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Studying the Dynamics of Spermatozoa in Relation to Sub-fertility

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Abstract

Male subfertility has been increasing for over 50 years, dropping by as much as 50% in western countries. Understanding the motion of sperm particles is important as it can help to expand our knowledge on how to combat this phenomenon. This project aims to explore the motility of a sperm cell. In order to better understand how to increase the chances of a sperm cell causing the fertilisation of an egg. A data set has been provided containing a collection of in-vivo footage of sperm particles moving through the reproductive systems of a group of mares, recorded using video microscopy. Processing this data, we captured individual sperm particles within the frames of the videos so that they can be tracked frame by frame. However, due to the nature of the videos the processing only allows for the largest or most visible particles to be tracked. Using the results of tracking the paths of the sperm particles and beads, a QQ-plot is created and a Shapiro-Wilks test is carried out to test whether the motion of a sperm particle is Brownian or not. The concentration of sperm along the reproductive system is found, giving an idea of where in the reproductive system the sperm are most prevalent. Finally, the velocity of the sperm tracks is modelled to determine the general direction of movement for each sperm particle and to further justify whether the movement of the sperm follows Brownian motion.

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1 Introduction

In recent history a colossal decline in male fertility has been observed, with men today exhibiting a sperm count of less than 50% that of men 50 years ago [1]. Naturally, such an astounding decrease in a fundamental component of the propagation of our species demands endeavours to understand and remedy the underlying causes of subfertility in men.

Sperm dysfunction is the most obvious, observable cause of rising fertility problems. While fertility problems as a whole are influenced by male reproductive systems and female reproductive systems, sperm dysfunction accounts for around 50% of fertility problems in the human population [1]. Therefore, we choose to isolate and study subfertility relating to spermatozoa, as statistically finding some way to improve this in particular will generate the most 'bang for buck' in treating subfertility.

Sperm dysfunction can be defined as the tendency of inability for spermatozoa to complete fertilisation. Motility is the ability of an organism (in this case sperm) to move independently using its own metabolic energy. Most notably, defects in the flagella directly impact sperm motility, often resulting in fertilisation failure [2]. Poor sperm motility is correlated with infertility [3], which is what gives CASA (Computer Aided Sperm Analysis) a purpose - as this means that motility can be visually assessed. If we can reach a firm understanding of the motility, and thus the impact on subfertility, we can potentially do something to remedy that factor of the subfertility problem.

In the effort to understand this, attempts at implementation of CASA have been going on for the last 20 years, but there hasn't quite been the progress that we would have hoped for. This is largely down to the complexity of spermatozoa itself, which gives rise to many possibilities that could be causing subfertility.

To illustrate this, consider that sperm motility can vary wildly over time instances between steady unidirectional movement, jolting movement in (seemingly) random directions or no movement at all [4]. This variation in efficient motility, combined with the many morphological variations that can lead to fertilisation failure (such as insufficient proteins or individual genetic deficiencies) gives rise to a host of potential sources that could be contributing to the subfertility problem. CASA can help us assess motility (and potential errors or problems), but morphological assessment requires other techniques. There is only so much we can achieve in analysing and diagnosing sperm defects that lead to the current observed decline in fertility.
Given this information, we look to analyse data sets of sperm imaging and give suggestions from our findings about the nature of the sperm's motility. The spermatozoa we are considering are from horses and donkeys, which are very similar in nature to human spermatozoa - and these similarities are observed to occur between many mammals. We hope to offer approaches to understanding the problem of subfertility in relation to understanding the motility, which could help in the journey toward effective diagnostics of subfertility and how to treat it, in particular more advanced and effective CASA.

1.1 Background Information

To begin, let us first look at what motility is. Motility in an organism such as spermatozoa is the ability for the organism to move independently using its own stored metabolic energy. What we want to determine is whether spermatozoa use their metabolic energy to drive themselves toward some goal, with the goal being the egg in order to complete fertilisation, or if they simply move aimlessly and the fertilisation is completed by chance of an individual sperm being in the right place at the right time.

By analysing a sperm cell's structure, we see what is known as a flagellum. This is a whip-like tail that aids the sperm in moving. A flagellum displaces fluid to propel the sperm in some direction, in a similarly fashion to the tail of a fish. Flagella are present in most motile bacteria [7].

It is clear then that the sperm does move independently and therefore has motility, but is the motion it exhibits reactionary to its environment or does it move randomly throughout its environment? This leads into the topic of Brownian motion.

Brownian motion is named after Scottish botanist Robert Brown, who is believed to have been the first to have studied Brownian motion in 1827. The motion can be described as any type of motion where quantities in a fluid

are undergoing random fluctuations in movement. The word 'random' is key here, as in relation to spermatozoa we are unsure if the movement is Brownian - but it could be.

Following on from sperm motility, just because spermatozoa are motile does not mean that they use metabolic energy to propel themselves towards some purpose. While it is possible that biological or chemical processes between the sperm and the contents of the fluid may occur which then direct the sperm towards the egg (this would imply purposeful movement), it is also possible that the sperm's movement is Brownian. Even though the spermatozoa are motile, they may move in a Brownian way so as to eventually disperse themselves evenly over the fluid through a process known as diffusion.

Diffusion occurs in Brownian motion because, given two equally sized sections of a given fluid A and B, if there are twice as many particles in section A as B, then if all particles experience Brownian motion (where the movement is random, evenly distributed over all directions and all velocities in a 3-D space) the chance of moving from A to B is twice that of moving from B to A [6]. Over time this probability function will tend to an even distribution (the particles are evenly spread out over the whole fluid). This is known as diffusion. The expected time to diffusion is based on the rate of particle transfer between sections A and B, which is based on average particle velocity (of the particles as a whole).

It is then reasonable to argue that if spermatozoa were follow Brownian motion, then fertilisation of an egg would occur eventually. By pure chance, fertilisation will be successful because a set of spermatozoa will eventually diffuse in such a way that one sperm cell occupies the same space as the egg (they combine). It may take a few 'tries', as has always been observed in mammals such as humans, but eventually a set of spermatozoa will diffuse in this way.

1.2 Previous Research

It is reasonable to argue that Brownian motion may be the primary method of propagation for spermatozoa, as the motion is seen in many other facets of nature [6]. That is to say, nature deems it as a successful method in it's development. One can also argue that Brownian motion will also work in fertilisation, as shown earlier. There is a clear common theme among previous works that the nature of motion of spermatozoa is not fully understood. While research has developed over the years, there still exists amibiguity, and no concrete conclusion appears to have been made.

In particular relation to this paper, D. B. Dunson's work in 1999 offers some interesting points. He outlines that plenty of research has been conducted in developing dynamic models of the movement of microorganisms (Okubo, 1980; Mandelbrot, 1982; Wiens 1989) with most methods being adapted from models for Brownian motion [5]. D.B. Dunson also goes on to argue that sperm do not move in a Brownian way, but rather explicitly purposefully, and suggests that the models are not directly applicable for this reason.

In a similar vain, K. Reynaud and Z.Schuss make interesting connections between Brownian motion observed in neurotransmitter molecules in the brain, and the observed motion of spermatozoa [10]. The numerical comparisons they make between the number of neurotransmitter molecules and the probability of finding a receptor bares strong resemblance to the suggestions outlined earlier in the paper regarding Brownian motion in spermatozoa and the fertilisation success rate being largely dependent on sperm count.

Motion aside, outright understanding of the morphology and ultrastructure of spermatozoa is yet to be attained. Across species, there is considerable diversity in morphology. It is suggested that the length of the sperm flagellum, especially the length of the midpiece, is a critical factor influencing sperm metabolism and velocity. However, understanding of the relationships between sperm ultrastructures and the sperm flagellar length is incomplete [8].

To conclude, the overarching issue currently is that while there is a huge amount of information still to be uncovered by way of an ideal analysis of spermatozoa, there means to enact such analysis does not currently exist. M. T. Gallagher outlines this, stating that if current methods were able to track the sperm flagellar waveform for a large number of cells, analysis of such tracking would yield a wealth of information [9]. Again, such feats could be possible if more efficient and advanced CASA's become available.

2 Methodology

2.1 Sperm In-vivo

Sperm in-vivo can be described as particles existing within an active fluid (bodily fluid). The dynamics of this fluid can change rapidly as the host bodies undergo various contractions - this can cause rapid changes in the movement of the sperm particles. In this study, the goal is to identify when the dynamic of the sperm are influences by other factors, such as chemotaxis and thermotaxis. Chemotaxis is when the dynamics of a system change due to the influence of a chemical gradient and similarity, whereas thermotaxis is caused by the changes in a temperature field.

2.2 The Data set

The primary data set of this study consists of a selection of in-vivo videos taken from various points within the reproductive system of Equine subjects. Corresponding with points locate in the uterus, vagina, cervix and different sections of the left and right uterine horns. The data consists of eleven different mares with unique code names - nine with horse semen and two with donkey semen in their reproductive system. The videos are recorded using video microscopy. Bead particulates were added as controls to the system to gauge the fluid dynamics of the system. Dye is applied to the system such that sperm particles and bead particles are recorded to the green and red channels of the video respectively (see figure 1).

A two dimensional image is taken every frame, this results in a loss of depth in the image. Sperm heads closer to the recording devise will appear larger and brighter and faster than particles more distant. Large particles can also be large collections of sperm moving together.

Each video can varies in activity, concentration, brightness and noise. Requiring individualised pre-processing before the particles can be tracked. The dye applied causes a significant degree of noise around the sperm heads, resulting in under defined boundaries between particles. The brightest areas correspond to the sperm heads, hence image segmentation of theses bright spots can be utilised to identify the centroids of the sperm heads.

2.3 Pre-processing the Data

The Data as previously stated has a large degree of noise, sperm and beads can also overlap each other to create larger bodies. This make pre-processing essential in ensuring that reliable tracks are recovered. Initially simply a threshold of minimum brightness were used to capture the majority of the particles in each frame. Much later in the study particle tracks from this data was found to be nonsensical, with very short and chaotic tracks. After significant attempts of adjusting parameters of the tracking model, it was determined that insufficient pre-processing was the cause. It was determined that the scope of behaviours that were attempted to be captured was too large. Larger particulates tend to be both brighter and faster than their smaller counterparts. These larger particles can be both large collections of particles or simply particles that were closer the recording devise (as there is no depth perception). Smaller particles were dimmer, slower, more tightly packed and harder to distinguish from the noise. Resulting in very different parameter requirements to successfully track the behaviours.

Thus segmentation of the larger particles would be prioritised as they are more simple to distinguish from noise. Taking a larger threshold to segment only the brightest particles was shown to not be sufficient, with this new stricter requirement the system became more sensitive to variation. As time progresses, movement of the fluid and contractions within the subject can cause sudden changing in the system, this can cause a abrupt change in brightness. Resulting in bodies dropping below the threshold and disappearing for a few frames and then later reappearing.

To combat this various binary image filters were trailed. Initially a ‘bpass’ was attempted. A ‘bpass’ is a spacial filter that pinpoints and sharpens round objects. Unfortunately, the method of microscopy used resulted in sperm heads becoming more elongated rather than round, resulting in minimal success from a ‘bpass’. A spacial filter that filters out objects smaller than a specified size was then trailed. This resulted in it being possible to use a much less strict threshold as smaller bodies where were being filters out. While this resulted in better results, it

did not account for larger objects undergoing a significant drop in brightness. A simple contrast filter showed very promising results, adjusting the contrast of the image so that over a certain threshold the brightness of the particles would be slightly boosted. A combination of these two filters allowed significantly better tracking of the particles. As each video in the dataset can vary vastly, each of these parameters must be adjusted for each set of data. This cannot be automated and can take a significant amount of time per dataset to find favourable conditions. Some videos had a significant amount of noise at a higher brightness level, a method was not found to sufficiently track the particle bodies in these cases.

Once the image has be sufficiently segmented, centroids of the sperm bodies could be identified by areas of consistent bright mass.

2.4 Tracking the Sperm Heads

Tracking the sperm heads after identifying the centroids is by nature very complex. The frame rate of the video results in relatively large gaps of movement between frames. This coupled with the complex dynamics of sperm moving through and passed each other, makes identifying the true trajectory of each particle unclear.

This study utilises methods and resourced provided by John C. Crocker and David G. Grier outlined in the paper "Methods of Digital Video Microscopy for Colloidal Studies (1996)" to track the particles. Each frame is compared with its successor. As by its nature each particles location can only be assigned to one future mapping. This requires a probabilistic analysis of the set of possible successors to successfully track the particles.

$$N = \{Set\ of\ possibles\ successors\} \tag{1}$$

Assumptions regarding the nature of movement must be made to determine the most likely location of each particle in the successive frame. Thus there is an assumption that the particles diffuse normally.[11] This does not restrict the model to only track normal particles, as in a most cases the closest successor will be chosen. It is only in the cases that there are multiple close viable successors that the model will be slightly bias towards normal movement. For a single particle the probability of diffusing distance δ is defined as,

$$P(\delta|\tau) = \frac{1}{4\pi D\tau} \exp\big(-\frac{\delta^2}{4D\tau}\big), \tag{2}$$

for time τ and self diffusion coefficient D. This can extended to set N particle such that,

$$P(\{\delta\}|\tau) = \big(\frac{1}{4\pi D\tau}\big)^N \exp(-\sum_{i=1}^{N} \frac{\delta_i^2}{4D\tau}). \tag{3}$$

Thus the successor is the location that has the maximum probability.

$$\max(P(\{\delta\}|\tau)). \tag{4}$$

Considering all locations found in the successive frame is a $\mathcal{O}(n!)$ problem. To tackle the computational complexity only particles within a specified range of movement is considered. This is an estimate of the maximum distance a particle can travel in a frame. [11]

One major flaw in this model is that it does not consider interactions between particles. Sperm movement can affect the dynamics of the fluid around it, this will affect the movement of other sperm particles in close proximity. The particles can also collide, stick together and fall apart all these dynamics are also not considered. While capturing such motions would be ideal, this would also increase the computational complexity even further of a model that already scales poorly. Thus the simpler nature of of this model is favourable for our data set that can consist a large number of particles per frame.

Before the particles can be tracked the maximum distance moved per frame will need to be determined. Initially a method was attempted which considered a faction of the average distance between particles, this did not show promising results. The only method that proved to be effective was to simply overlap frames and visually recording the average movement. Bodies within the system can have very different behaviours thus a compromise is required such that most behaviours can be captured. These values can also vary greatly for each dataset.

The initial tracks were not very promising, with tracks on average being 3 frames of length. This was thought to be due to an insignificant max distance. As supervised observation did not seem to work and full parameter sweep was attempted. This varied values form a max distance of 0.1 to mean distance between particles. After this sweep was done over multiple sets of data it was determined that the max distance was not the source of insufficient results. It was concluded that poor prepossessing was the cause.

After sufficient altercations were applied to the pre-processing method significantly higher particle tracks were found. Averaging from 35-55 frames tracks per particle depending on the dataset, ideally a much higher track length would have been preferable but the lengths of this degree are sufficient for analysis. Very small tracks of length 1-15 were removed as they were deemed too short for analysis. Very low tracks of length 1-5 can most likely be attributed to noise.

Due to the large degree of prepossessing applied to the dataset the number of eligible bodies to track had been significantly reduced. This makes temporal analysis of the system less reliable as in certain time steps the number of data points can minimal or non existent. To convert the data tracks into a form suitable for temporal analysis, the data was split into intervals of 50 frames, the average velocity of each particle within said interval is taken. The average of these values were then calculated to determine the general velocity at each time step. This was done with both sperm heads and bead particles. Figure 5 shows the average velocity of the beads particle in a dataset. It clearly shows how variant the system is and that the fluids do not show any consistent direction or overall trend. To determine the dynamics of the sperms head which are no caused by movements of the fluid. The sperm velocity relative to the average velocity of the beads at each time interval can be considered. If no external influences exist then the sperm data should now show signs of Brownian motion. If Brownian motion is not present then it can be determined that other factors are influences the movement of the sperm. This can include chemo-taxis, thermo-taxis or an any unknown factor.

2.5 Brownian Motion

After tracking the paths of the sperm and beads, we then consider the overall motion of the sperm. Initially, we looked at the average change in position of the sperm in each frame. There was a clear change in overall position and that the sperm were moving in a certain direction. However, it did not indicate whether the motion was normal.

To assess whether the motion is Brownian, two different methods were utilised. The first was a normal distributed QQ-plot. Finding the variance and mean of the sperm and beads positions allowed a normal distribution of the sperm to be plotted against their x or y position. This outputted a visual representation of where each sperm would be if it was normal distributed as a line. Five sections were found for each normal distributed line. The difference between the quantiles and there respective x or y position was used to form a second line with the area shaded in to show the difference in that position respective to their normal distribution. The Shapiro-Wilks test was used when it was difficult to see if it was Brownian and the data set was small.

3 Results

3.1 Concentration Along the Reproductive System

By using the same function that was used for reading the sperm videos, it is also possible to find the concentration of sperm particles in each area of the reproductive system. This is achieved by reading through time intervals of 30 frames for each video and counting the number of particles present in the image for each frame. Taking an average of this then gives the average amount of sperm for the area of the reproductive system that the video is recording. Using the available videos for the mares with horse semen in their reproductive tracts, and using an appropriate brightness threshold for the visibility of the sperm cells, running the concentration code provided this data showing the average sperm concentration in the different areas of the reproductive system for each mare:

By taking the total average for each area of the reproductive system, it is evident that the highest concentration of sperm is within the uterus, with the lowest concentration being found inside the right uterine horn.

Average sperm concentration (1dp)										
Area of RS	Mare 1	Mare 2	Mare 3	Mare 4	Mare 5	Mare 6	Mare 7	Mare 8	Mare 9	Avg
Cervix	415.0	2059.0	84.7	949.2	748.9	373.5	744.7	1621.1	N/A	874.5
Lower uterine horn (right)	331.8	1911.6	218.4	1936.8	536.6	282.9	1071.9	1173.9	466.2	881.1
Lower uterine horn (left)	113.4	1695.0	185.1	1287.8	1366.0	179.4	518.5	769.0	79.3	688.2
Upper uterine horn (right)	105.0	1916.5	13.0	1360.2	744.3	138.0	282.7	1562.5	1149.3	807.9
Upper uterine horn (left)	727.7	2003.6	67.5	1287.2	969.6	122.7	1504.1	1583.3	119.3	931.7
Middle of uterine horn (right)	226.0	1448.7	100.6	166.4	426.2	465.4	632.1	1022.2	627.3	568.3
Middle of uterine horn (left)	N/A	1903.7	103.9	1682.2	113.1	89.3	188.0	814.7	56.8	619.0
Uterus	1638.6	1549.5	194.2	1762.7	693.0	355.2	455.1	1088.8	1296.6	1003.7
Vagina	995.8	1781.7	223.7	1372.4	802.5	806.0	706.2	1671.1	63.0	935.8

Table 1: Table of average sperm concentrations in the reproductive systems of nine different mares

3.2 Sperm Tracks

Overlaying the tracked sperm centroids over a still frame can give a gauge of the quality of the particle tracks. Figure 2 shows an example of sperms tracks of a data set for a upper uterine horn. Only the largest particles were targeted for tracking which is evident by the large jumps between tracks. While a relatively low amount of sperm were tracked an average track length of 35 was found. Figure 3 shows an track example of another Upper Uterine Horn sample. Significantly more particles were successfully tracked in this sample, but the tracks are shown to be more chaotic will a less clear directional path.

The same method was applied to data sets all across the reproductive system for multiple samples, producing similar results.

3.3 Velocity of Sperm Tracks

Figures 6 and 5 shows the velocity of sperm and bead particles for the left lower uterine horn . It clear that particles tends to accelerate in a certain direction and then abruptly change. This is can attributed to contractions in the system which cause sudden changes in the fluid dynamics.

Figure 8 shows the average velocity of a sperm head when the velocity of the beads are removed. If no other factors affect the movement of the sperm then the movement should be found to be Brownian. This strongly relies on the reliability of the bead tracks, and as stated previously constancy is an issue with some time steps having minimal tracks. Ideally Strong behaviours in the velocity of the beads should also be visible in the sperm dynamics. A comparison of bead and sperm velocities can bee seen in Figure 7, this clearly shows that the beads and the sperm do not show this mirrored behaviour. This could either be due to insufficient data or simply tracking the average velocity per period of time may not be sufficient. Which implies that there may be competing fluid flows within the space of the frame. This spacial aspect has not be investigated in this study.

3.4 Determining Brownian Motion

Multiple different data sets considering various areas along the reproductive tract were assessed using Shapiro-Wilks and the Normalised QQ-plots to determine which of them are normal.

Type Of Data	Amount	Non-Brownian	Brownian
Sperm	14	14	0
Beads	14	12	2
Velocity	12	5	7

Table 2: Positions and Velocities of data found to be Brownian

The Sperm and Beads category refers to the x and y positions of the particles. It's expected that both the sperm and the beads to be not be normally distributed due to sperm movement towards fertilisation and as the beads move due to the flow caused by the sperm movement. The Velocity category is velocity of sperm beads relative to the average bead velocity as the time varies. This was expected to be to be normal. This was not the case. This may be due to the incomplete data sets. Two sets of data are missing due to limited amount of data in each set

making too little to determine Brownian motion.

Figures 9 show an example of what the normalised QQ-plots would be. The straight line running through the middle shows the normalised values of x and y positions. The section in red is the difference between what the position of the particles would be if they were normal distributed.

3.5 Analysis of Results

The advantages of our sperm track data are that they successfully managed to visualise the movement of sperm particles within at varying time intervals for each area of the mares' reproductive systems, and successfully tracked both larger sets and smaller sets of sperm in a graphical manner. Additionally, we have successfully managed to convey through these tracks that the sperm's movement is random in direction, and that most sperm particles do not make it beyond the uterus – this is shown by the concentration data which clearly visualises the highest concentrations of sperm particles to be within the uterus, with further areas of the reproductive tract having significantly lower concentrations of sperm. Furthermore, our analysis of the sperm's movement has shown evidence that sperm does not follow Brownian motion.

However, the results from our methodology may not be entirely reliable as we only used short sperm particle tracks from our data meaning that our results may not be fully representative of the full movement that each particle takes within the reproductive system and therefore unsuitable for a full analysis. Additionally, the different brightness thresholds that we have used in our modelling may not be entirely accurate in distinguishing each individual sperm particle from either noise within the reproductive tract or other nearby sperm, so an analysis of the movement for each individual sperm proved to be quite difficult to compute.

4 Discussion

4.1 Merits and Limitations to the Model

The proposed model gives a cheaper alternative to typical tracking methods of sperm. An example is a Computer Assisted Semen Analysis (CASA). Usually, CASA costs can be in ten of thousand of pounds. Our method allows an individual/organisation without large funding to tell if the sperm is functioning properly. Giving them a platform to conduct various forms of analysis for example, a Fourier analysis can be utilised to determine the most dominant taxis affecting the sperm. Overall, being much cheaper then the alternatives.

Our model focuses on tracking the overall dynamics of sperm movement. While good at giving a general guide of what is occurring in the entirely of the reproductive system, it does not consider the interactions between sperm. As a result, it does not consider the forces acting between sperm and how it affects fluid motion. Giving a limited spatial analysis of the movement of the sperm. This can be seen in figure 2. Generally, the sperm are moving in a certain direction, but individual sperms acting differently.

When tracking the data we focused on the larger and brighter bodies (2.3). Getting longer tracks allows for a better analysis and cuts out a lot of the background noise. However, cutting out a lot of the dimmer particles results in large loss of information. In addition, the tracking method and data only consider two dimensions while sperm move in a three-dimensional motion in the fluid. Resulting in them moving closer and further away. The tracking method would break these movements into separate tracks separate as they cross the threshold boundary, causing much larger tracks to be cut into multiple short ones. As can be in seen figure 4. The majority of the tracks that were detected had a length less than 20, when in reality the majority of tracks should exceed 50-100. For a better analysis of the sperm movement and its causes the track lengths need to be much higher to allow a methods such as a Fourier transform to find the dominant frequencies. The limitations of focusing on larger tracks means smaller tracks were cut out and only parts of much longer tracks it could detect were registered. This ultimately cuts out large amount of data.

The data was recorded using video microscopy, a two-dimensional recording method of equine sperm in the reproductive system, a three-dimensional system. As mentioned earlier, recording the data in two dimensions removes

the depth of the reproductive system. Many of the sperm with shorter tracks are sperm that are moving faster due to being closer, while sperm further away are constantly crossing into and out of the threshold. Furthermore, distant sperm that are not bright enough are considered just part of the surrounding noise and are not tracked. In addition to this the frame rate of recording is low. This results in many gaps in the data cutting apart different tracks and not enough sperm in many time steps causing many sperm tracks to disappear.

Tracking each sperm particle require to follow a single green body from the data frame as can be seen in figure 1. However, due to the dimensionality, multiple sperm can overlay in a single spot in a frame. This method cannot distinguish between them and considers them a single track until they diverge. This results in some tracks being cut and a decrease in overall track size length.

Another problem is the heterogeneous nature of the data. Resulting in our method being heavily supervised. This is due to how data was recorded. Both for tracking of the data and calculating the concentration movement a threshold was needed per data set (2.3). This meant a larger amount of time and resources was needed to fine tune the parameters for every data set to make sure it is correctly detecting the tracks. This is a highly subjective method making it possible for different individuals to have alternate results for the same data set. Ultimately, this decreases the reliability of this method.

4.2 Further Developments

A further step to improve our results is to find a way to record the tracks of the sperm particles in three dimensions. This would require finding a new set of data to analyse with the program looking at the particles from different angles within each area of the reproductive tract within the same time frame, therefore the z velocities and directions can be recorded as well as the x and y components. Another way to achieve this would be adjusting the sperm tracking program to be able to recognise the change in depth of the sperm particles. However, this would be difficult to achieve as it would require precise use of brightness thresholds as well as there being no real way telling how far a sperm particle has travelled by just observing the change in depth.

As previously stated the model considers average sperm velocity per interval of time. This does not consider each particles individual location within each interval. This has been attributed as the most probable cause for the inconsistencies found in the comparison between sperm and bead particles. The recorded area, if sufficiently large can have varying fluid behaviours dependant on different regions within the frame. Thus a more segregated analysis where the frame is split into small regions should produce more favourable results. A larger density of tracks per frame will be needed to implement this which would require a revised pre-processing method that can capture more behaviours. This can even be extended further to a more detailed spacial analysis that could also account for particle interactions. Sperm cells cause local fluid fluctuations in the areas closest to them, this affects the dynamics of both other sperm cells and bead particles nearest to them. Resulting in the bead behaviours closest to sperm cells being less representative of general fluid behaviours. Thus, a model that considered weighted bead and sperm velocities dependant to their proximity to other particles would give a better representation of the fluid and sperm dynamics.

Behaviours of large and small particles vary largely and require significantly different pre-processing. A model that separately considers both small and large particles then later combines the results would be ideal. This would significantly increase the time commitment of the supervised prepossessing, and considering that many data sets need to be processed, renders current method being unpractical. Therefore the pre-processing method would need to be revised to a method that requires less supervised attention. While this may reduce the quality of data being produced, the increased quantity of data points can be considered as a favourable trade off.

Another development would be to apply Principal Component Analysis (PCA) to our findings. This would require finding and choosing parameters that affect the sperm particles' trajectory, such as the straight-line and curvilinear velocities of the particles, and then performing PCA on said parameters to find any patterns or correlation between them. Additionally, another logical step to take with our findings would be to develop our analysis of sperm particle movement through the reproductive system by modelling the chemotaxis and thermotaxis of the particles. This has been put into practice for testing sperm motility by Alquézar-Baeta et al. [13] as well as by Pérez-Cerezales et al. [14] and would provide a more ironclad conclusion on whether the motion of the sperm

particles can be found to be Brownian or not.

5 Conclusion

In conclusion, with male fertility rates declining at a rapid rate, understanding the dynamics of sperm movement within the human body becomes increasingly more desirable. Each sperm particle undergoes a complex and perilous journey along the reproductive tract with a multitude of factors affecting their movement. Utilising video microscopy, sperm and bead particles were tracked at various points along the reproductive system. Temporal analysis of average sperm movement produced inconsistent results, implying that that a more detailed spacial analysis of individual sperm is required to yield more reliable data. Despite this, it is clear that the dynamics of sperm particles are significantly more complex than particles simply undergoing Brownian motion within a dynamic fluid, implying that modelling chemical and thermal influence is crucial to understanding sperm dynamics.

COVID-19 Lock-down Mitigation

The lock-down and early closure of university facilities had a number of repercussions on this project. The primary effect was reduced access and availability of our supervisor/specialist on sperm dynamics. The COVID-19 lock-down occurring relatively early in the time frame of this project, resulting in increased difficulties in determining a foundation for the project. The inability to sit with our supervisor in person and easily discuss our methods and difficulties resulted in many early aspects taking significantly longer than they would have otherwise. While the project consisted of image processing, we had made multiple initial incorrect assumptions regarding our data set resulting in a large portion of time being wasted - these would have been easily picked up if we had more access to our supervisor in person. While we were able to have online meetings, availability was limited in the early months due to increased staff workload and our findings were harder to visualise in an online setting.

Another main issue was the lack of access to university resources, as due to the lock-down we were restricted to our personal laptops and devices. The data set we were working with was extremely memory intensive, as our scripts had to load multiple versions of the large sample videos. This tended to overload our systems, resulting in each data set taking multiple hours to process with a high chance of our systems crashing. This when fine-tuning a sensitive model often resulted in days where no progress was made. Under normal circumstances, we could have either received access to better systems from our supervisors or found better resources ourselves. This resulted in a significantly down scaled attempt to track the sperm behaviours, tracking only the largest particles and ignoring behaviours of smaller particles which was significantly less memory intensive. Furthermore, due to the COVID-19 outbreak we were unable to take part in an actual poster session as a group either, so we experienced more difficulty in the development of the poster as there was less immediate access to guidance or examples of what makes for a good poster session.

As previously stated image processing requires a lot of fine-tuning and is very memory intensive, meaning that it was impossible to run a screen share with other group members while fine tuning parameters. This made it increasingly difficult to work efficiently with other members in the group, where otherwise members could have sat together and resolved problems. This resulted in individuals tackling large and important aspects in the project essentially alone, making any progress more time consuming.

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Appendix

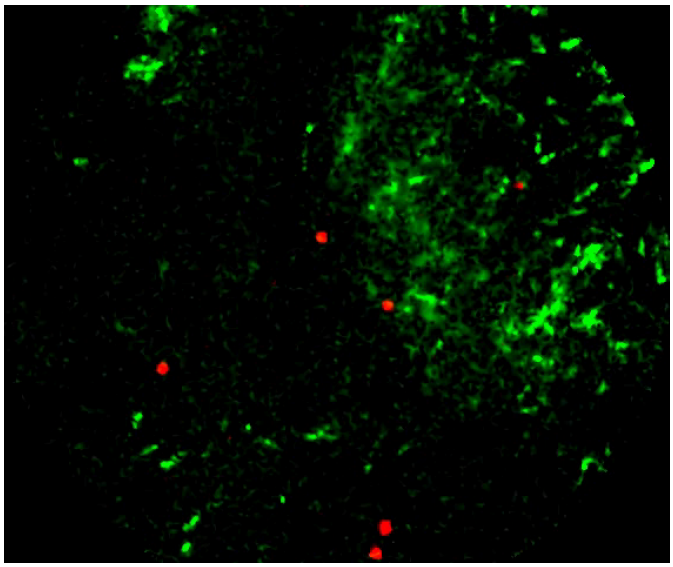

Figure 1: Example of a single frame for data recording sperm activity in a Uterus. The brightest green spots correlate to sperm heads, and the red spots correlated to control bead particles. The slight haze around each particle comes from noise caused by the green and red dye.

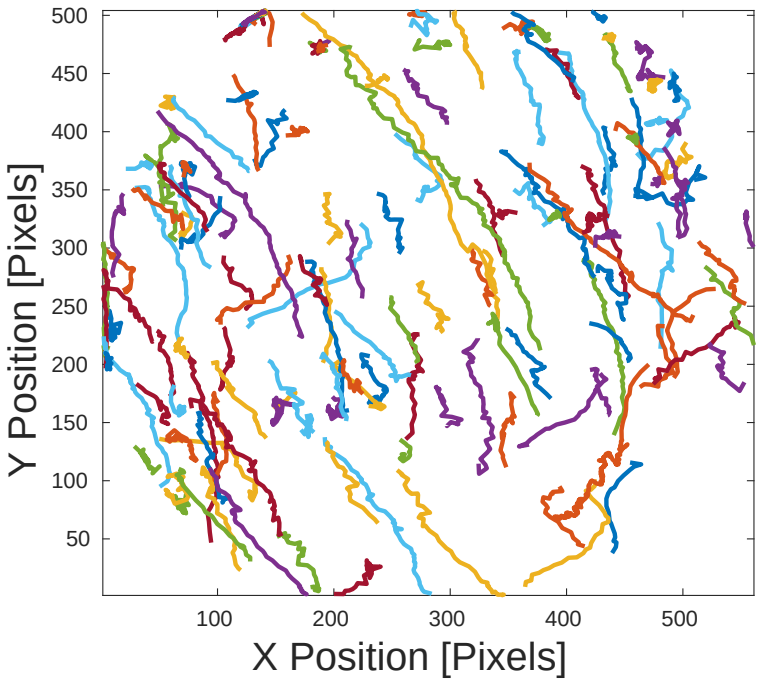

Figure 2: Sperm tracks of large bodies in an upper uterine horn. Where larger sperm bodies were targeted for tracking. This is also an example of how few particles were successfully tracked for some data sets.

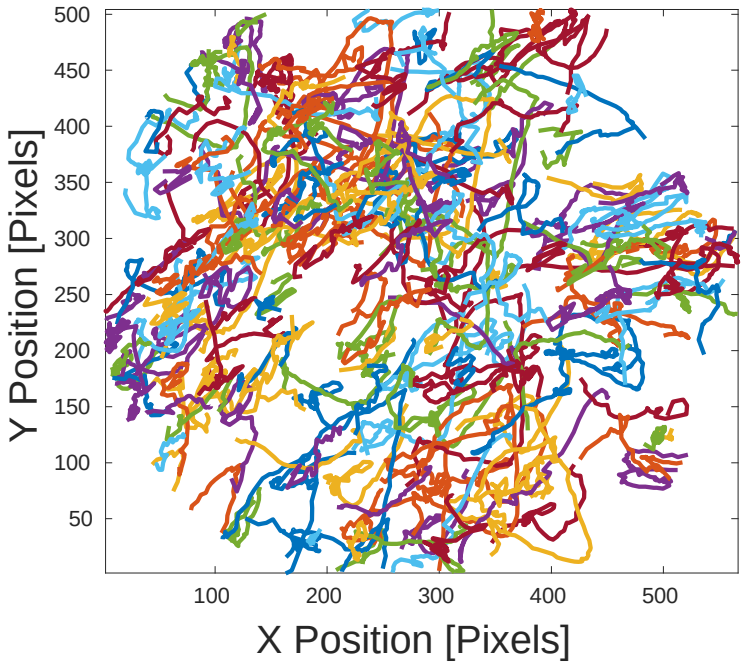

Figure 3: Sperm tracks of large bodies in another upper uterine horn. Where larger sperm bodies were targeted for tracking. This is also an example of how few particles were successfully tracked for some data sets.

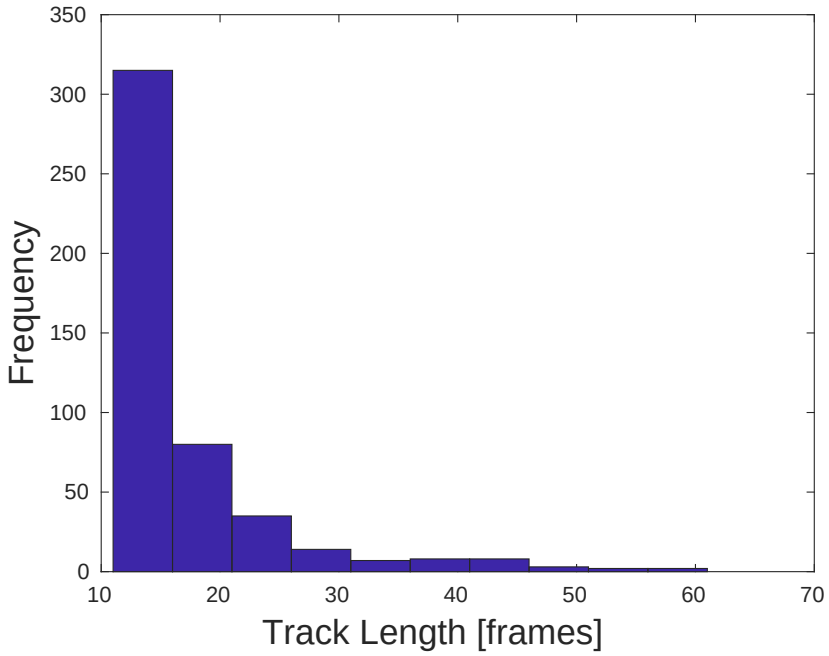

Figure 4: Distribution of the particle track length for data corresponding to the lower uterine horn. While the total frequency can vary greatly for each data set the general shape of the distribution is consistent for all data sets.

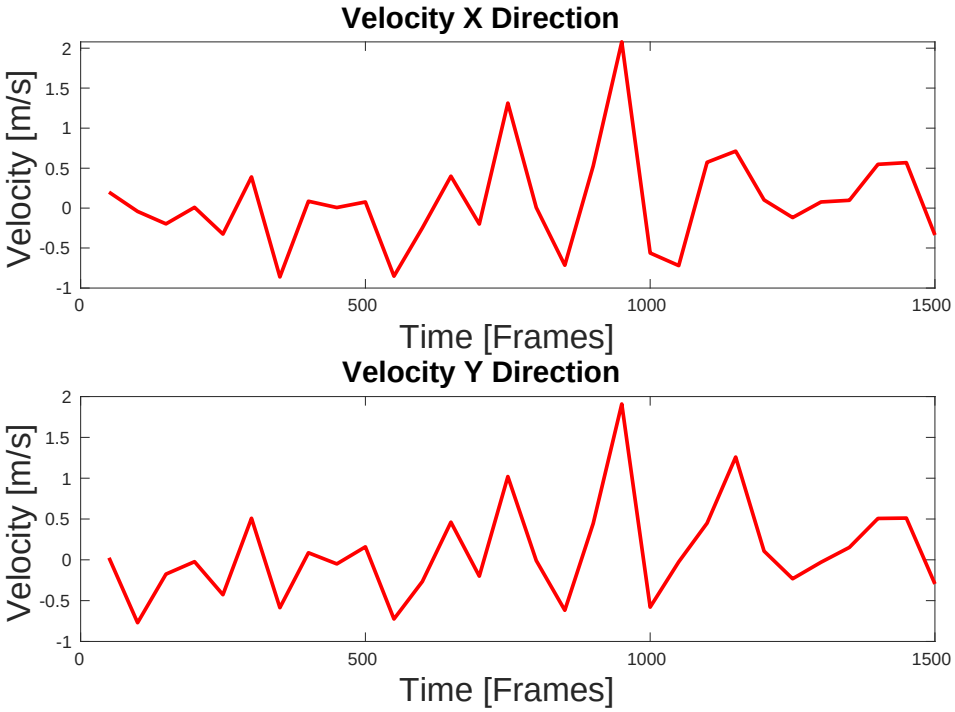

Figure 5: Bead velocity in X and Y directions for a lower uterine horn data set. Average velocity taken of all sperm velocities in time intervals of 50 frames.

Figure 6: Sperm velocity in X and Y directions for a lower uterine horn data set. Average velocity taken of all sperm velocities in time intervals of 50 frames.

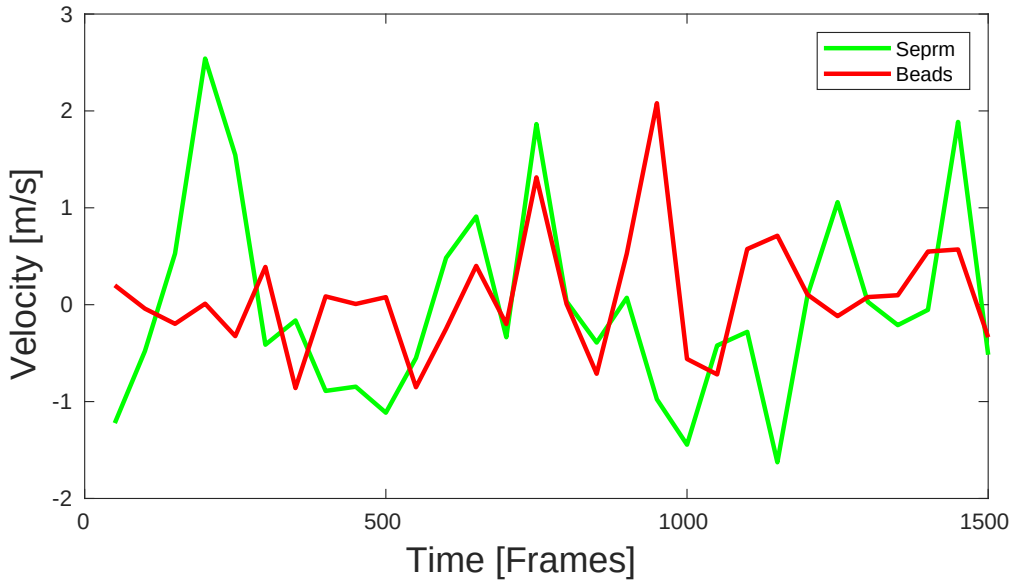

Figure 7: Sperm and bead velocity in X direction for a lower uterine horn dataset. Average velocity taken of all sperm velocities in time intervals of 50 frames.

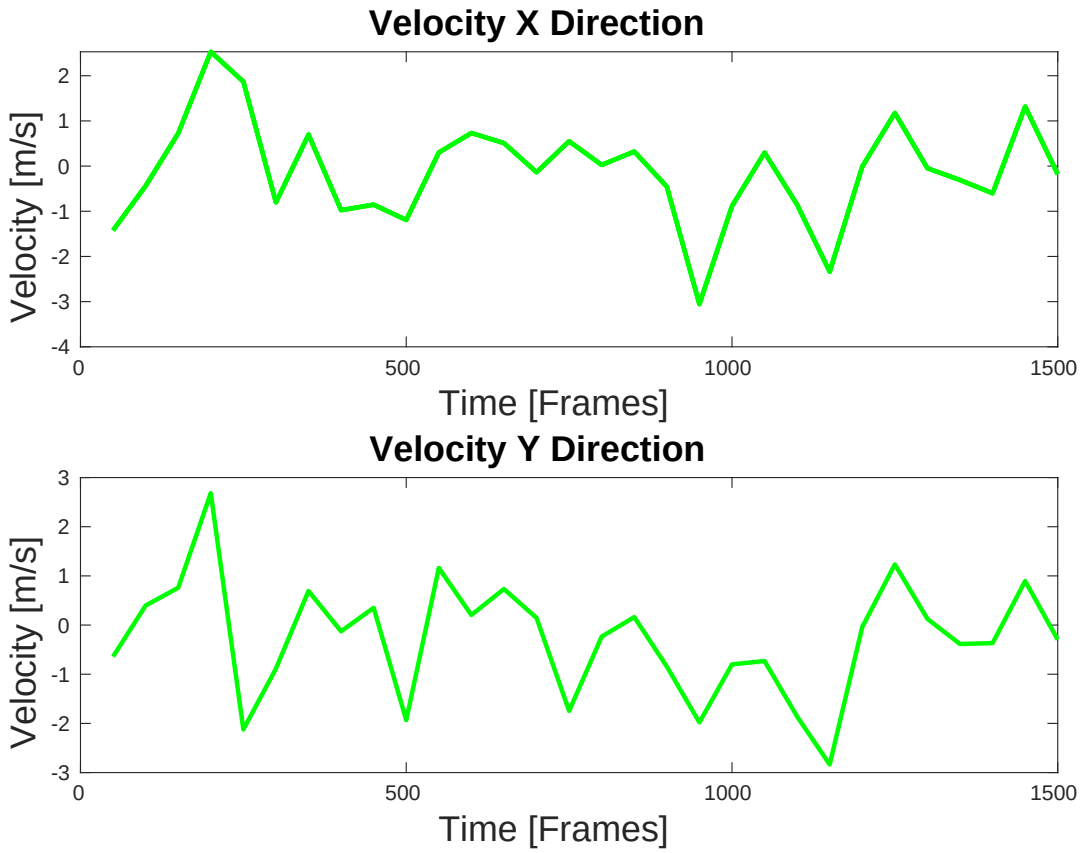

Figure 8: Sperm velocity with bead dynamics removed in X and Y directions for a lower uterine horn dataset. Average velocity taken of all sperm velocities in time intervals of 50 frames.

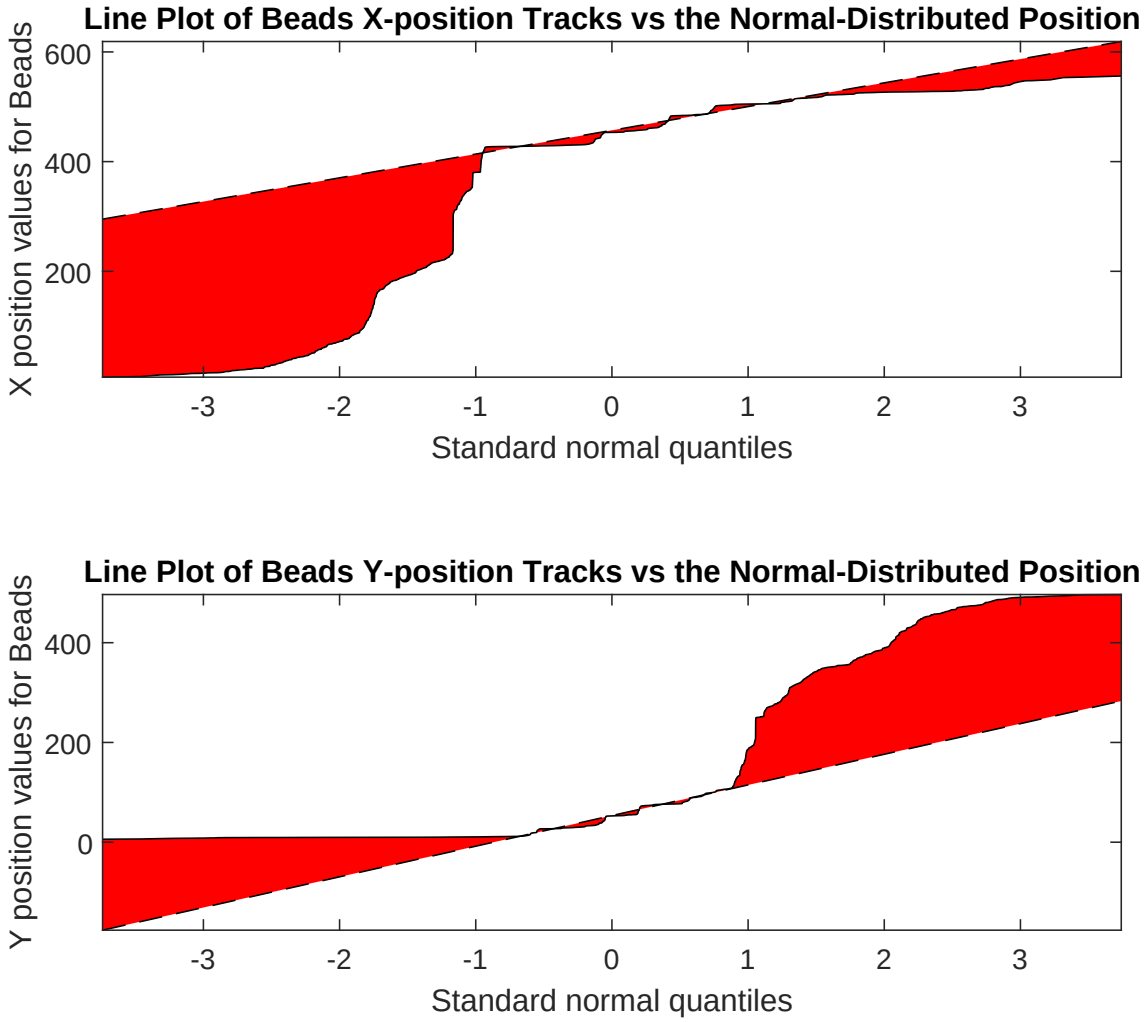

Figure 9: Line plot showing the normalised position of the beads in the lower left uterine horn. An example of how different the tracks are compared to where they be if they were normally distributed