# *Short-term Exposure to Radio Frequency Radiation Appears to Have Little Effect on Sperm Cell Function*

**By**

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**ABSTRACT**

Microwave spectrum radio frequency fields (RF) are about as ubiquitous as oxygen in today's society; whether from cellular phones, wifi routers, or cellular towers, we are constantly bombarded with this type of RF exposure. Recently a number of groups have suggested cellular phones are associated with several disease states; however, the potential ill effects of microwave spectrum RF on reproduction are not so well understood or studied. The present study aims to complement the minimal research already present in the field by examining a piece of spectrum not typically studied, that of the wifi router and its effects on reproductive cells ex vivo. Semen samples were acquired from five human donors. Each sample was divided into a control and exposed aliquot with the control receiving no RF from a wifi source and the exposed aliquot receiving continuous RF from a wifi source for 24 hours. Samples were evaluated at set time points of 1, 2, 3, 6, 9, 12, 15, 18, and 24 hours using the World Health Organization (WHO) standard for measuring standard semen parameters of motility, progressivity, straightness, and linearity. As expected, all semen parameters decreased over time ($p < 0.001$) regardless of treatment group. However, the data suggest no difference in any of the semen parameters due to RF exposure ($p = 0.392$). Unlike previous studies in other tissues, short-term exposure to RF radiation appears to have little effect on sperm cell function. Further studies are needed to assess long-term exposure on cell function.

**ACKNOWLEDGMENTS**

The undertaking of an undergraduate research project was a daunting challenge with an unexpectedly tedious journey. The resolution of my work, however, could not have been realized or polished to the degree that it is were it not for the truly dedicated and patient efforts of my lab and project partner Dr. Amy VanGheem; my thesis director Dr. Samuel Prien; my thesis reviewer Dr. Leslie Thompson; Dr. Lindsey Penrose with The Texas Tech University Health Science Center Obstetrics and Gynecology Department; Dr. Marjean Purinton and Donna Srader with the Honors College; Colleen Sonnentag, Scott Stover, two good friend without whose input and proof reading this piece would not flow as it does; and all the donors that made this project possible in the first place. Additionally, I feel a special sense of gratitude to the Texas Tech Health Science Center's Obstetrics and Gynecology lab and staff for letting me use their equipment and supplies and for always being available for questions, even those that were surely one too many.

This thesis is the culmination and pinnacle achievement of my college career and because of the graciousness of others I succeeded far beyond anything I ever expected or dreamed.

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## CHAPTER 1: INTRODUCTION

The field of reproduction and fertility is an obviously important field of study to the future of humanity and its development. Cellular phones and the microwave spectrum radio fields (RF) they use and emit have become as ubiquitous and indispensable as a standard #2 pencil, yet the effects of the RF they emit on gametes and reproduction are virtually unstudied. At best, the topic of study is emerging. By completing a scientifically and statistically significant research study with accompanying data, Dr. Samuel Prien and I will help to demystify a relevant and pertinent question to the current generations of habitual cell phone users and any potential side effects that adult men may face.

The fields created by the use of electrical and radio equipment became a topic of investigation in the scientific community after a study demonstrated a possible linkage between electromagnetic fields and childhood cancer (Wertheimer, Leeper). Multiple reports since then have concluded conflicting reviews regarding the effects of exposure to fields produced by electrical, mechanical, and radio equipment including a 1992 review by Chernoff that stated available data did not conclusively support the theory that electromagnetic fields induce adverse effects, notably regarding reproduction (Chernoff, et al.). Another study by Chung in 2003 demonstrated no connection to maternal toxicity or developmental toxicity in fetuses following exposure of female Sprague-Dawley rats to 60 Hz electromagnetic fields during gestation (Chung, et al.). Effects of similar fields on dams, F1 offspring, and F2 fetuses were examined in a study by Chung in 2004, which revealed no significant effects in exposed groups then either (Chung, et al.). Two further reviews conducted by Juutilainen in 2003 and 2005 have concluded that electromagnetic fields have no strong adverse effects on development,

although both studies did note some increase in skeletal alternations in several experiments (Juutilainen). Therefore research into the topic is mired in a confusion of conflicting results and conclusions. Additionally, research into radio fields specifically created by cellular phones and their frequencies is minimal and at best is developing.

As many as 15% of adult couples in the U.S. are infertile, with at least half being attributed to male infertility (American Urological Association 2) and so out of a million adult couples, 150,000 are unable to conceive, and for approximately 75,000 of the males involved, it is due to some sort of complication regarding their sperm. Out of those 75,000 men, around 10% are idiopathically infertile (American Urological Association 3) or infertile for unknown reasons. When the scale is taken into account it becomes a huge problem where one in 100 men is infertile for unknown reasons. One potential explanation that has been getting a large amount of attention lately has been cellular phones and the radio fields they emit.

Cell phones use frequencies that are considered to be in the microwave frequency range (between 300MHz and 300,000MHz). Typical cell phones in the U.S. operate in frequencies between 700MHz and 2,200MHz, similar in range to what household microwave ovens use to cook food (~2,450MHz). Microwave ovens heat through a process called dielectric heating, essentially heating molecules by increasing the speed and frequency with which they "knock" into the surrounding molecules, thus increasing kinetic energy, the energy of motion. As kinetic energy increases, so does temperature and, thus, dielectric heating.

Using this premise, it is therefore reasonable to conclude that dielectric heating could very well arise from the frequencies used by cellular phones. In order to test this hypothesis that dielectric heating is a potential culprit for idiopathic infertility, we used a typical 2,400MHz

wifi router to emit frequencies in the range of cellular phones and microwave ovens. We placed the wifi router inside a standard laboratory incubator with the router plugged in and operating, but not connected to a Local Area Network (LAN) line. The LAN was left disconnected because of the technical limitations of getting an internet connection line into the incubator. The router was used instead of a cellular phone because of the limitations of a cellular phone to emit constant waves and because of the problem of battery life and having to change out a battery. Also, it was believed the router would be able to withstand the 95% humidity requirements of the incubator for the 24 hours of exposure time.

Based on examination of the correlation between microwave ovens, cellular phones, and dielectric heating, it stands to reason that cellular phones and the radio fields (RF) they emit are a plausible explanation for idiopathic infertility. I propose that one of the mechanisms that cause the infertility is decreased sperm cell motility caused by the exposure to RF. Previous studies have indicated that cellular phones do in fact negatively affect human sperm cell motility (Agarwal, et al.; Erogul et al.; Fejes, et al.). Studies have also demonstrated that rat sperm cell motility is negatively affected from exposure to cell phone RF (Mailankot, et al.; Yan, et al.). This study will serve to supplement, recreate, and, if possible, expose the pathway by which the negative effect on motility is caused.

## CHAPTER 2: METHODOLOGY

After obtaining Institutional Review Board (IRB) approval from the Texas Tech University Health Science Center (see appendix) and obtaining signed informed consent forms from each of our five paid volunteer donors ranging in age from 20 to 24, a one-time semen sample was obtained from each donor. The donors were not screened or chosen in any manner other than by volunteer basis.

The samples were collected by masturbation into a standard collection cup and immediately incubated under standard conditions for human embryo incubation, 37°C and 95% humidity. Each sample was then washed with sterile incubator temperature Hams media using a standard Assisted Reproductive Technology (A.R.T.) lab protocol within two hours of collection. This standard required each donor's sample to be centrifuged with Hams media in order to obtain a sperm cell pellet. Each sample was centrifuged twice with fresh Hams media to try to eliminate everything but the actual sperm cells in the sample. After each centrifugation, the cells were vortexed with the fresh Hams media to resuspend the cells in media. After two full wash and vortex cycles, each sample was then split into two equal portions with volumes dependent upon the donor's initial donation volume.

One of the portions from each donor was labeled "Control #" and the other "Exposed #" with the "#" corresponding to the donor number (1-5). Each sample was then placed in an incubator, with the control samples going into incubator A with no RF source other than background RF and the exposed samples going into incubator B which contained the wifi router as the RF source. Both incubators were set and maintained to a constant 37°C temperature and 95% humidity (standard incubation settings for human semen). The samples were placed in

standard test tubes and capped in standard test tube racks in both incubators. In addition to the sperm cells themselves, each sample set of control and exposed spermatocytes were suspended in Hams media along with penicillin at 0.001 g/100ml of media to prevent bacteria growth.

The control and exposed groups were examined and data from the four parameters of interest, motility, progressive velocity, straightness, and linearity were recorded over a 24-hour period at 1, 2, 3, 6, 9, 12, 15, 18, and 24 hours. The times were chosen in order to allow us to view data most efficiently when graphed and without measurements that would show little change between each other. It was expected from examining the relatively short life cycle of the sperm cell that changes in semen parameters would be rapid at first and then slower over time, especially when taking into account the limited energy supply that sperm cells possess.

Analysis at each time point required all of the control aliquots to be sampled once and then evaluated using an approximately 10-µL droplet of solution in the Integrated Visual Optical System (IVOS), an instrument used in standard A.R.T. labs to count, examine, and provide data on human sperm cells. Using this instrument, 200 sperm cells were counted as prescribed in the WHO standards. The IVOS automatically tabulated how many cells were motile out of the 200 and gave a percentage. It is possible to use the IVOS to examine morphology as well; however, for the scope of this project, morphology data were not collected. Rather the focus was the motility, progressivity, straightness, and linearity of each sample set's control and exposed groups at every time point.

After the variables were observed and calculated for each control sample, the exposed samples were observed and data were obtained. Data sheets for all samples along with the

pertinent identifying information were collected so that data could be compiled and analyzed at a later date. Conducting data analysis at a later date allowed researchers to focus on adhering to strict sterile protocol and technique.

The router used in this study was a Belkin T5D7230-4 single antenna b/g residential wifi router that operates in the 2,400MHz spectrum. An active internet connection was not supplied to the wifi router due to technical limitations. The router's RF field was measured using an RF meter each time the exposed sample set was removed from the incubator for a reading. The actual numerical values of the RF field strength varied greatly by the second and were never consistent; therefore, the RF meter was used only to verify that there was, in fact, a measureable RF field being emitted.

Following the completion of the time set and data collection for the samples, all contaminated disposables and human waste were disposed of properly, according to state mandates. All non-disposable equipment was washed and decontaminated following normal A.R.T. lab procedures.

Each donor was a confirmed cellular phone owner and due to the difficulty of locating donors who do not own cellular phones, it was impossible to take into account the potential effects of exposure to RF prior to ejaculation. Therefore the focus in this study was to examine RF exposure post ejaculation. This determination led to the conclusion that if little effect was seen in sperm cells post ejaculation, then the probability that pre-ejaculation exposure is harmful is small.

## CHAPTER 3: RESULTS

Figure 3.1 represents the motility for the control and exposed groups for the first sample JM-01 over a 24 hour period. JM-01 presented a surprisingly highly motile and progressive sample. After compiling the data into graphical form, it was observed that the exposed group performed slightly better overall, as opposed to the control group as would have supported the original hypothesis. Over every single data point there were slight, but consistently better motility measurements by the exposed group.

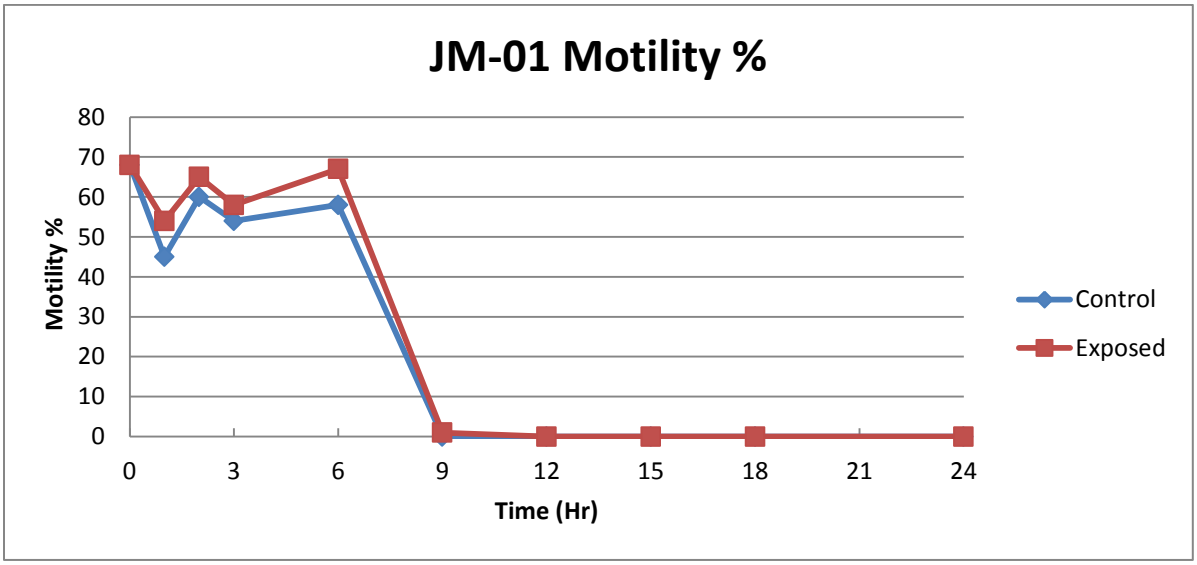


**Figure 3.1: Percentage of motile sperm cells while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-01.**

The motility represented the portion of the sample that the IVOS registered as in motion. Somewhat better measurements for the purposes of this study were the percentages that were actually progressive, or forward moving. The progressive percentage measurement was a much stronger indication of fertility as it denoted sperm that were moving in a non-circular motion. Figure 3.2 represents the percentage of progressive sperm cells within the control and exposed groups of JM-01. In figure 3.2, with the exception of two data points the

exposed group measures a better progressive percentage. When examining the two data points on the exposed trend line in particular, it seems like the measurements were somehow incorrectly taken because the data experienced an extreme and unnatural fluctuation. It is possible that the tubes were not adequately mixed before removing the 10-µL aliquot. In the event of this issue, the measurement would have been inaccurate because the sperm cells would have settled to the bottom and not have been evenly distributed in solution.

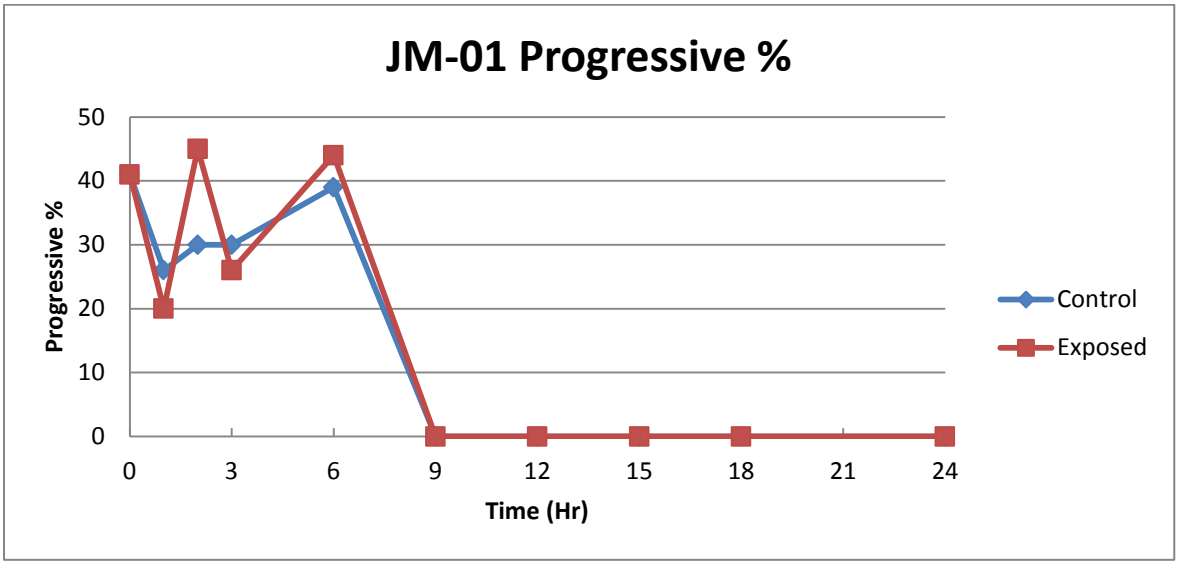


**Figure 3.2: Percentage of progressive sperm cells while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-01.**

Straightness and linearity were linked in that both were calculated using linear velocity as part of the equation. Straightness was calculated by dividing linear velocity by average path velocity (Welch 12). Linear velocity was, as the name would imply, the velocity of straight forward motion exhibited by the sperm cells, whereas average path velocity was a measurement of the velocity of the average path a sperm cell travels. Linearity was linear velocity divided by curvilinear velocity. Curvilinear velocity was a measurement of the sum of the straight lines in regard to the position of the head of the sperm cell (Welch 12). Straightness

and linearity were significant seminal factors because they directly demonstrated a sperm cell's ability to exhibit progressive motility, the type of motility that is pivotal to getting the sperm cell to the egg cell. Figure 3.3 represents the percentage of sperm cells in the control and exposed groups of JM-01 that demonstrate straightness. The exposed group's measurements were all slightly more superior to the control group's measurements except for hour two where the exposed group takes a slight momentary dip. One other data point that is noteworthy is hour nine for the exposed group which demonstrates a 60% increase over the respective control data point. Since it was only one data point, however, its statistical significance is low.

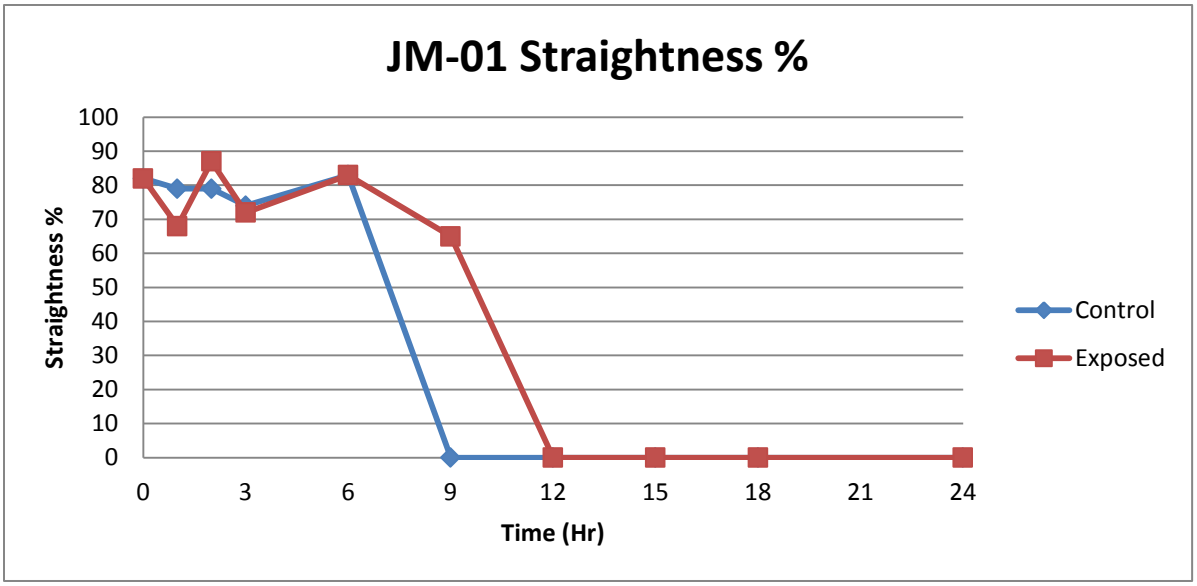


**Figure 3.3: Percentage of sperm cells that exhibited straightness while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-01.**

As figure 3.4 helps to demonstrate when compared to figure 3.3, the graphs for straightness and linearity are different in actual values, but demonstrate nearly identical graphing patterns as would be expected. Figure 3.4 shows the exposed group having superior measurements in all but two data points, hour two and hour six. However, the exposed data point on hour six is only ever so slightly below its control counterpart. The exposed point on

hour two has a deficit of about 8% less sperm exhibiting linearity. The exposed group performs significantly better at hour nine, as seen in figure 3.3.

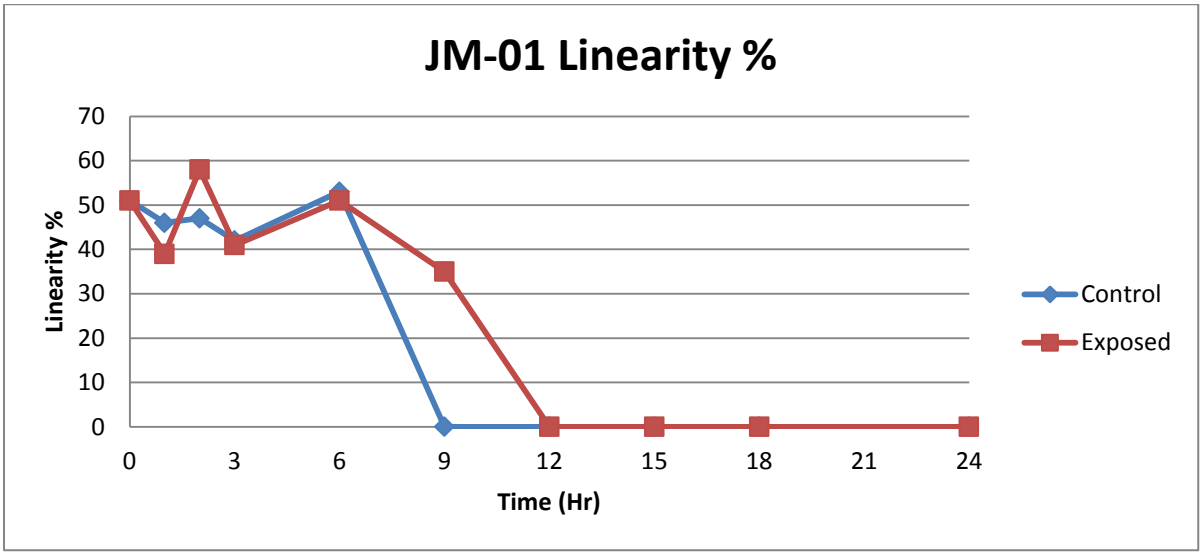


**Figure 3.4: Percentage of sperm cells that exhibited linearity while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-01.**

Figure 3.5 represents the results for JM-02's motility percentage for both the control and exposed groups. Results were a little different when compared to figure 3.1 with JM-01's motility percentage in that the control group has multiple data points where it performed better than its exposed counterpart. Thus far, hour nine in figure 3.5 demonstrates the most superior measurement when compared to the exposed group with a nearly 10% difference, in favor of the control group. Around hour 12 the data points become similar and remain so from then on.

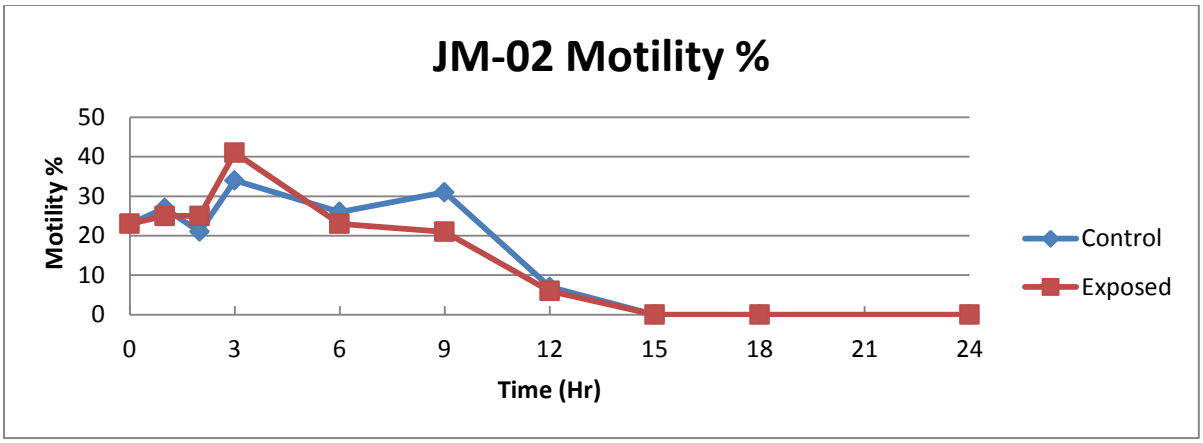


**Figure 3.5: Percentage of motile sperm cells while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-02**

Figure 3.6 represents the progressive percentage for JM-02 and it shows the control group performing slightly better, with fair consistency, than the exposed group. This trend was what would be expected were the hypothesis supported; however, the margin with which the control group performed better is notably small. Overall, the trend lines of both control and exposed groups in figure 3.6 are similar to data shown in the preceding figures. This result supports the idea that the difference between exposed and control groups is small.

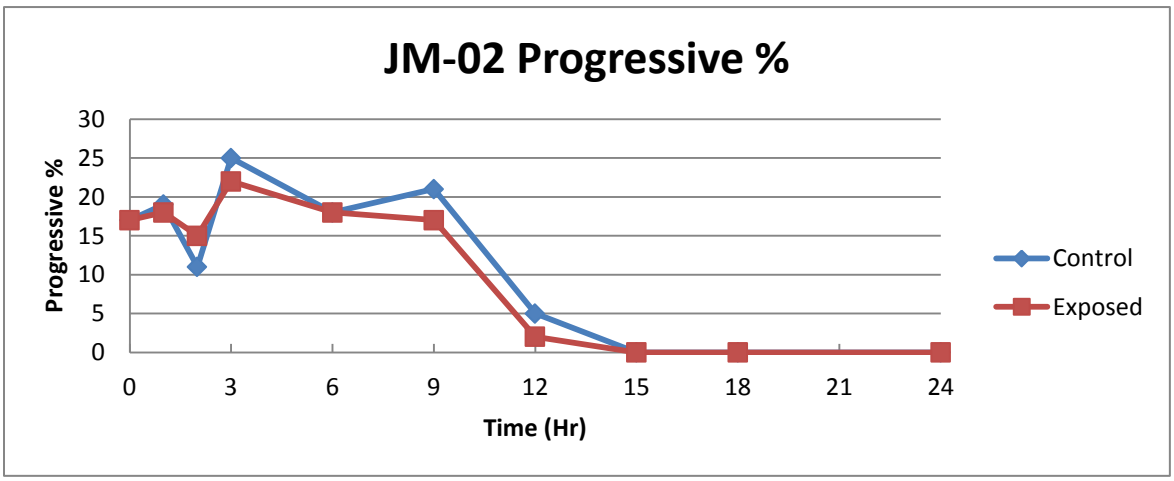


**Figure 3.6: Percentage of progressive sperm cells while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-02.**

Figure 3.7 represents the data points for the percentage of sperm cells that exhibited straightness for JM-02. Overall the control and exposed groups demonstrate similar trend lines in figure 3.7. The control group demonstrates a significant but momentary advantage over the exposed group over hour 12.

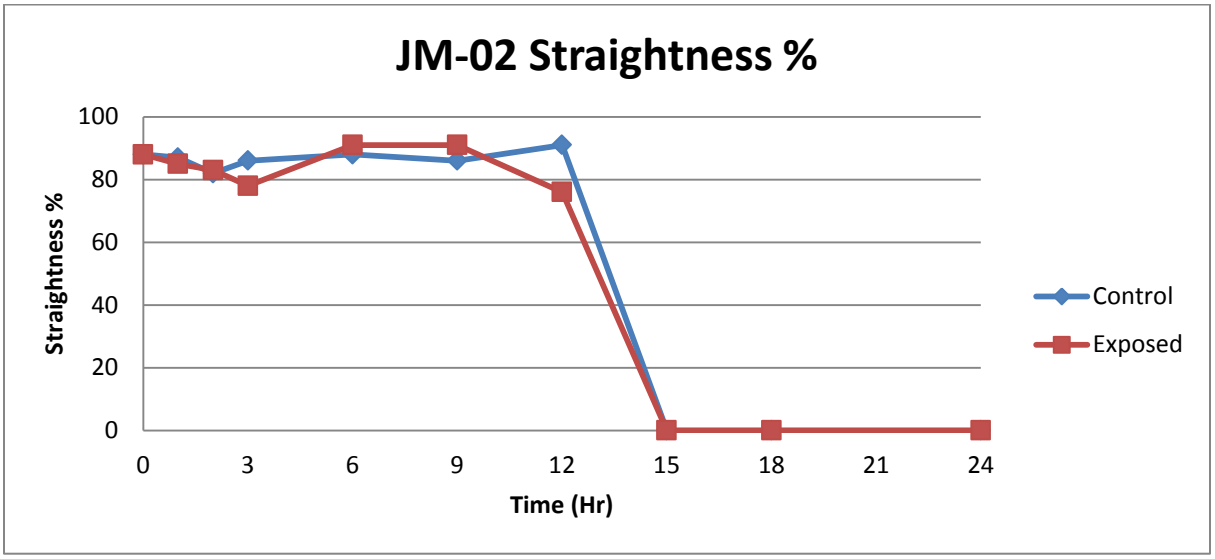


**Figure 3.7: Percentage of sperm cells that exhibited straightness while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-02.**

Figure 3.8 shows the data points for the percentage of sperm cells that exhibited linearity for JM-02 in both the control and exposed groups. The differences between the control and exposed groups at hours three and nine are markedly different, with hour three having demonstrated a superior measurement for the control group and hour nine having demonstrated a superior measurement for the exposed group. Once again as with figure 3.3 and 3.4, 3.7 and 3.8 ultimately share different data points, but the graphs follow a similar graphical path.

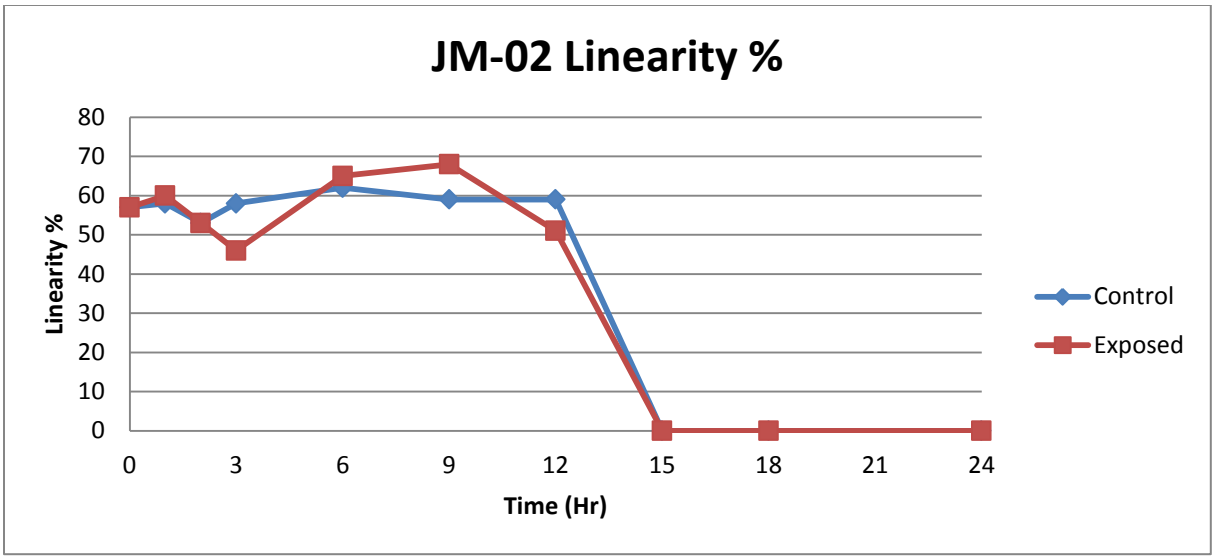


**Figure 3.8: Percentage of sperm cells that exhibited linearity while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-02.**

Figure 3.9 represents the percentage of motile cells for the exposed and control groups for sample JM-03. The exposed group in figure 3.9 did slightly better from hours one through three; however, it took a steep dive after hour three. Hour six demonstrates a nearly 10% difference between the exposed and control trend lines, favoring the control group. There was a momentary but significant spike in exposed data for hour 12; however, it did not follow the natural trend line and for this reason it seems that the data probably was an outlier, or so far away from average that it is considered an accidental occurrence and not statistically significant.

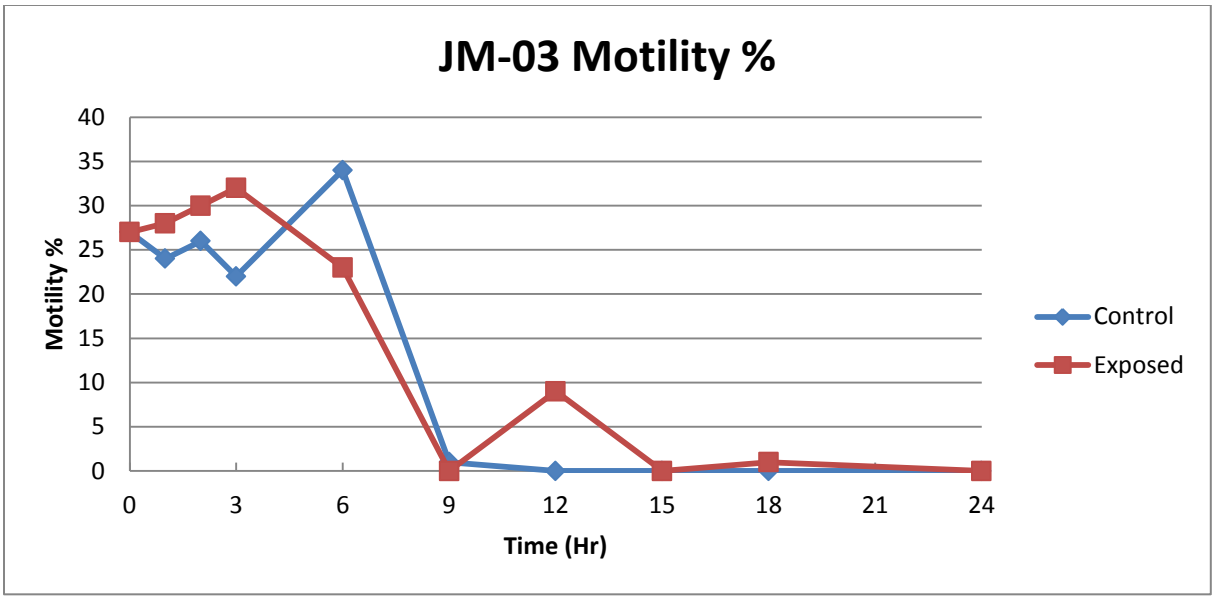


**Figure 3.9: Percentage of motile sperm cells while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-03.**

Figure 3.10 represents the percentage of progressive sperm cells for the control and exposed groups for JM-03. The trend lines for figure 3.10 are very similar to those in figure 3.9; however, one specific point of interest is the control group at hour three. It took an unnaturally large dip down that was not sustained. More than likely this was an outlier caused by not mixing the sample tube well enough before collecting the 10-µL aliquot for analysis in the IVOS. Also, the outlier on the exposed group for hour 12 is still apparent, but slightly less obvious, in figure 3.10 than it is in figure 3.9. The control group point at hour six is also significant because it shows a rather large but unsustained increase in percentage of sperm cells that are progressive, an entire 10% higher than the exposed group's equivalent point. It could potentially be an outlier.

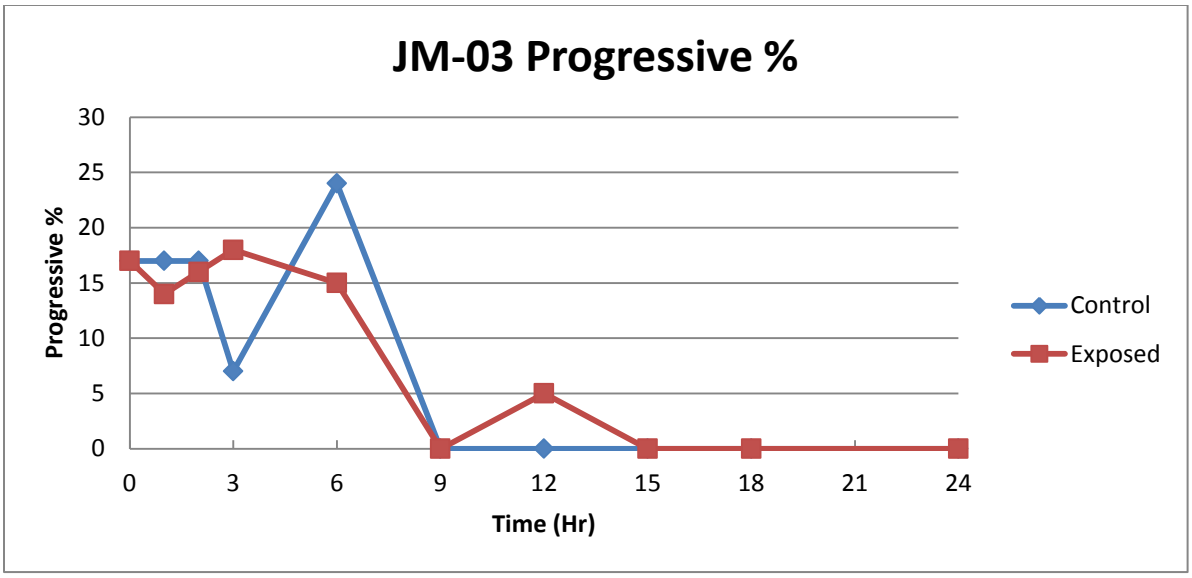


**Figure 3.10: Percentage of progressive sperm cells while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-03.**

Figure 3.11 represents the percentage of sperm cells that demonstrate straightness in their paths of travel for the control and exposed groups for sample JM-03. Minus the obvious outlier with the exposed group at hour 18, the straightness and linearity graphs followed the same pattern as does figures 3.3 and 3.4 and 3.7 and 3.8. Within these two parameters, however, JM-03 does not seem to exhibit a consistent advantage in either the control or exposed group. Both trend lines exhibit momentary crests above the other group's line.

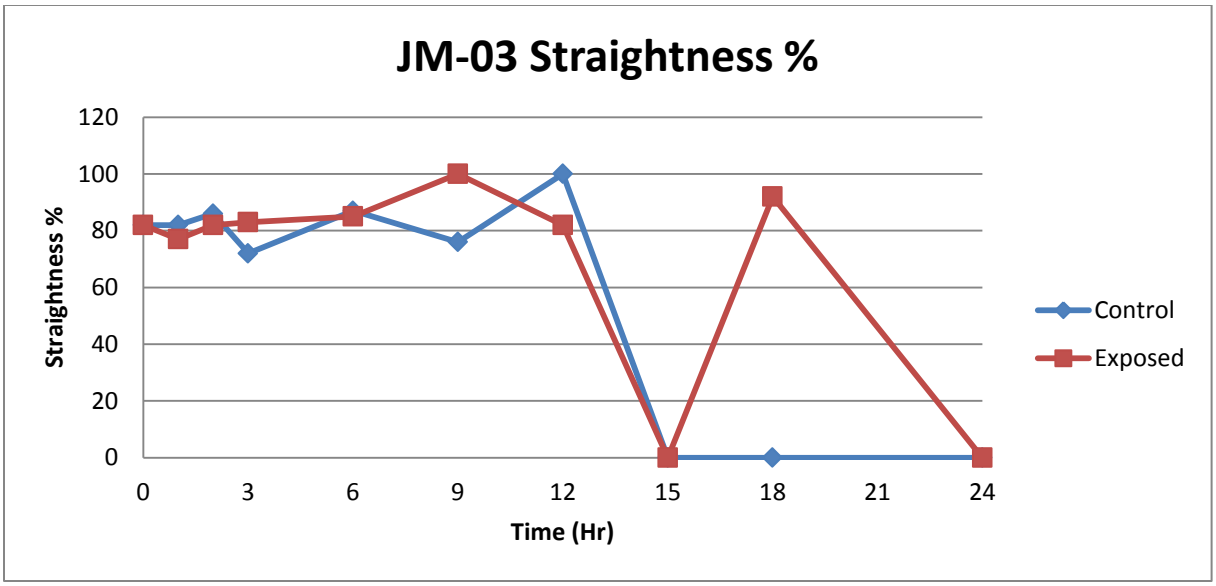


**Figure 3.11: Represents the percentage of sperm cells that exhibited straightness while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-03.**

Figure 3.12 represents the percentage of sperm cells that exhibited linear motion in the control and exposed group of sample set JM-03. The outlier from figure 3.11 in the exposed group on hour 18 still is apparent; however, another potential outlier presents itself in the control group's trend line at hour 12. The peak at hour 12 for the control group is significantly higher than the equivalent exposed point. Additionally, the climb which the hour nine control data point made for the hour 12 data point is notably high and suggestive of an unnatural trend.

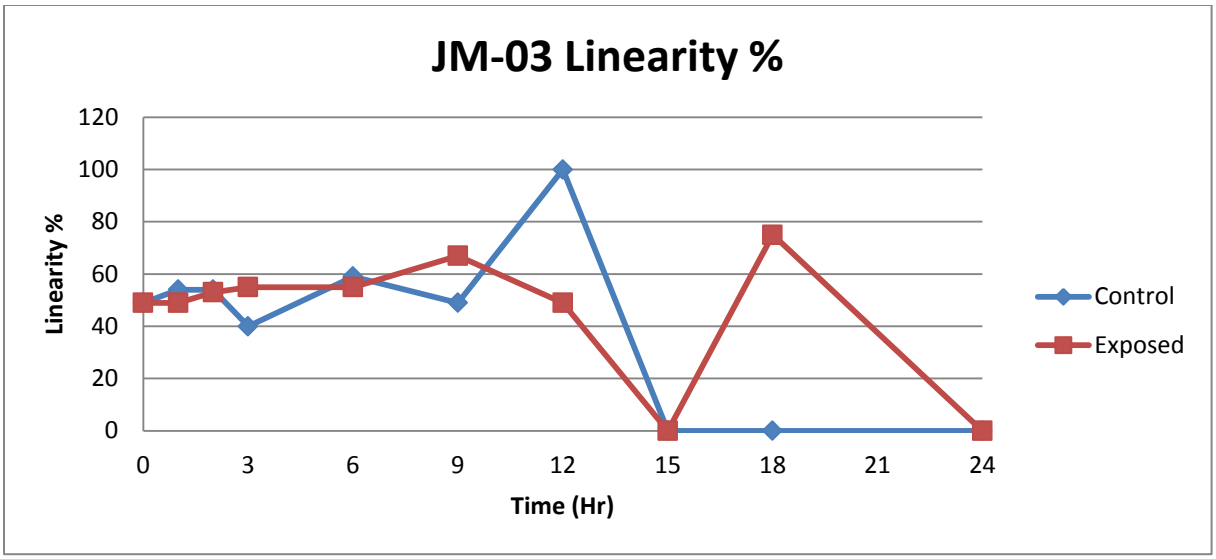


**Figure 3.12: Percentage of sperm cells that exhibited linearity while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-03.**

Figure 3.13 represents that percentage of motile cells in the control and exposed groups of sample JM-04. The overall trend lines for the control and exposed groups in figure 3.13 are similar to the trend lines in figures 3.1, 3.5, and 3.9. An exception in figure 3.13 is in the latter hours where the exposed group performs notably better than the control group. This could potentially suggest the opposite of the proposed hypothesis and that there, in fact, may be a benefit to sperm cell function post wifi radio frequency exposure. The gains that the exposed trend line exhibit are not large; however, they were sustained which increases the chances that they were indeed statistically significant.

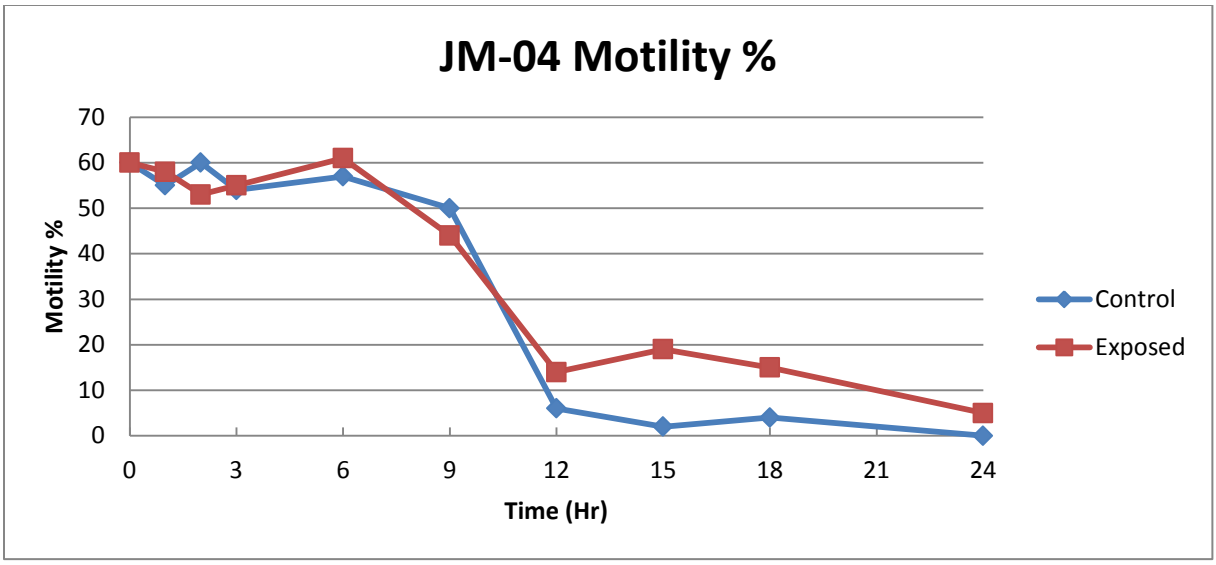


**Figure 3.13: Percentage of motile sperm cells while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-04.**

Figure 3.14 shows the percentage of progressive sperm cells in the control and exposed groups of sample set JM-04. Figure 3.14 is interesting especially when taken into comparison with figure 3.13 in that the gains exhibited in the latter hours of the study by the exposed group are no longer seen when evaluating the progressive percentage curve. In fact, figure 3.14 demonstrates a much more consistent mix of control points being higher than exposed and vice versa, which suggests that, at least as far as the progressive percentage parameter is concerned, there is not a statistically significant difference. Figure 3.13 in comparison to figures 3.14, 3.15, and 3.16 may suggest that potentially some aspect of wifi radio frequency exposure increases motility without affecting the other parameters. When examining the graphs for motility of the other sample sets this same trend was not seen, therefore decreasing the probability that these data are different in a statistically significant way.

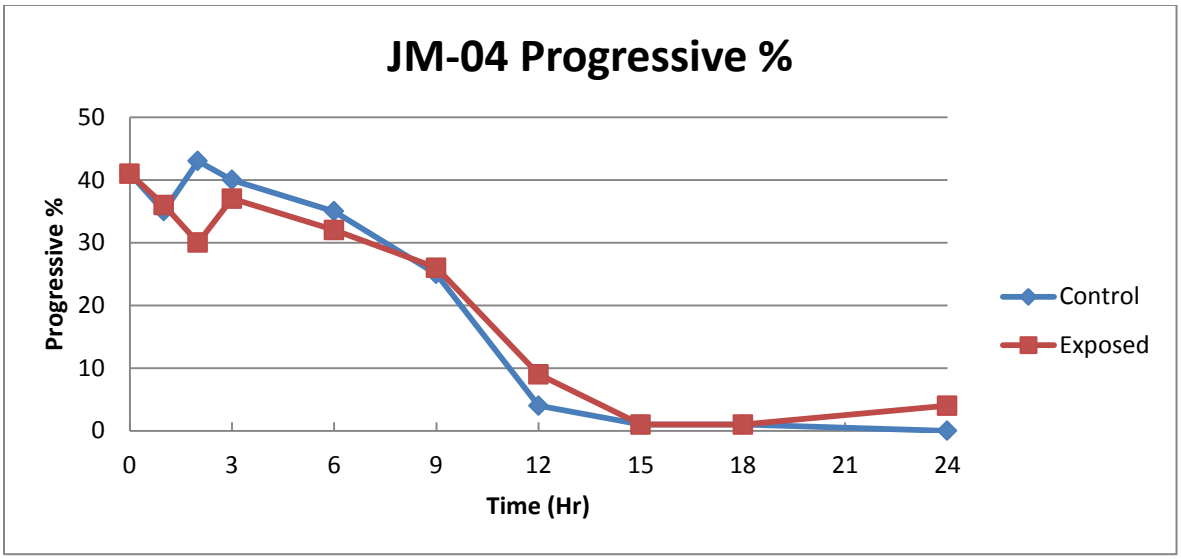


**Figure 3.14: Percentage of progressive sperm cells while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-04.**

Figure 3.15 shows the percentage of sperm cells that exhibited straightness in their motion for both the sample and control groups of JM-04. Until hour 18, both the control and exposed groups exhibit almost indistinguishable data points in figure 3.15. Because of the drastic nature of the drop off represented by hour 24 in the control group in figure 3.15, it is deemed an outlier as it did not follow a natural pattern.

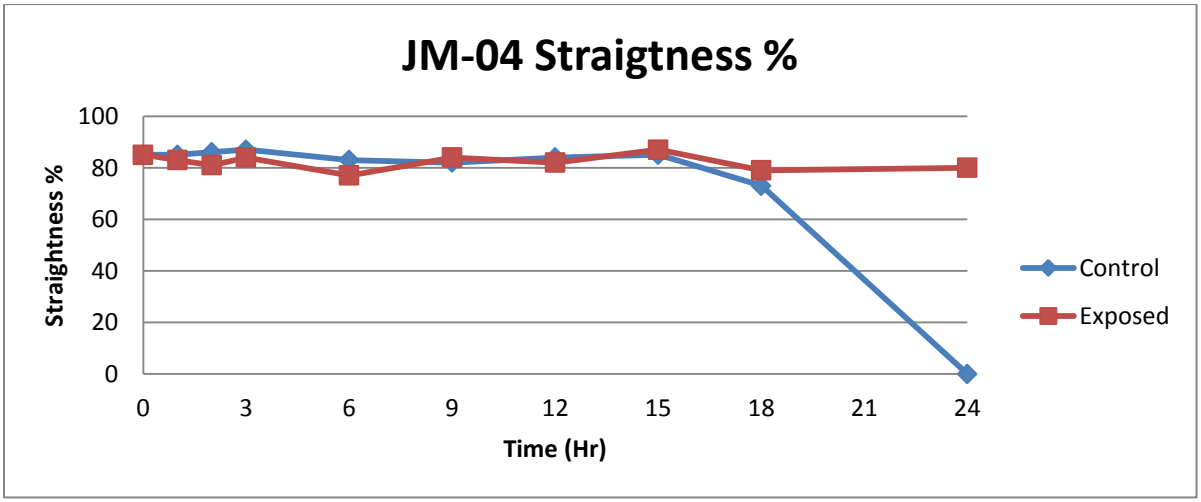


**Figure 3.15: Percentage of sperm cells that exhibited straightness while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-04.**

Figure 3.16 represents the percentage of sperm cells that exhibited linearity for both the control and exposed groups of sample set JM-04. The same pattern represented in figures 3.3 and 3.4, 3.7 and 3.8, and 3.11 and 3.12 is also represented in figures 3.15 and 3.16; the data trend lines for straightness and linearity are nearly the same. Both the control and exposed groups in figure 3.16 have moments where they surpass the opposite group's equivalent points; however, the trend bounces back and forth. The recognized outlier from figure 3.15 for hour 24 is evident in figure 3.16 as well in the control group.

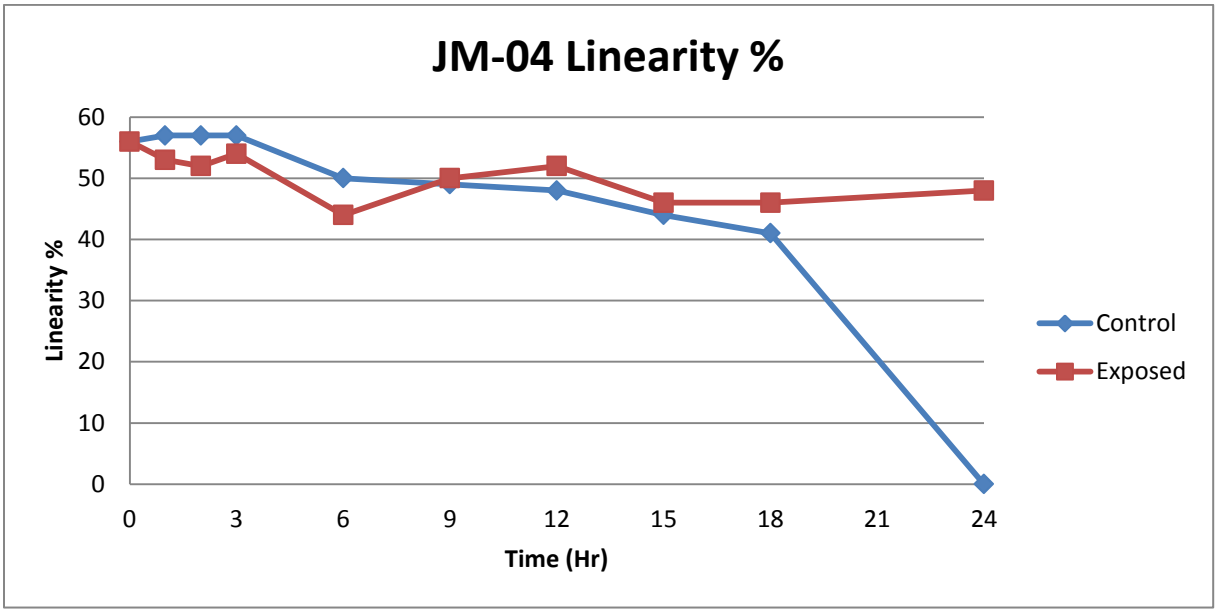


**Figure 3.16: Percentage of sperm cells that exhibited linearity while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-04.**

Figure 3.17 represents the percentage of motile sperm cells in the control and exposed groups in the JM-05 sample set. The motility curve shown is indistinct as compared to the other motility curves in figures 3.1, 3.5, 3.9, and 3.13. Some points of the control group present higher than the exposed group and some vice versa.

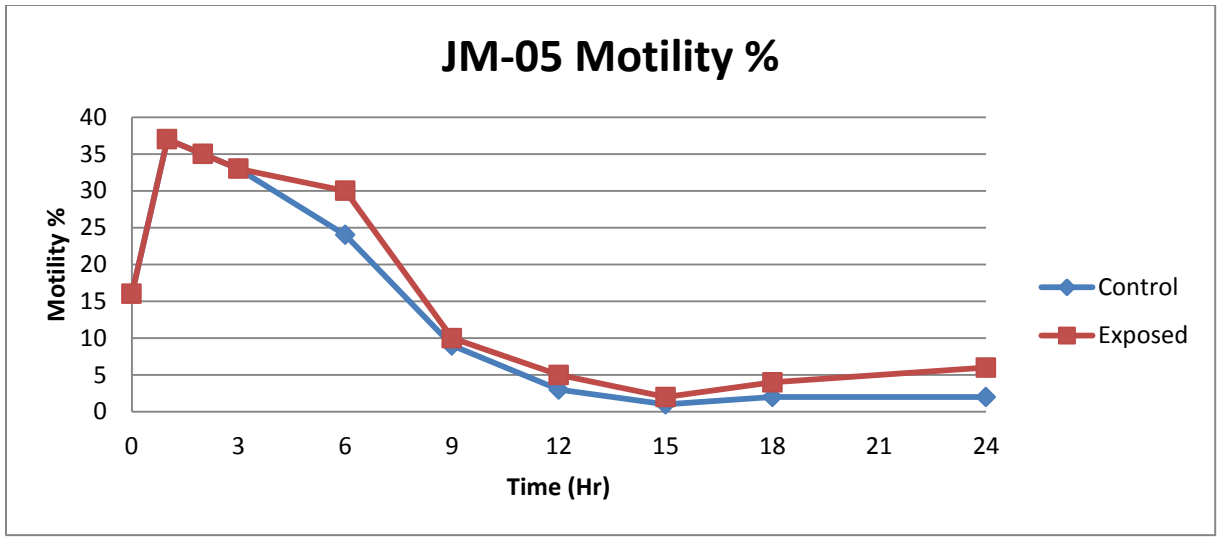


**Figure 3.17: Percentage of motile sperm cells while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-05.**

Figure 3.18 shows the percentage of progressive sperm cells in the control and exposed groups of the JM-05 sample set. The progressive percentage curve in figure 3.18 is similar to the motility curve in figure 3.17; however, it also presents like the curve in figure 3.14 in that the progressive curve does not show the gains for the exposed group as were expressed in the motility curve. Other than that, the data points for figure 3.18 are indistinct.

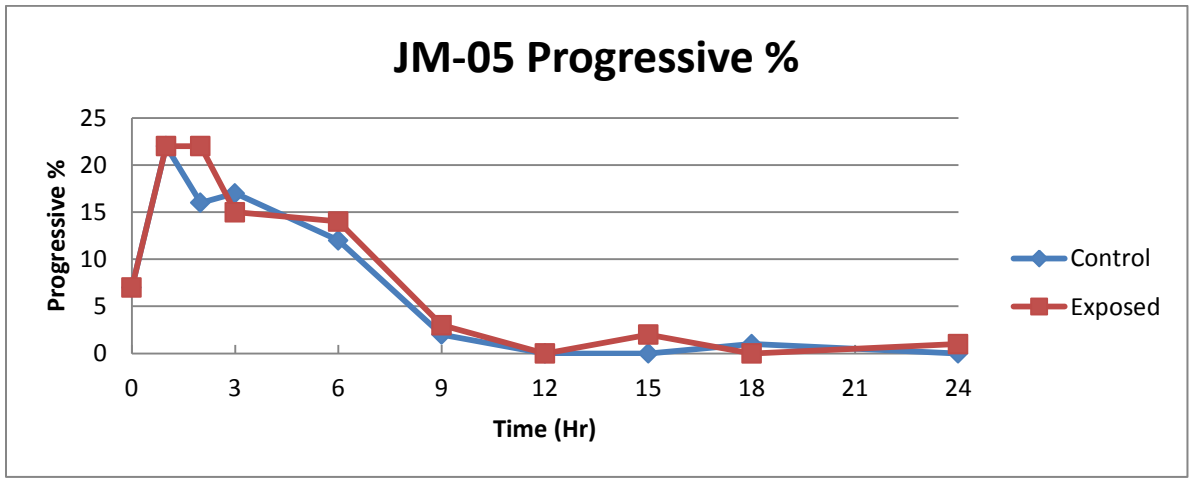


**Figure 3.18: Percentage of progressive sperm cells while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-05.**

Figure 3.19 represents the percentage of sperm cells that exhibited straightness in both the control and exposed groups in the sample set JM-05. Over most of the data points, neither group demonstrates an advantage in figure 3.19 except at the 24 hour data point where the exposed group edges out a noteworthy advantage over the control group. This could potentially suggest a statistically significant difference between the control and exposed groups over time.

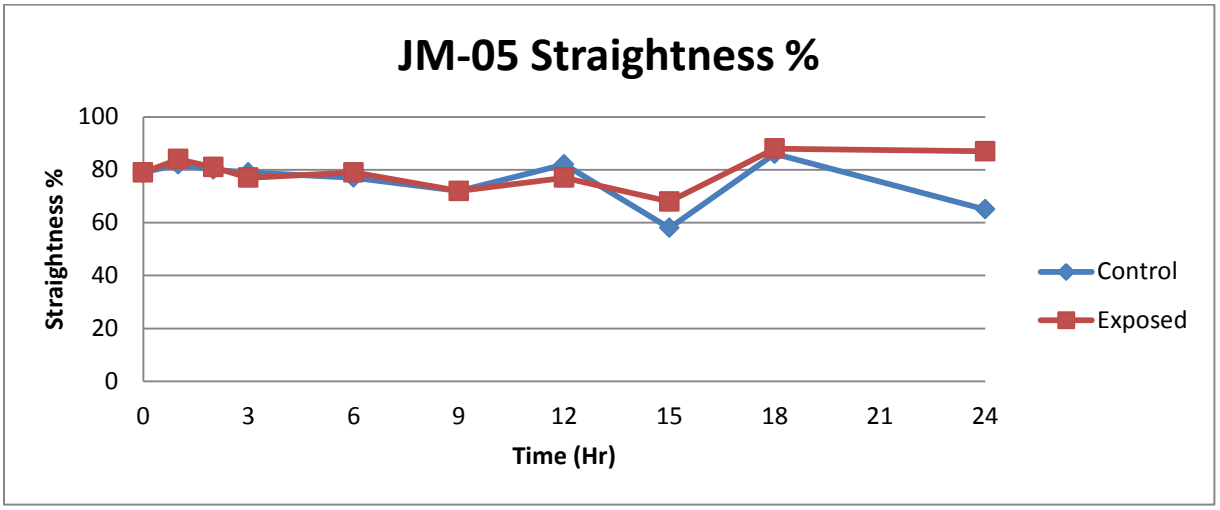


**Figure 3.19: Percentage of sperm cells that exhibited straightness while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-05.**

Figure 3.20 represents the percentage of sperm cells that exhibited linearity in their motion in both the control and exposed groups for sample JM-05. Figure 3.20 continued to represent the graphical pattern between straightness and linearity when compared to figure 3.19, and the previous straightness and linearity graphs in figures 3.3 and 3.4, 3.7 and 3.8, 3.11 and 3.12, and 3.15 and 3.16. The hour 24 data point for the exposed group also demonstrates a large advantage over the control data point. This evidence in figure 3.20 suggests that the exposed group potentially possessed an advantage over the control group that was statistically significant.

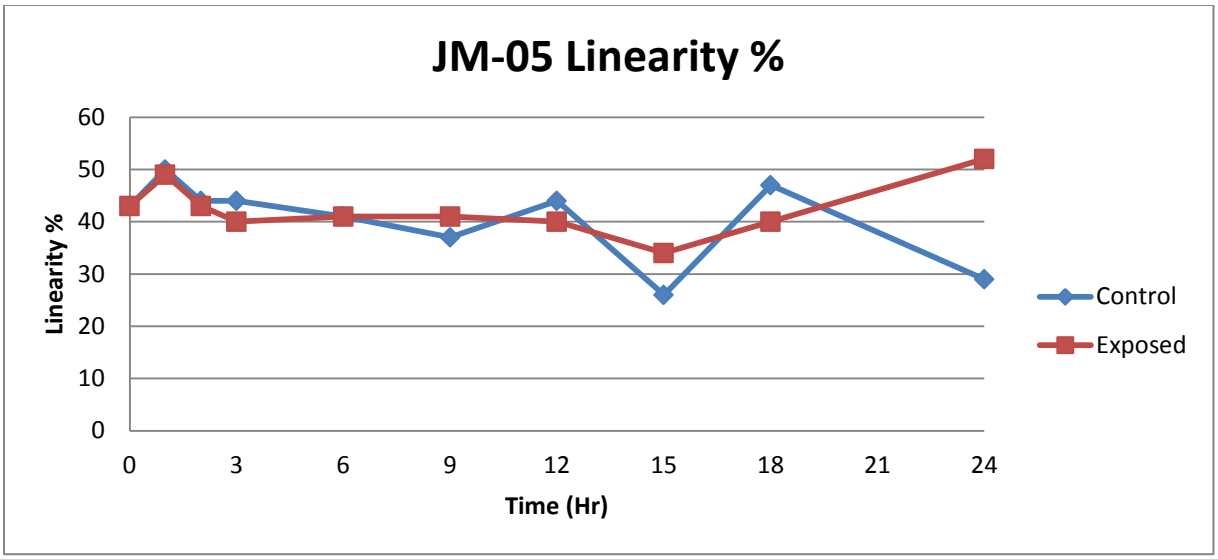


**Figure 3.20: Percentage of sperm cells that exhibited linearity while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period for JM-05.**

## CHAPTER 4: DISCUSSION

Figure 4.1 represents the average overall motility of all samples, both control and exposed groups. When examining figure 4.1 the overall average motility for all control and exposed samples over all time periods, the differences become almost indistinguishable. Since the data are nearly indistinguishable it is highly suggestive that, at least for the small sampling encompassed by this study, the data differences between the control and exposed groups are minimal and not statistically significant. This would reject our hypothesis that wifi radio frequency exposure negatively effects sperm cell function.

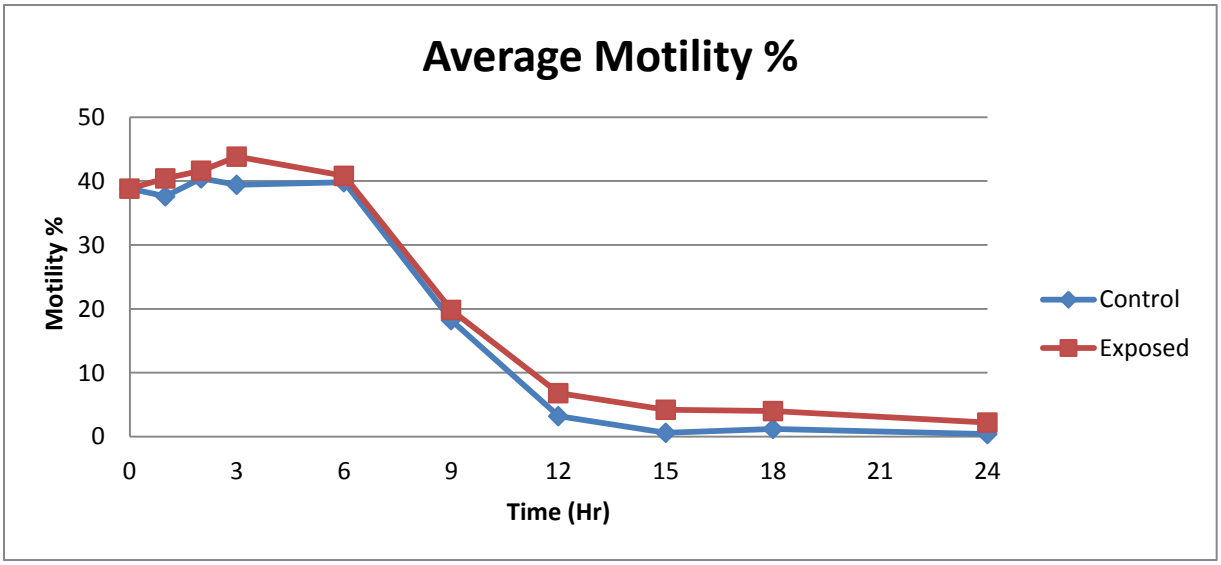


**Figure 4.1: Average percentage of motile sperm cells while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period.**

Figure 4.2 shows the average percentage of progressive sperm cells of all control and exposed groups in all sample sets over the 24 hour period. The nearly identical trend lines in figure 4.2 for the control and exposed groups strongly suggests that the hypothesis is rejected and that post ejaculatory sperm cells are not affected by short-term radio frequency radiation

exposure in the 2,400MHz spectrum in a readily measureable way. Neither group maintained a significant advantage over the other for a sustained period of time.

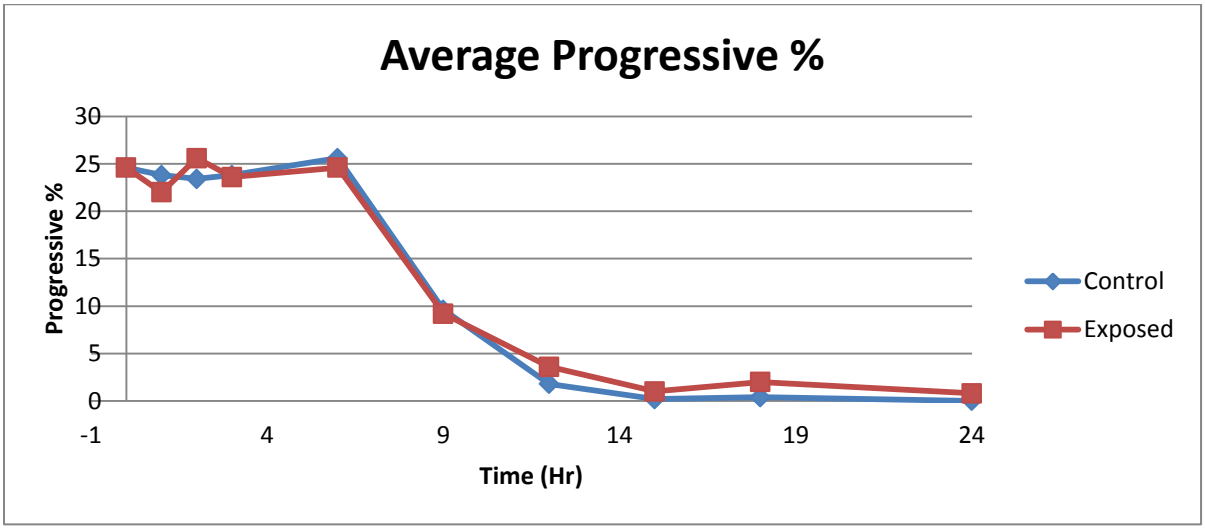


**Figure 4.2: Average percentage of progressive sperm cells while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period.**

Figure 4.3 shows the average percentage of sperm cells in all groups in all samples that exhibited straightness in their paths of motion over the 24 hour period. Figure 4.3, as do figures 4.1 and 4.2, also supports the rejection of the initial hypothesis. The differences between the control and exposed data trend lines are minimal. The data suggest a high probability that the differences between the control and exposed groups for progressive percentage are not statistically significant. The 24-hour data point for the exposed group is the only data point that exhibited a notable gain over the opposite group, but because it is the only data point it can be dismissed as statistically insignificant.

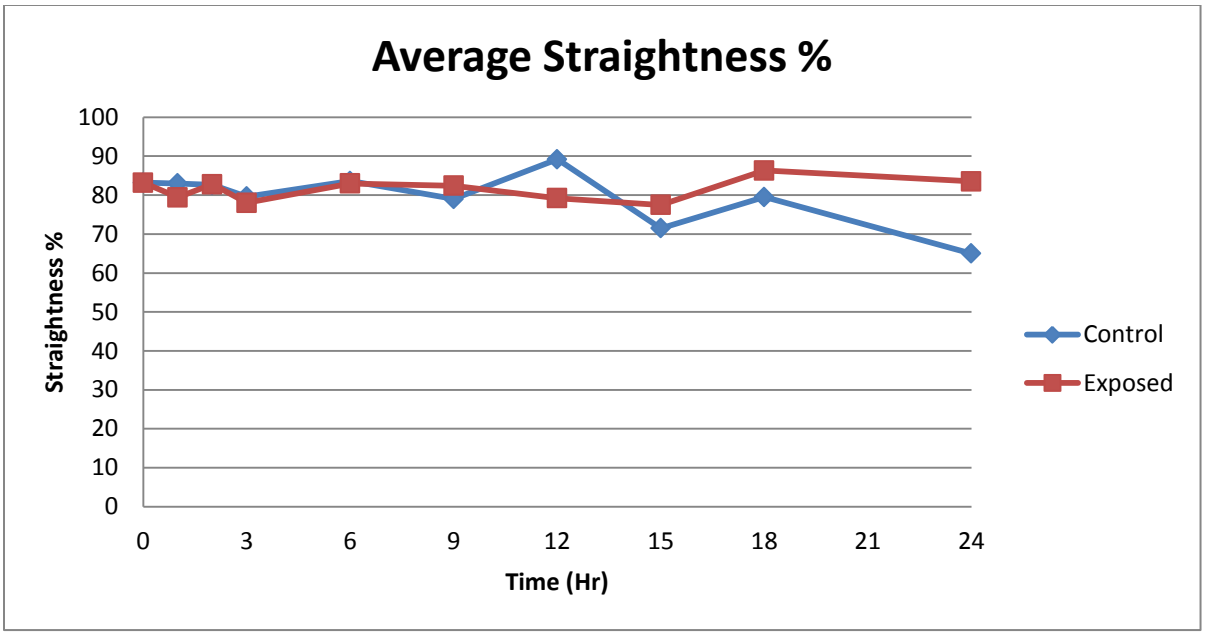


**Figure 4.3: Average percentage of straightness of all sperm cell paths while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period.**

Figure 4.4 represents the average percentage of sperm cells that demonstrated linearity over all groups in all samples over the 24-hour time period. Figure 4.4 presents a few data points of interest. Hour 12 for the control group presents an abnormal data trend pattern that gave it a nearly 10% advantage over the exposed trend line; however, because of the drastic decrease represented in the hour 15 data point for the control group compared to hour 12, it is discernable that the data point for the control group at hour 12 is an outlier and, therefore, not statistically significant. Hours 18 and 24 in figure 4.4 present potentially the most statistically significant data that the opposite of the hypothesis is true; that sperm cells exposed to wifi radio frequency are actually benefitted. At hour 18 the exposed group demonstrates a 10% advantage over the control. This advantage increases to 20% at hour 24 for the exposed group.

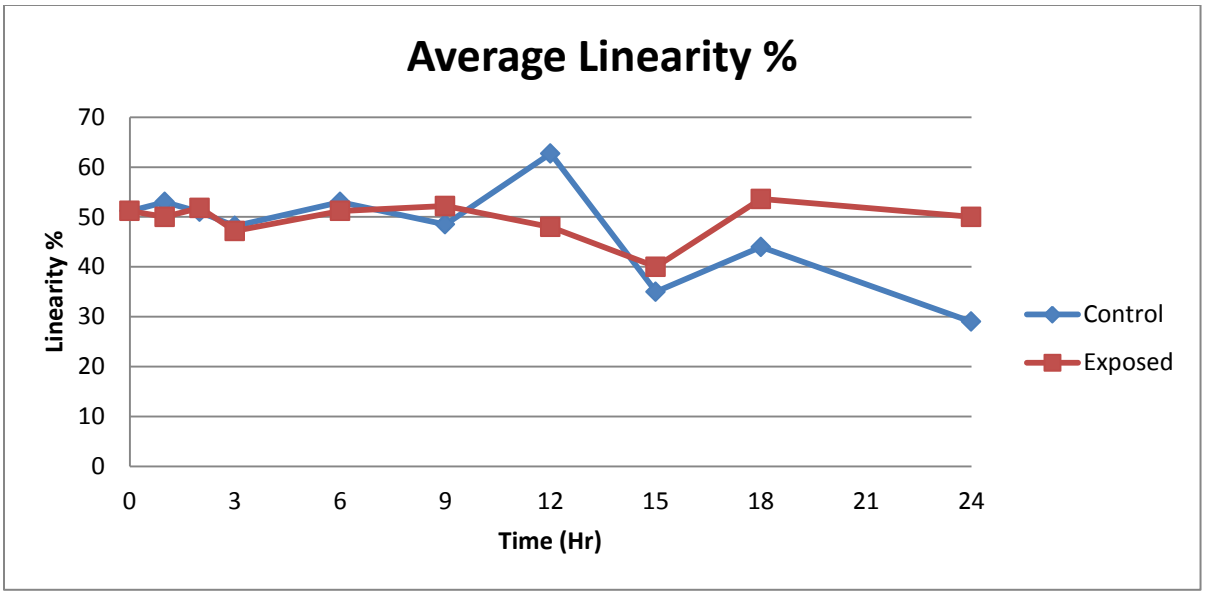


**Figure 4.4: Average percentage of sperm cells that exhibited linearity while exposed to radio frequency fields from a wifi router or non-exposed controls over a 24-hour period.**

Figures 4.1, 4.2, and 4.3 are strongly indicative that there are no statistically significant differences between the control and exposed group for the sample population used. Figure 4.4, for the majority of the graph, also seems to support that there are no statistically significant difference between the exposed and control groups until the end of the graph. The last two reading hours for average linearity demonstrated what could have been statistically significant data.

One potential dilemma with determining whether the end data on figure 4.4 is statistically significant is that the sample size is small for the scope of this study. In reference to other studies, this study was designed specifically to examine whether or not post ejaculatory sperm cells were affected by radio frequency exposure in the cell phone frequency range. The few studies that have been performed have been performed in multiple frequency ranges so it is entirely possible that different frequencies affect sperm cells differently. Also, maintaining consistency with the intensity of the signals was difficult. With the consistency issues on top of

the limited sample size of the study, it was most nearly suggestive of only one strong conclusion: more studies are needed examining the potential ill effects of exposure to technology and the wireless energy these products use and transmit.

However, as far as this study is concerned, the data results strongly suggest that there is no statistically significant difference between post ejaculatory sperm cells exposed to radio frequency radiation in the 2,400MHz range and those that are not.

## CHAPTER 5: CONCLUSION

Over time, the exposed group did not demonstrate a statistically significant difference with the control group because all semen parameters decreased over time ($p < 0.001$) regardless of treatment. Further and larger studies are needed to confirm this outcome; however, it appears that short-term exposure to radio frequency radiation, specifically in the 2,400MHz spectrum, has no noticeable effect on sperm cell motility and function. The proposed mechanism of dielectric heating seems highly unlikely as 24 hours should have been ample time to notice effects if any were present. One potential reason that effects were not seen could be that because the life cycle of post ejaculatory sperm is so short, defects do not have time to arise. Another potential explanation is that intensity of the radiation emitted was not strong enough to induce dielectric heating. The wifi router used in the study was a single dipole residential router. A more powerful commercial or an industrial multiple input multiple output router may produce different results. Although the set up was not able to include this variable, it is also possible that providing a live internet line to the router would produce different results as well. What the data suggests, however, is that the 24-hour window of exposure from wifi radio frequency radiation does not affect sperm cells in a readily detectable way. A larger and more long-term study is needed in order to evaluate the effects of long-term radio frequency radiation exposure on male fertility and sperm cell formation within the testes.

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# APPENDIX

TEXAS TECH UNIVERSITY
HEALTH SCIENCES CENTER

**INSTITUTIONAL REVIEW BOARD FOR THE PROTECTION OF HUMAN SUBJECTS**
**FWA # 00006767 LUBBOCK/ODESSA IRB #00000096**

**NOTIFICATION OF CONTINUING REVIEW APPROVAL**

June 21, 2010

**IRB #:** 01005

**STUDY:** STUDIES ON IMPROVING MALE FERTILITY USING PAID DONOR SAMPLES (L-OB/GYN-01005)

**PRINCIPAL INVESTIGATOR:** Samuel D. Prien, Ph.D.

**SUBMISSION REFERENCE #:** 033232

**TYPE OF REVIEW:** EXPEDITED

**APPROVAL DATE:** 6/21/2010 **RISK ASSIGNMENT:** Expedited

**REVIEW PERIOD:** 12 Months **EXPIRATION DATE:** 6/20/2011
*(based upon date recommended for approval)*

**SUBJECTS APPROVED AT THIS SITE:** 200

This submission was reviewed in accordance with 45 CFR 46.109 (e).

**SPECIFIC INFORMATION PERTAINING TO THIS APPROVAL**

REVIEWER COMMENTS: The study is actively enrolling subjects. The numbers reported in this report are incorrect. According to last year's review, the PI reported the total number of subjects enrolled to be 39. He has enrolled 7 subjects since the last review.

Number of subjects approfved by the IRB 200
Number of subjects at last continuing review: PI reported 30 (should be 39)
Number of subjects since last continuing review: 7
Total number of subjects enrolled since study began: PI reported 37 (should be 46)
Number of subjects that have completed protocol requirements: PI reported 27 (should be 46 since last CR was 39 and 7 additional subjects have been enrolled and the intervention is just collecting a semen sample)
Number of subjects that are still on study intervention: PI reported 10 (should be 0)

**IRB Recommendations:**
1. Approve continuation of this study, expedited/minimal risk, annual review.
2. **The IRB asks that in the future the PI please double-check subject enrollment numbers before submitting to the IRB.**
3. **Note that this project lacked IRB approval between 6/10/2010 and 6/20/2010. Federal regulations and institutional policy prohibit any research activity from taking place during the time of the lapse in IRB approval.**

**Approval Period:** This approval is for a period of 12 Months. You should receive electronic notification 45 days prior to the expiration of this project's approval. *However, it is your responsibility* to insure that a Continuing Review Submission Form has been submitted by the required time.

**Consent Form:** The currently approved and stamped consent form must be used when enrolling subjects. You are responsible for maintaining signed consent forms for a period of at least three years after study completion. NOTE: A HIPAA authorization form is required at the time of obtaining initial consent and whenever the purpose of the study is revised or changed.

**Reporting:** The principal investigator must report to the IRB any serious problem, adverse effect, or outcome that occurs with frequency or degree of severity greater than that anticipated. In addition, the principal investigator must report any event or series of events that prompt the temporary or permanent suspension of a research project involving human subjects.

**Modifications:** Changes or modifications in a research project **must have approval** by the IRB prior to initiation. When modifications are deemed necessary to prevent immediate harm to a subject, changes or modifications must be reported to the IRB within 24 hours.

**Study Completion:** If this project is completed within the approval period, you are required to submit a Study Update indicating "Final Closure". The study project is considered completed when:

1. Investigators will not contact subjects for further information related to this project
2. Access to subject health care records are no longer required for information related to this project
3. All IRB requests for information have been completed and no longer require an investigator response
4. A summary report has been completed. This must be attached as a Supporting Document in the Study Update submission.

## GENERAL INFORMATION

The Texas Tech University Health Sciences Center Institutional Review Boards are duly constituted (fulfilling FDA requirements for diversity), allows only those IRB members who are independent of the investigator and sponsor of the study to vote/provide opinion on the study, has written procedures for initial and continuing review, prepares written minutes of convened meetings, and retains records pertaining to the review and approval process; all in compliance with requirements defined in 21 CFR (Code of Federal Regulations) Parts 50 and 56, and ICH (International Conference on Harmonization) guidance relating to GCP's (Good Clinical Practice).

The Texas Tech University Health Sciences (TTUHSC) Center Policies and Procedures are available for reference on the TTUHSC Human Research Protection Program Website (http://www.ttuhsc.edu/research/hrpo/irb/).

**TTUHSC Lubbock/Odessa Institutional Review Board**
**3601 4th Street STOP 8146**
**Lubbock, TX 79430**
**806-743-4753**