfall might be premature exiting of the job, an insufficient amount of data storage, and possibly using GPUs resources instead of CPUs to accelerate the job process. It is possible to reduce simulation time, but it is not recommended because the system requires a minimum amount of time to reach equilibrium.

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