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Could the p75 Neurotrophin Receptor p75NTR Help Unlock the Mysteries of Infertility?

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Abstract

Histology is the study of microscopic tissue structures that examines the correlation between structure and function. This technique was utilized when analyzing the biological processes of folliculogenesis and oogenesis. These processes study the maturation of the ovarian follicle and production of an ovum, respectively. The p75 neurotrophin receptor ($p75^{NTR}$) has been identified to contribute to the maturation of ovarian follicles, but the role of $p75^{NTR}$ in ovulation remains unknown. To investigate the role of $p75^{NTR}$ in ovulation, an animal study analyzing the histology of the mouse ovary was conducted. In this study, the number of primary follicles, secondary follicles, antral follicles, and corpus luteum were analyzed in wild type mice and then compared to the follicles present in genetically modified mice that lacked the $p75^{NTR}$ gene (p75 knock out (p75KO) mice). When the mice in this study reached eight weeks of age, the mice were killed, and their reproductive tissues were collected. Upon harvest, the tissues were processed for sectioning and then stained for analysis. Microscopic analysis revealed that there was a significant relationship between the number of corpus luteum present in the wild type mice compared to the knockout mice ($p=0.006$). Indicating that wildtype mice are ovulating at higher rates than p75KO mice. This data supports that $p75^{NTR}$ is playing an active role in the ovulatory process.

Keywords: histology, folliculogenesis, oogenesis, wild-type mice, p75 knockout mice, ovarian follicles, p75 neurotrophin receptor

Table of Contents

List of Figures

List of Tables

Acknowledgements

I would like to extend a special thank you to my mentor, Dr. Oliver Oakley, without whom this project would have not been possible. Thank you for volunteering your time, resources, and knowledge for this project to be a success. Thank you for teaching me, encouraging me, and cultivating my interest in research.

Introduction

1.1 Histology

Histology is the study of microscopic structures of tissues, including their role in the body, their relationship to anatomy, their interaction with the body systems and the ways tissues are affected by disease (Musumeci, 2014). In particular, this field of study is crucial for understanding how specific biological processes work in the body. Histology aids in determining the function that specific tissues, proteins, and receptors play in these biological processes, as it examines the correlation between structure and function. Folliculogenesis and Oogenesis are just two examples of the biological processes that can be analyzed utilizing this field of study.

1.2 Folliculogenesis

Folliculogenesis describes the growth, differentiation, and development of the ovarian follicle. The ovarian follicle is a densely packed shell of somatic cells that contains an immature oocyte. There are 46 chromosomes, 23 pairs, that exist in the human genome. Somatic, or body, cells exist in the diploid state, meaning that all 23 pairs of chromosomes are present within a cell. An oocyte is found within the ovarian follicle and it is classified as an immature ovum, or egg.

The adult ovary is a multifunctional, dynamic organ that varies according to its physiological status (Findlay, 2003). During folliculogenesis, ovarian follicles go through a series of functional stages from a primordial follicle leading up to ovulation. Primordial follicles are surrounded by a single layer of flattened follicular epithelial cells, also known as granulosa cells. These primordial follicles are very small, typically found close to the outer edge of the ovary. Upon stimulation, the primordial follicle develops into a

primary follicle. At this stage, the oocyte enlarges, and the cells surrounding the oocyte divide, creating two layers of granulosa cells. It is at this stage that the stroma forms, which creates a capsule around the follicle.

The primary follicle then develops into the secondary follicle. At this stage, the oocyte enlarges, and the follicle grows and becomes much larger than a primary follicle. In secondary follicles, an antrum, or fluid filled space, begins to form. Next, an antral follicle forms. Antral follicles are much larger in size compared to secondary follicles; it is at this stage that the oocyte enters into its second meiotic division. Additionally, at this stage, the antrum becomes much larger and surrounds the cells within the antral follicle. When the oocyte enters into its second meiotic division, it is then expelled from the follicle, marking that ovulation has occurred. Once ovulation occurs and the ovum has been released, the corpus luteum is formed from the remnants of the follicle. Upon ovulation, the antral follicle collapses and fills with a blood clot. This, in turn, forms the corpus luteum. The corpus luteum is an enlarged structure that becomes vesicular and it is indicative that ovulation has occurred.

Follicles can be divided into two morphological categories based on the presence or absence of an antrum, or a fluid filled cavity (Findlay, 2003). Pre-antral follicles are classified as primordial, primary, or secondary. Antral follicles are large, mature follicles that are classified as ovulatory. Folliculogenesis is a continuous process, meaning that at any time the ovary contains follicles in many stages of development (Curry, 2018). At birth, the ovary contains around 400,000 primordial follicles which contain primary oocytes. In each ovarian cycle, about 20 primordial follicles are activated to begin maturation (Andersen, 2017).

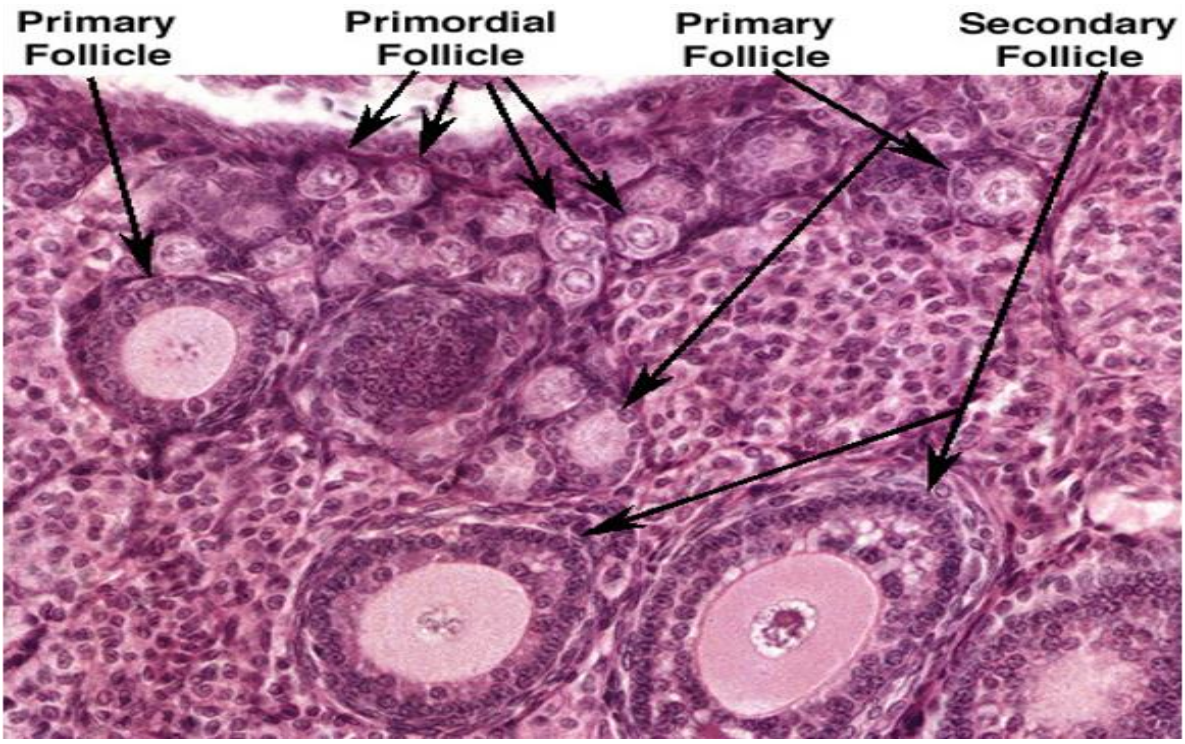


Figure 1. Histology of primordial, primary, and secondary follicles in the ovary (Dissen, 2009).

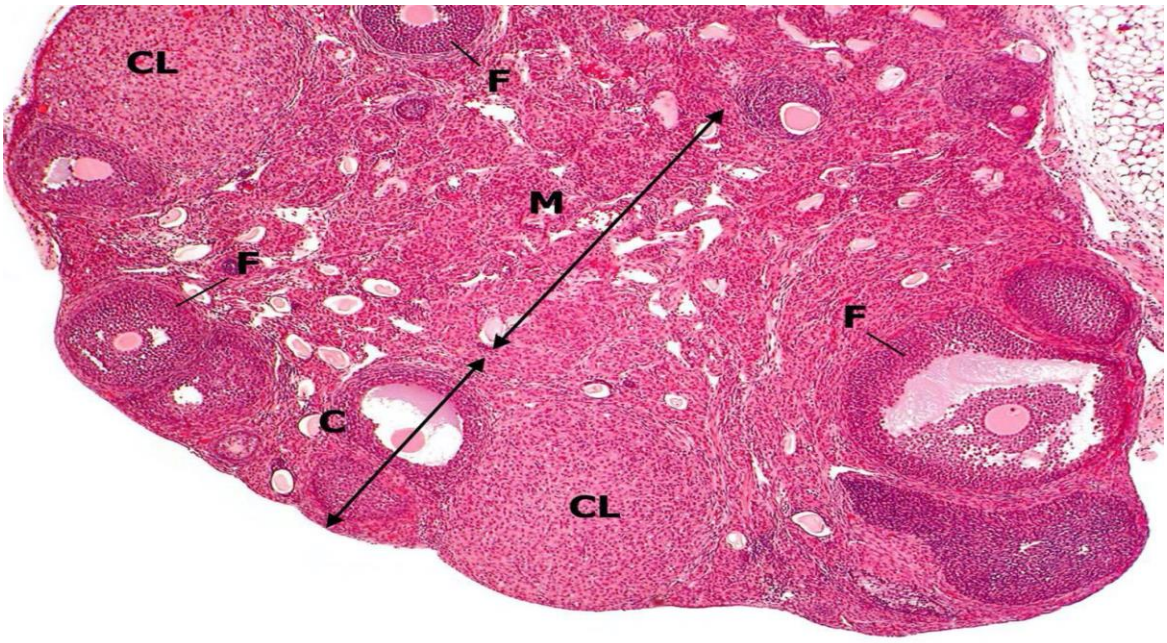


Figure 2. Histology of mature follicles (F) and corpus luteum (CL) in the ovary.

1.3 Oogenesis

As an ovarian follicle matures from a primordial follicle up to ovulation, the oocyte contained within the follicle matures as well. The oocyte undergoes a process known as Oogenesis. Oogenesis is the process in the female reproductive system in which the primary egg cell or oocyte, matures into an ovum, or egg. During oogenesis, the egg grows and undergoes cell division. The egg remains as a primary ovum until it is time for ovulation. At the time of ovulation, the egg undergoes its second mitotic division. At this point, the chromosomes contained in the ovum split. Half of the chromosomes form one cell and the other half of the cells for a different cell. During this division, one cell that is produced is larger than the other, termed the ovum. The smaller cell that is produced is termed the polar body (Gilbert, 2000.) Once ovulation occurs, the ovum matures in the ovary and eventually is carried into the fallopian tubes. At this stage, the ovum is ready for male fertilization. Studies suggest that a large number of proteins and receptors play crucial roles in the development of the oocyte (Sánchez, 2012). In contrast to folliculogenesis, oogenesis is a discontinuous process, meaning it is a process that happens in stages with intervals between the stages.

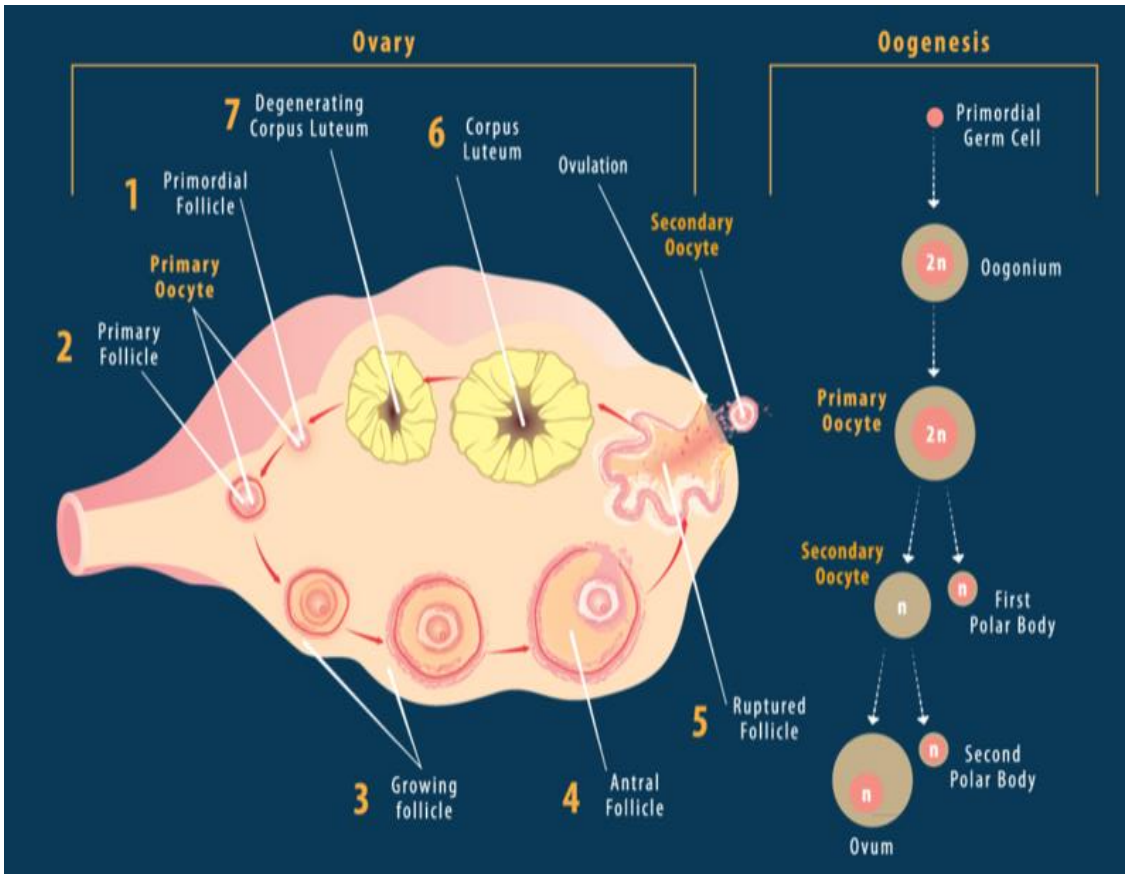


Figure 3. Depiction of folliculogenesis and oogenesis; the maturation of the ovarian follicle and maturation of the primary oocyte into an ovum, respectively.

1.4 Hormones Regulating Ovulation

Hormones produced by the pituitary gland are responsible for stimulating ovulation in women each month. Ovulation and the maturation of the ovarian follicle is largely regulated by fluxing gonadotropic hormone levels (Holesh, 2020). The hormones involved in ovulation include, gonadotropin-releasing hormone (GnRH), gonadotropin hormone, follicle stimulating hormone (FSH), estrogen, luteinizing hormone (LH), and progesterone. The cells surrounding the oocyte, the granulosa cells, and the cells that appear as the follicle develops, theca cells, mature and develop in response to these

specific gonadotropic hormones. A surge in the luteinizing hormone initiates follicle maturation by activating responsive second messengers in preovulatory follicle cells (Lee-Thacker, 2020). Hormones have been thought to exclusively contribute to follicular development and oogenesis; however, new data suggests that follicular development and ovarian maturation is controlled by more factors than these specific hormones.

One example of this phenomena is identified in research by Oakley and collaborators where a correlation between the spleen and follicular development was identified. In this study, leukocytes, or white blood cells, were identified to traffic from the spleen to the ovary. This study suggests that the spleen may serve as a main reservoir of leukocytes (Oakley, 2011). Chemicals that trigger an inflammatory response attract leukocytes to the site of injury or infection. It is known that ovulation is a consistent cycle of inflammation and repair. Interestingly, this research explains that infiltration and distribution of leukocytes in the ovary are correlated with hormonal changes associated with estrous cycles, indicating that reproductive hormones can elicit inflammatory responses in the ovary. The results of this study showed that the removal of the spleen in mice caused a decrease in leukocytes and thus follicular development. Building from this research, it is possible that neurotrophins may play a connecting part in this infiltration of leukocytes from the spleen to the ovary.

Neurotrophins

The neurotrophins (NT's) are a family of soluble growth factors. NT's control the development and maintenance of discrete neuronal populations (Fariñas, 1999), but also of some non-neuronal tissues, including endocrine and immune cells (Tessarollo, 1998). Neurotrophins act on cells through two distinct classes of receptors. The high-affinity

signal receptors are Trk proteins; TrkA, TrkB and TrkC. Trk proteins have tyrosine-kinase activity (Levanti, 2005). A member of the tumor necrosis factor family, $p75^{NTR}$, serves as a p75 neurotrophin low-affinity receptor (Dechant, 2003). $p75^{NTR}$ can be utilized by similar ligands as Trk A and Trk B. However, the functional roles of $p75^{NTR}$ are complex.

The mammalian ovary contains detectable levels of both NTs and NT-receptors, identified at the mRNA or protein levels (Dissen, 2002). Moreover, studies on mice carrying mutations in genes codifying for these molecules have contributed to clarify its role in follicular development and ovulation (Dissen, 2001).

1.5 p75 Neurotrophin Receptor ($p75^{NTR}$)

$p75^{NTR}$ is a neurotrophin receptor. It is a transmembrane glycosylated receptor that elicits an array of biological functions through its ability to interact with ligands and coreceptors (Alsheri, 2017). A neurotropin is a protein growth factor that interacts with specific neurotropin receptors and it sends signals between cells (Reichardt, 2006). Neurotrophins were previously thought to be exclusively involved in the regulation, maintenance, development, and function of the vertebrate nervous system. However, neurotrophins have been identified to exhibit different functions from those already identified in the nervous system.

Specific neurotrophins have been found in the ovaries and have been documented to contribute to the maturation of ovarian follicles (Kerr, 2009). Kerr's research highlights the fact that the ovaries of several species including rodents, primates, and humans produce neurotrophins and express the $p75^{NTR}$ required for signaling. The neurotrophin receptor, $p75^{NTR}$, has been identified in the developing mouse ovary. $p75^{NTR}$ is

documented to be involved in oogenesis and folliculogenesis (Dissen, 2009). However, the specific role of $p75^{NTR}$ in ovulatory function is not fully understood. Despite the long list of functions described for $p75^{NTR}$, its mechanism of activation and signal initiation remains unclear.

It is known that $p75^{NTR}$ alone, or with other coreceptors, can mediate several important cellular functions including cell death, survival, maturation, and inhibition (Chao, 2003). $p75^{NTR}$ is thought to modulate cellular responses to the neurotrophins by enhancing the sensitivity of the Trk receptors (Meeker, 2014). The p75 receptor is more widely expressed in the central nervous system during development than in the adult (Yan, 1988), suggesting that this receptor may have a special role during development. A comprehensive study of $p75^{NTR}$ reports that NGF receptors ae required for the growth of primordial ovarian follicles, a process known to occur independently of pituitary gonadotropins (Dissen, 2002).

1.6 Wild Type Mice v. p75 Knock-Out Mice

In the field of biology, mice are often utilized in experimental animal studies. Mice are often used as models for studies because their biological and behavioral characteristics closely resemble humans, so they are appropriate to answer many research questions (Perlman, 2016). In this particular research design, two types of mice were studied; wild type (WT) mice and p75 knock out (p75KO) mice. Wild type mice are mice that have not been genetically modified. P75KO mice have the p75 gene removed or "knocked out" of them and it is replaced with artificial, non-coding DNA. Knocking out the activity of the p75 gene provides valuable information for how that gene behaves in the body. The gene produces a protein, without the gene there is no protein produced. So,

comparing wild type mice ovaries to p75KO ovaries allows for the function of $p75^{NTR}$ in ovulation to be analyzed directly.

1.7 Objective and Purpose

This experiment focused upon analyzing the number of primary follicles, secondary follicles, mature follicles, and corpus luteum that were present in wild type mouse ovaries compared to the number of follicles found in the p75KO ovaries. The ovaries from both wild type and p75KO mice were harvested from the mice when the mice were eight weeks of age. This is the optimal time to harvest reproductive tissues for accurate analysis. The purpose of this study is to understand the role that $p75^{NTR}$ is playing in folliculogenesis and ovarian maturation. This in turn broadens the understanding of fertility in mice which correlates directly to fertility in woman. Infertility is an issue that affects millions of women in America each year. There are current treatments for infertility including IVF and other hormonal therapies. However, none of these treatments are actively targeting neurotrophins or neurotrophin receptors. So, based upon what we know about $p75^{NTR}$ and the critical role that it may play in the maturation of ovarian follicles, studying the mechanism and effects of $p75^{NTR}$ could prove to be very beneficial in this field of research.

Methods and Procedures

2.1 Harvesting Tissues

To begin, the wild type mice and the p75KO mice were housed in an environmentally controlled room on a 12-hour light and dark regulated photoperiod. Animals were provided with food, water, care, and kept in accordance with the Institutional Animal Care and Use Committee (IACUC) protocols and regulations. The mice were subjected to natural ovulation. The mice began to ovulate at 13 to 14 days of age. At the age of 8 weeks, or 56 days, the mice were euthanized. The mice were euthanized using carbon dioxide inhalation, followed by cervical dislocation. Upon euthanization, the reproductive tissues of the mice, the uterus and ovaries, were harvested. Ten ovaries from five different wild type mice were obtained and ten ovaries from five different knock out mice were obtained. Upon harvest, tissues were fixed in 10% paraformaldehyde solution for 24 hours and then placed in phosphate buffered saline (PBS).

2.2 Embedding

The tissues were then quickly embedded in paraffin wax in a process known as embedding. This process hardens the tissue which makes it much easier to cut sections from. The paraffin wax was mounted onto cassette's that were then labeled and stored in the fridge at 38º Fahrenheit.

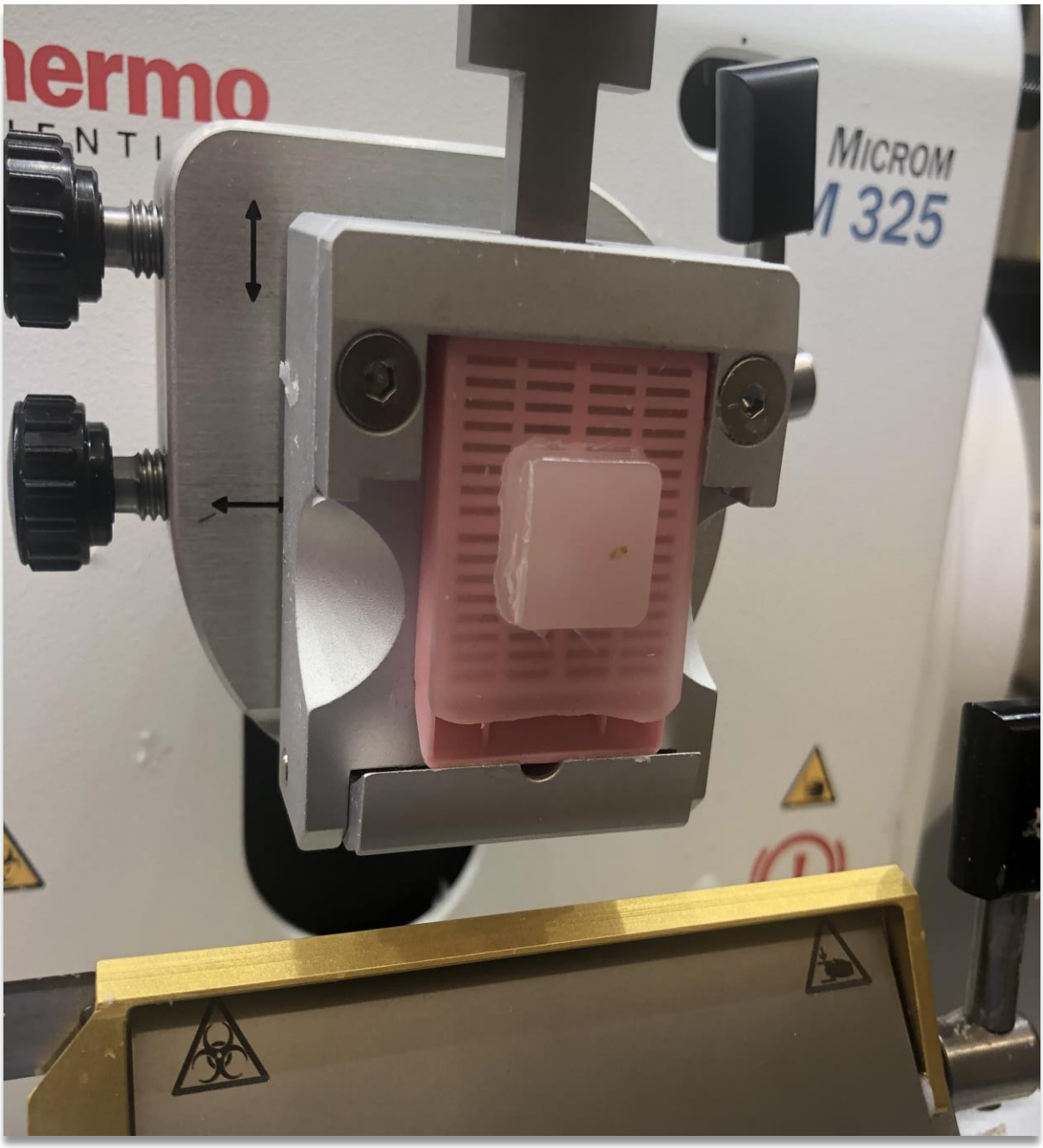


Figure 4. Fixation of ovarian tissue sample placed in microtome ready for sectioning.

2.3 Sectioning

Next, the tissues were sectioned using a microtome. A microtome is an instrument that is used in the lab to cut extremely thin slices of material, known as sections. As the lever of the microtome is rotated, the microtome moves the tissue samples over a small blade, producing micrometer sectioning's. Three sequential, five micrometer sections were obtained from each ovary that was harvested. In total, thirty tissue samples were obtained.

Figure 5. Microtome instrument utilized to section ovarian tissue samples.

2.4 Wet Mount

Following sectioning, the tissues were then transferred to the Leica HI1210 water bath. The water bath was set to 48º Celsius. This temperature is slightly lower than the temperature of the wax, 55º Celsius. This water bath is used to stretch the tissue section, which prevents wrinkling of the section. Each section was placed in the water bath for approximately three seconds. After three seconds, the tissue sample was then mounted onto a microscope slide. The microscope slides utilized in this experiment were Poly-L-Lysine coated. These coated microscope slides have shown to be most effective in promoting adhesion of sections. To mount the tissue onto the slide, the slide was inserted under the tissue at a 45º angle. Once the top of the tissue was attached to the slide, the microscope slide was tilted to a vertical position and was lifted upright out of the water in one motion. The outer edge of the water bath machine provides a thermal surface. The slides were then left overnight on the thermal surface of the water bath to dry.

Figure 6. Leica HI1210 water bath with a surface that provides high thermal conductivity rates.

2.4 Staining

In order to deparaffinize and stain the tissue samples, a twenty-step staining method was performed. To begin this technique, the tissues were placed in an incubating oven at 55º Celsius for 10 minutes to slightly increase the temperature of the wax. To deparaffinize the slides, the slides were submerged in three separate Histoclear baths. The slides were submerged in the first Histoclear bath for 5 minutes, the second Histoclear bath for 4 minutes and the third Histoclear bath for 4 minutes. Following the Histoclear submersions, the slides were rehydrated by incubating them in decreasing EtOH baths. In order to do this, the slides were placed in 100% alcohol for 1 minute and then submerged in a second bath of 100% alcohol for 1 minute. Next, the slides were submerged in 95% alcohol for 1 minute. After this, the slides were placed in a water bath for 1 minute and 30 seconds. The type of water used was tap water.

To stain the tissue samples blue, the staining agent Hematoxlin 560 was used. The tissue samples were placed in the Hematoxlin stain for 2 minutes. Following this, the slides were placed back into the water bath for 1 minute. After the water bath, the slides were submerged in Define™ for 1 minute. The concentration of Define™ used was 1 capful of the Define™ with 250 milliliters of 70% EtOH. Next, the tissue samples were submerged in the water bath for 2 mins. Following this water bath, the slides were submerged in another staining agent, Blue Buffer 8, for 1 minute and 30 seconds. The concentration of Blue 8 used was 2 grams of blue 8 with 100 milliliters of 70% EtoH. Following this the slides were placed into the water bath for 1 minute and 30 seconds. The slides were then placed in 80% alcohol for 1 minute.

Next, the slides were submerged in alcoholic Eosin Y 515 for 1 minute. After the Eosin submersion, the slides were submerged in 95% alcohol for 1 minute, followed by a

submersion in 100% alcohol for 1 minute. The slides were submerged in a second bath of 100% alcohol for an additional minute. The slides were then submerged in two final Histoclear baths; the first Histoclear bath for 1 minute and the final Histoclear bath for 1 minute. Histoclear is used in this technique to stain the nuclei of the cells blue. Eosin is used to stain cytoplasmic components pink. Eosin is also used to stain erythrocytes bright red and other eosinophilic structures red, orange, and pink. All dyes and concentrates utilized were from the brand Leica™.

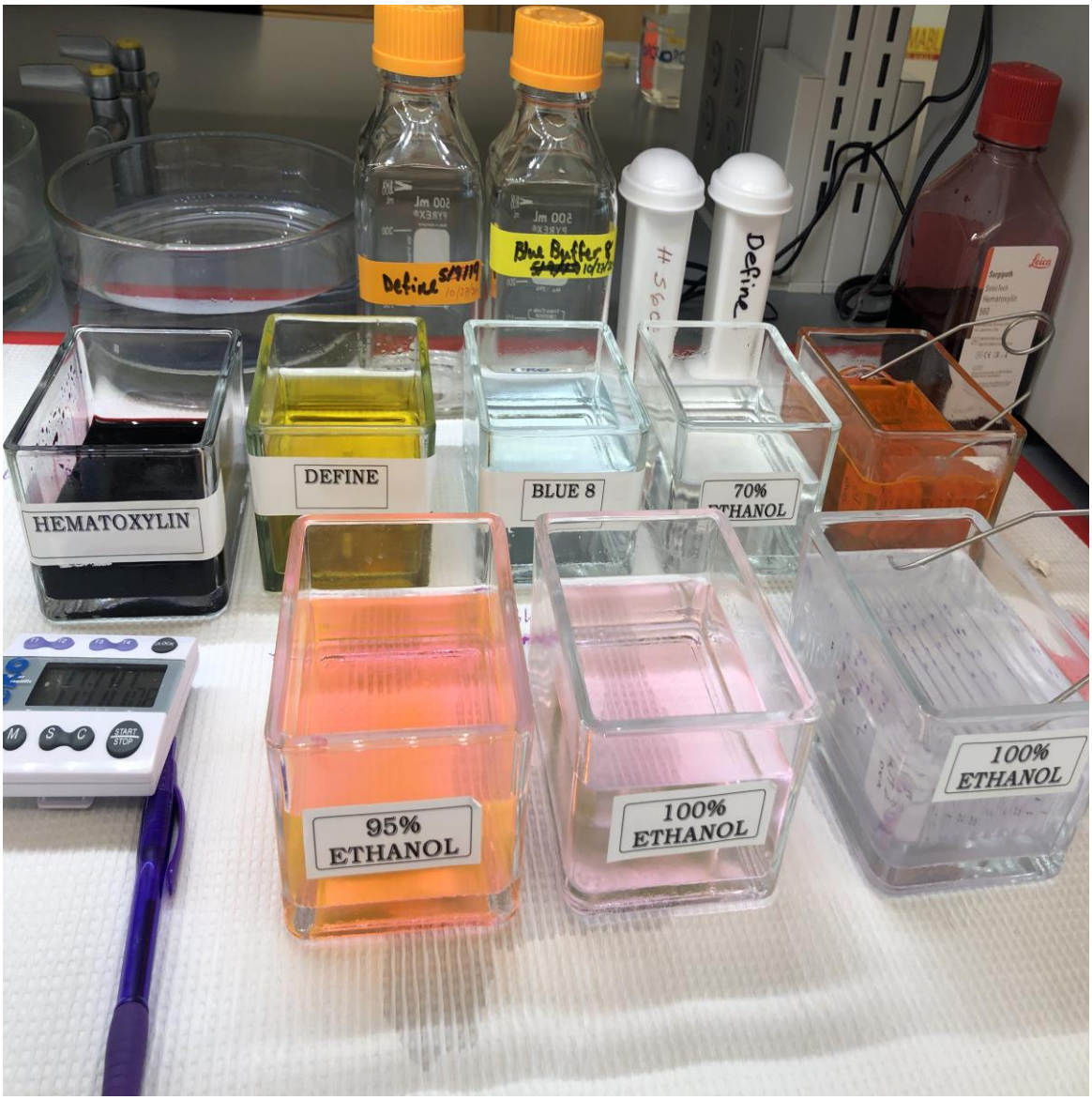


Figure 7. Depiction of the dyes and solutions used to deparaffinize and rehydrate the tissue samples.

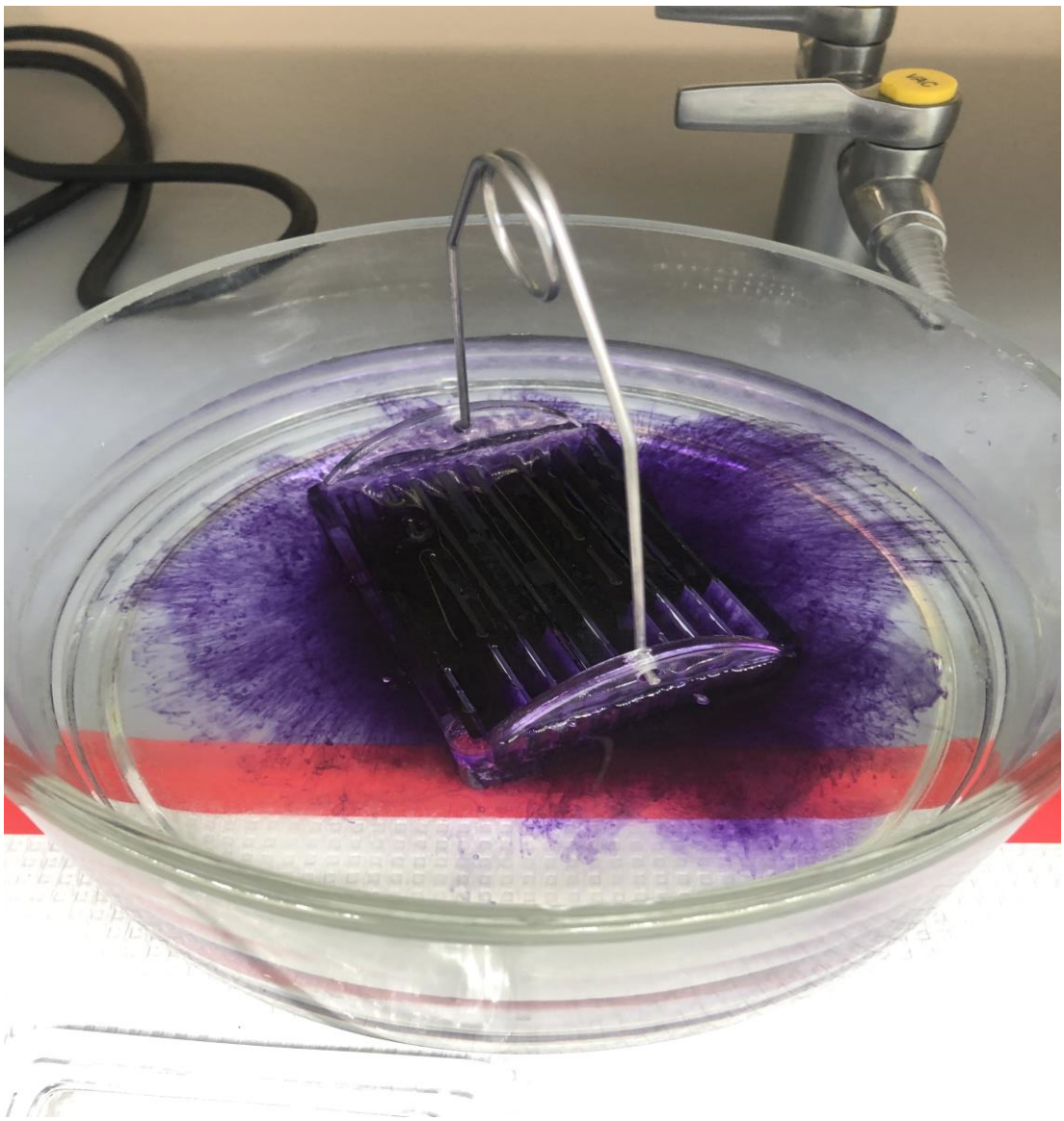


Figure 8. Depiction of the slides in the water bath after Hematoxlin submersion. This step is used to rinse the dye and rehydrate the tissues.

2.5. Mount in Permount and coverslip

Finally, Permount was used to secure a microscope slip over the microscope slides. This mounting medium allows for rapid mounting and long-term storage of slides. To secure a microscope slip over the slides, one drop of permount was placed on each tissue sectioning; a total of three drops of permount per microscope slide. Once the permount was placed onto the tissues, a microscope slip was placed at an angle and lowered slowly

onto the surface of the microscope slide. This technique prevents bubbling under the microscope slip. The microscope slides were left untouched for 48 hours to allow the permount to fully dry before microscopic analysis.

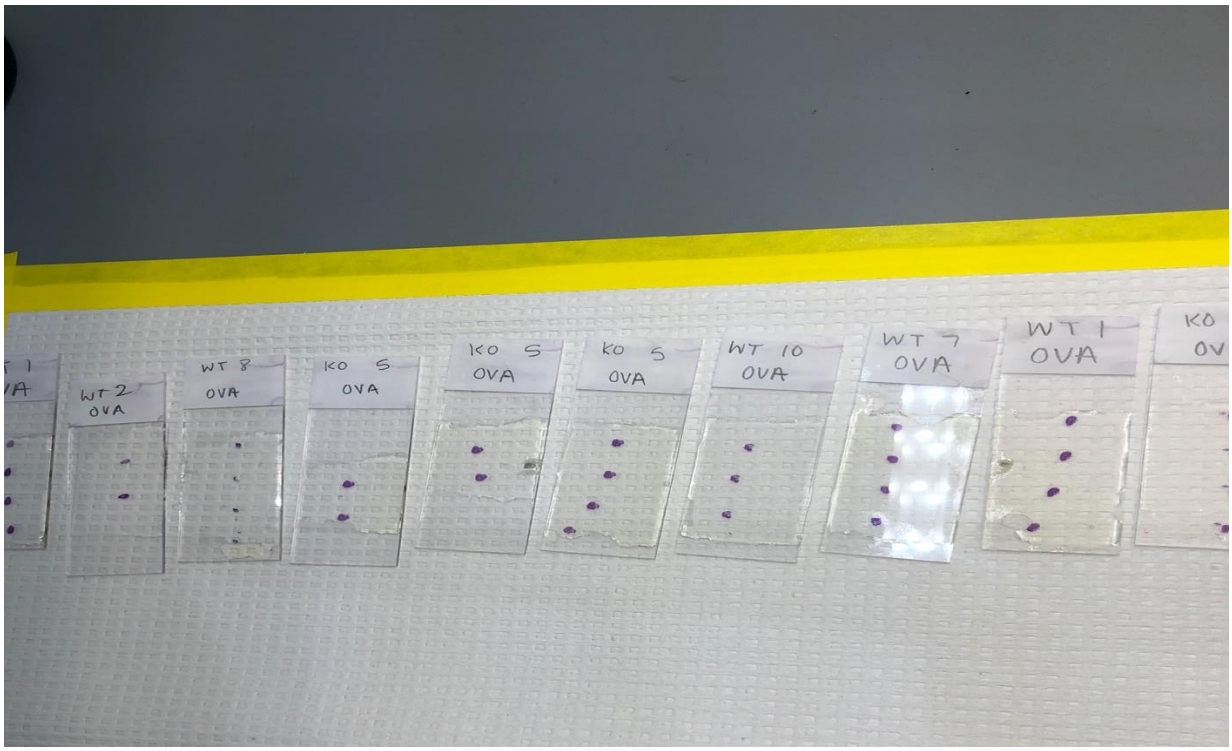


Figure 9. Microscope slides mounted in permount, ready for microscopic analysis. Permount is depicted in the photo as the liquid surrounding tissues.

2.6 Microscopic Analysis

Once the permount fully dried, the slides were ready for microscopic analysis. Slides were analyzed using an electron light microscope. A microscope camera was employed to obtain a better visualization of slides and for data collection.

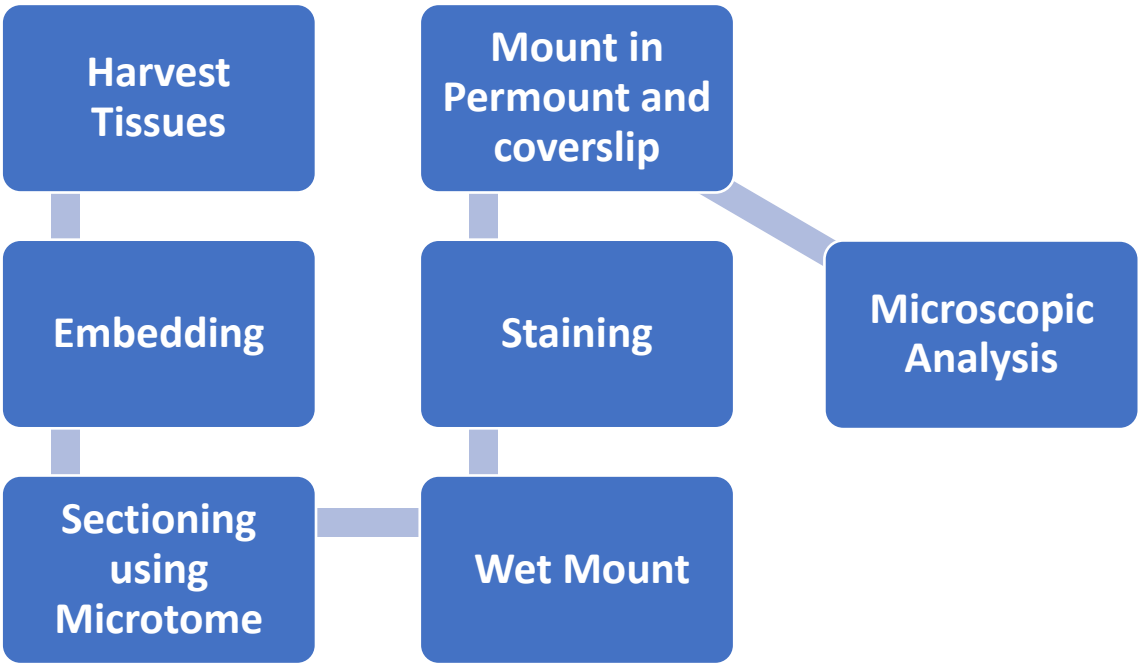


Figure 10. A comprehensive chart of the process of methods taken to reach microscopic analysis of ovarian tissues.

Results

3.1 Follicles Identified in Wild Type Ovary

Five different wild type ovaries were analyzed under a microscope. The number of primary follicles, secondary follicles, antral follicles and corpus luteum was analyzed. The histology of these structures were analyzed as well.

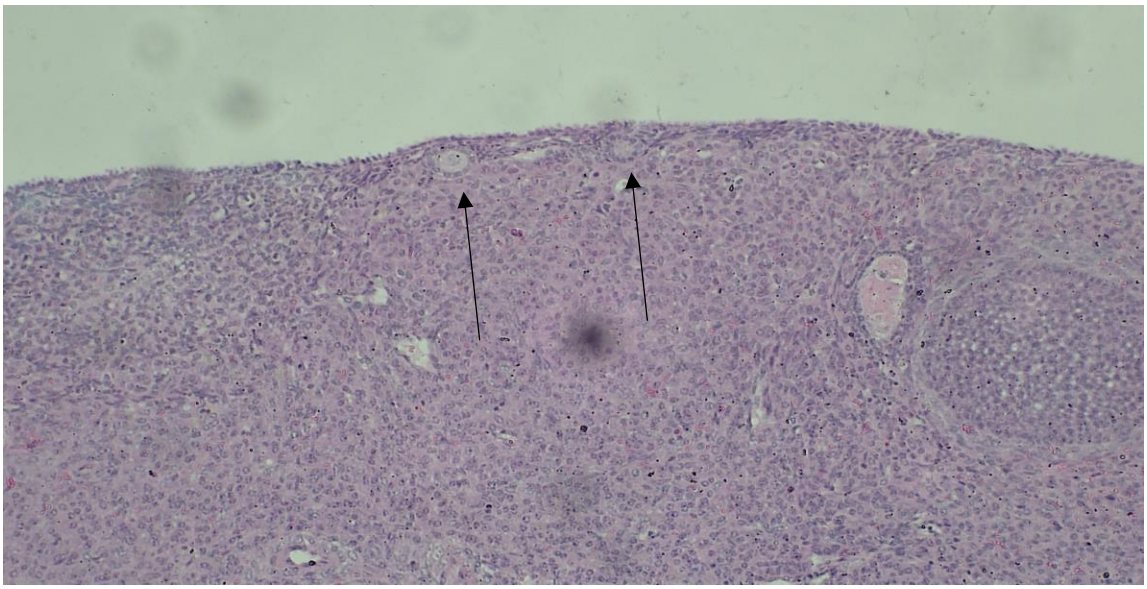


Figure 11. Example of primary follicles identified in the wild type mouse ovary.

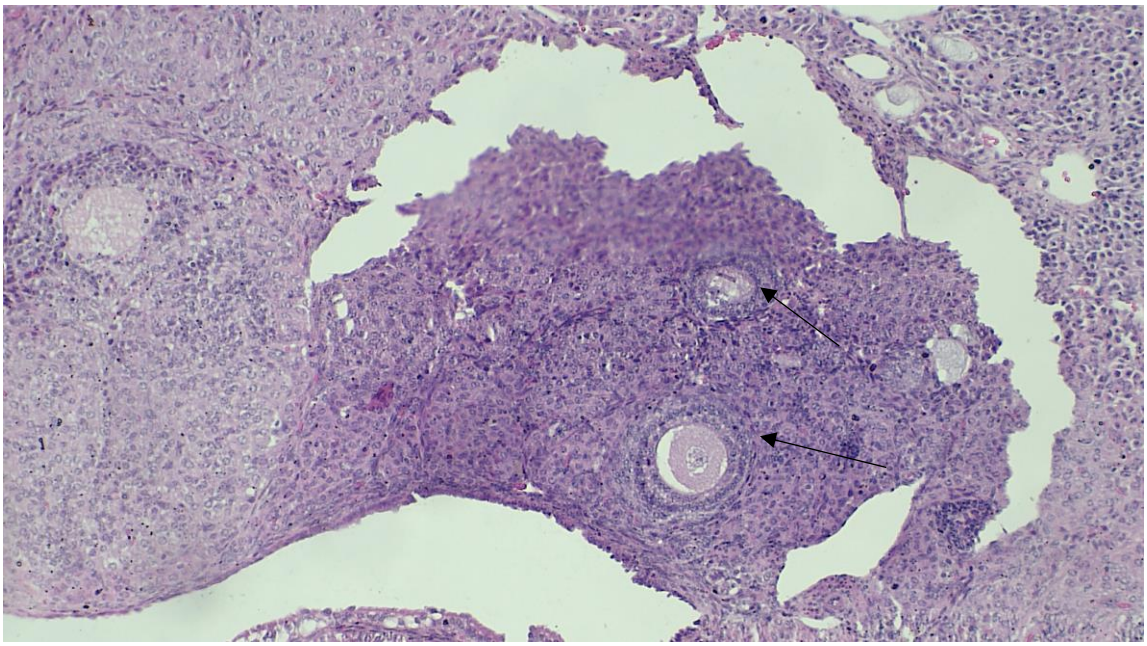


Figure 12. Example of secondary follicles idenfied in the wild type mouse ovary.

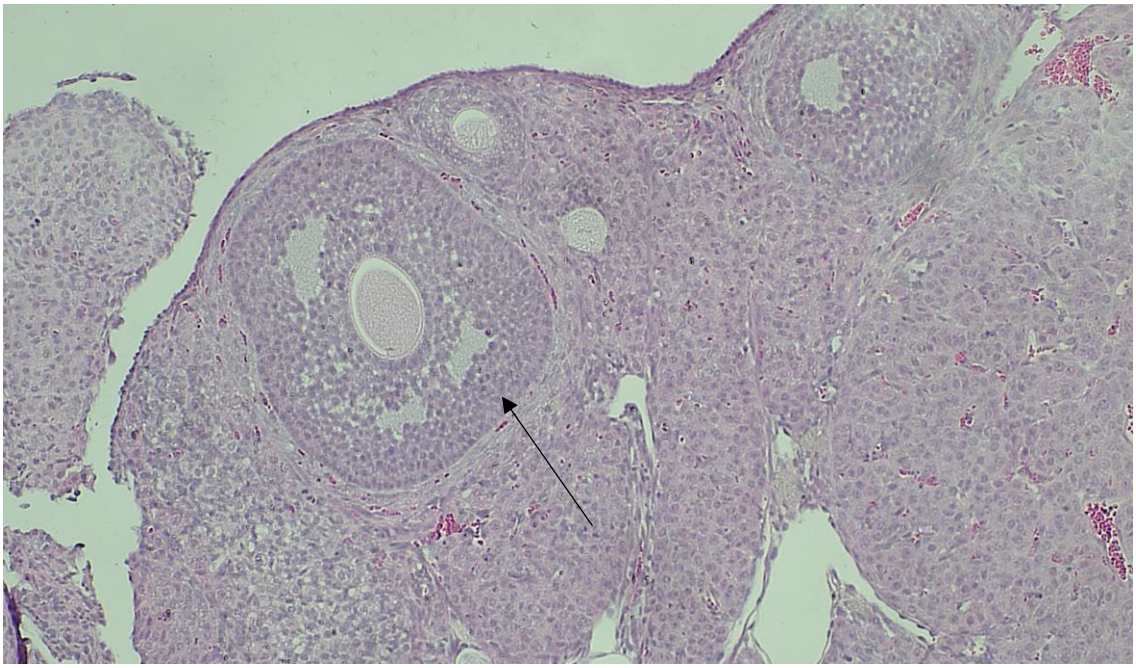


Figure 13. Example of an antral follicle identified in the wild type mouse ovary.

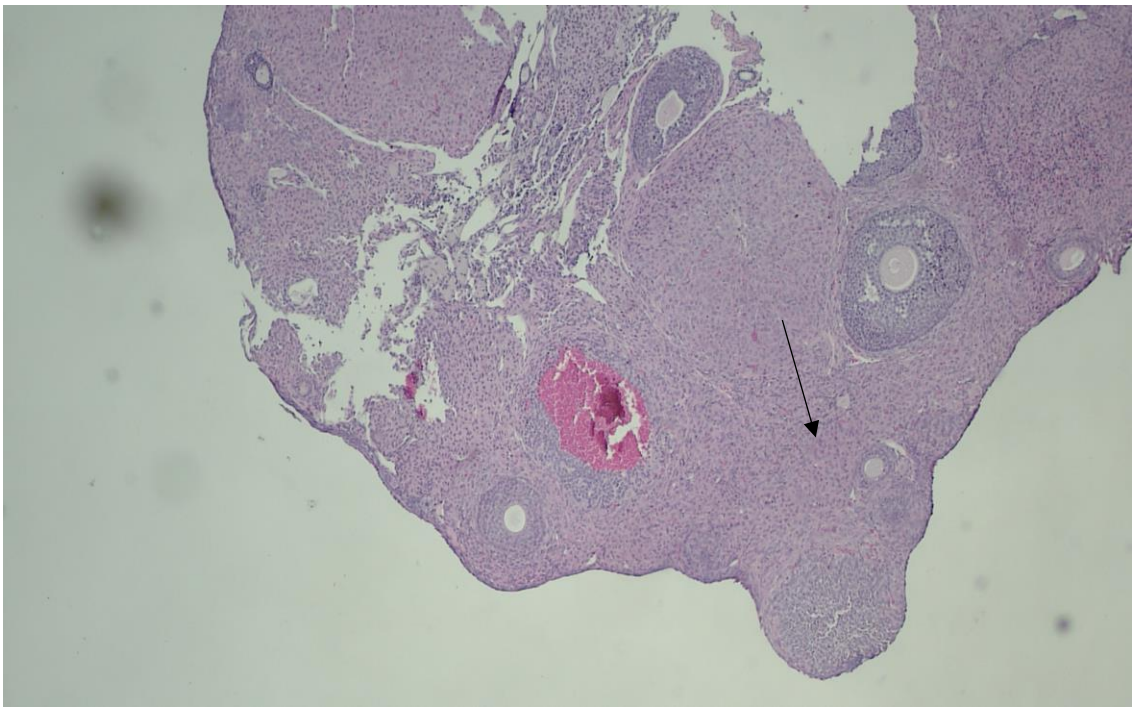


Figure 14. Example of a Corpus Luteum identified in wild type mouse ovary.

3.2 Average Follicles in WT Ovary

The data collected indicated that on average, there were 3.37 primary follicles, 5.11 secondary follicles, 0.81 antral follicles, and 2.39 corpus luteum in the wild type mouse ovary sectionings (Table 1).

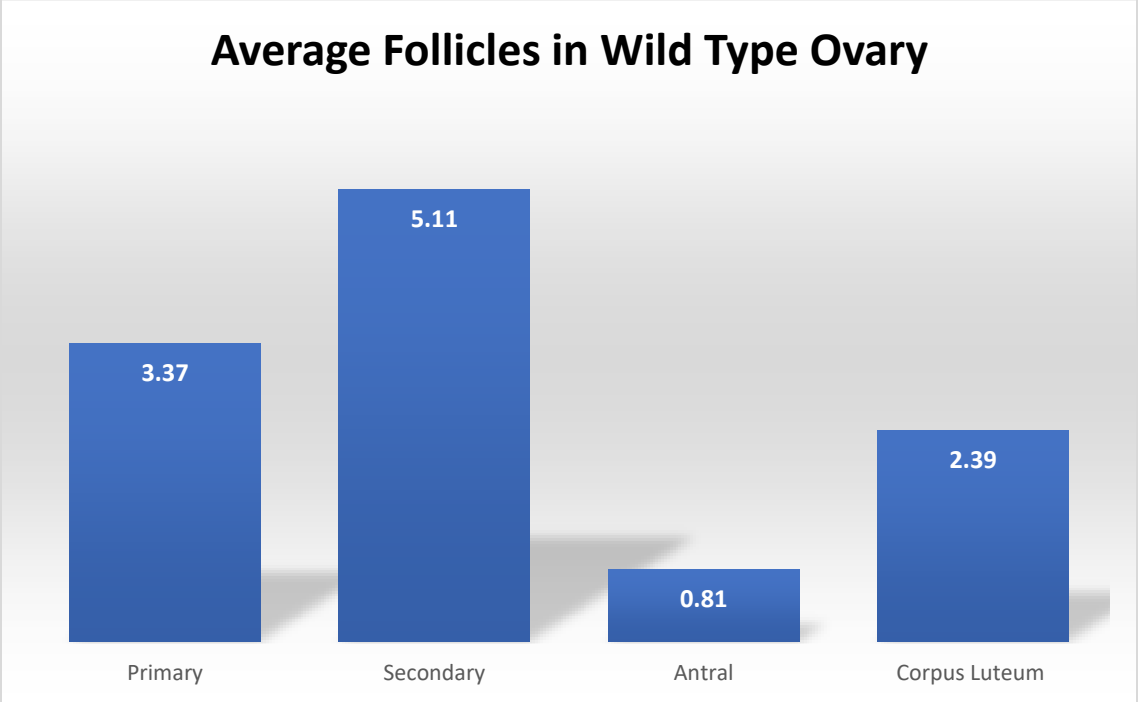


Table 1. Average number of follicles found in the wild type mouse ovarian samples.

3.3 Follicles in KO Ovary

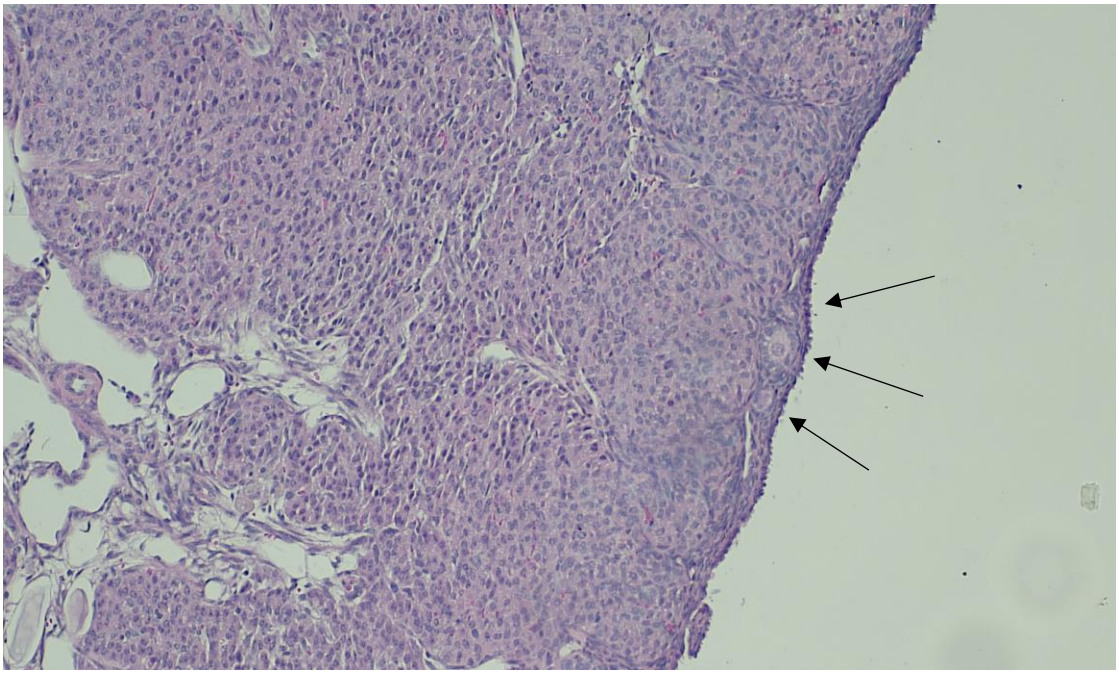


Figure 15. Example of primary follicles identified in the knock out mouse ovary.

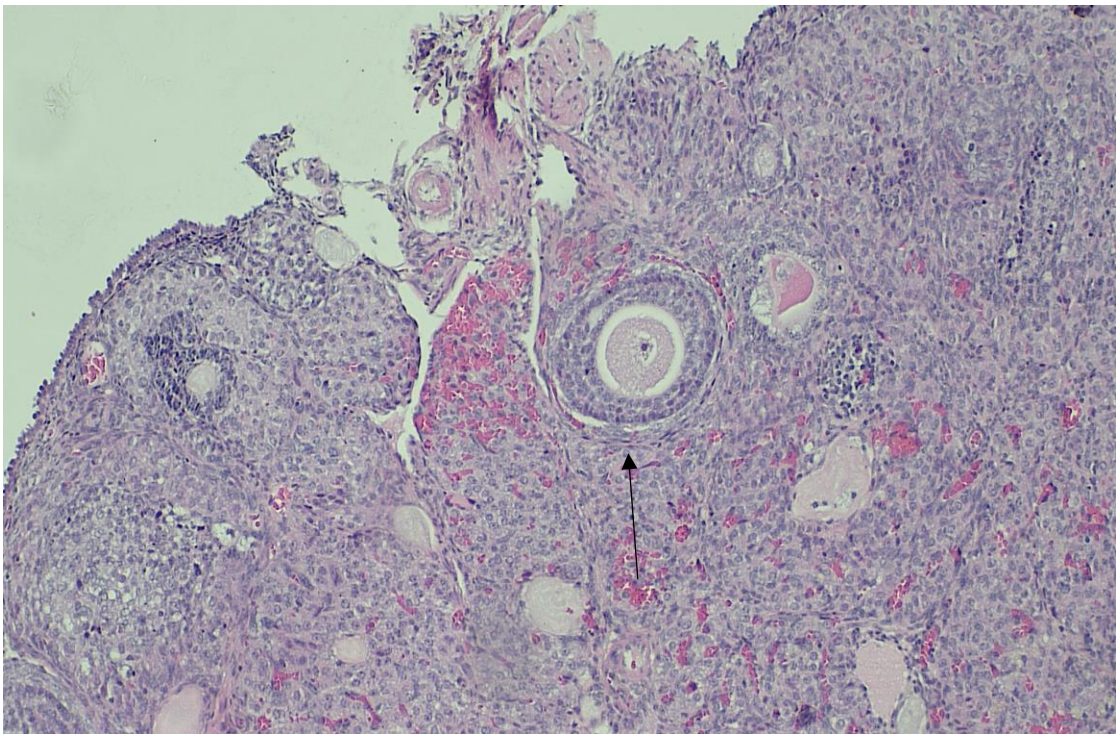


Figure 16. Example of secondary follicle identified in the knock out mouse ovary.

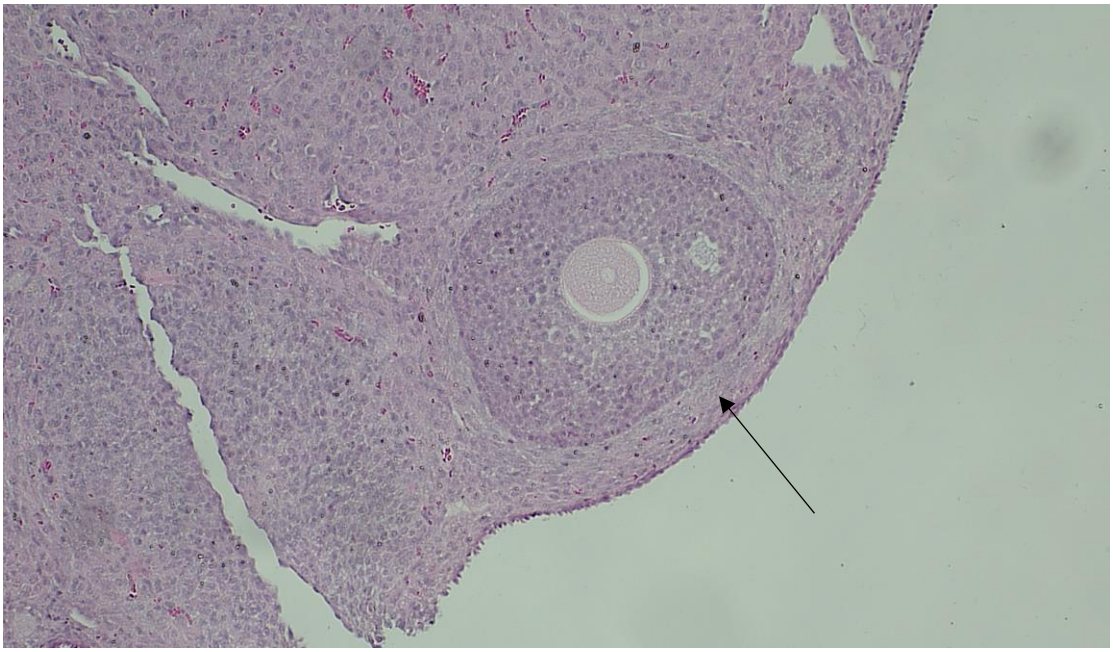


Figure 17. Example of an antral follicle identified in the knock out mouse ovary.

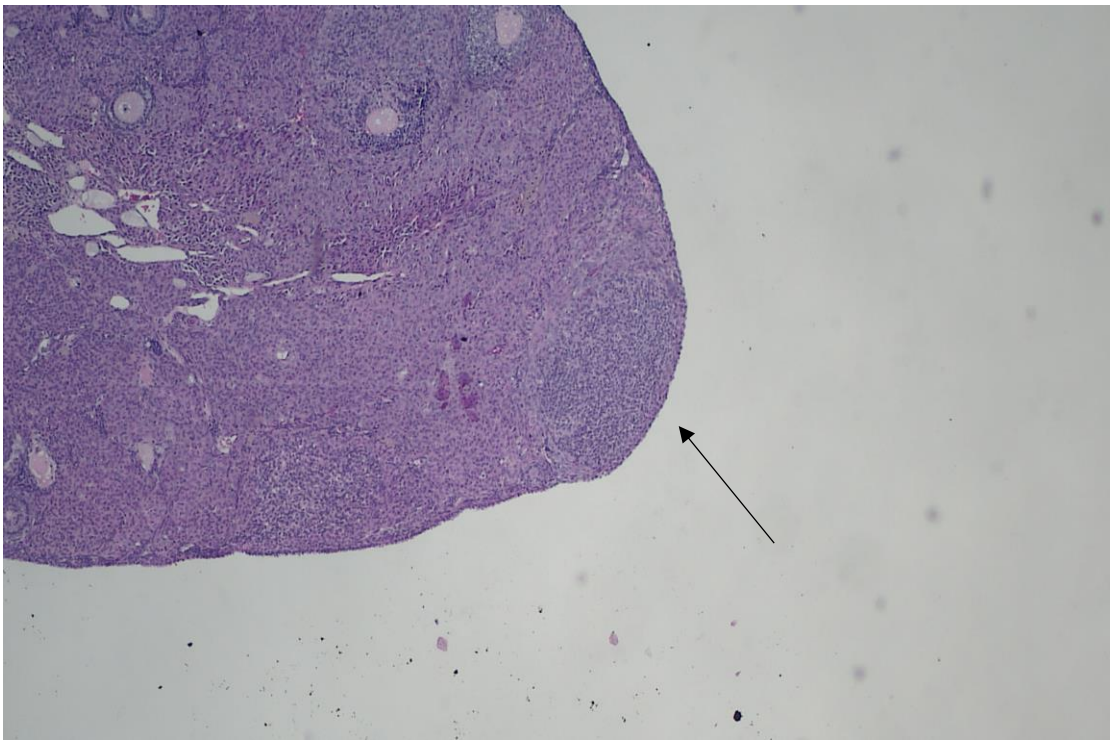
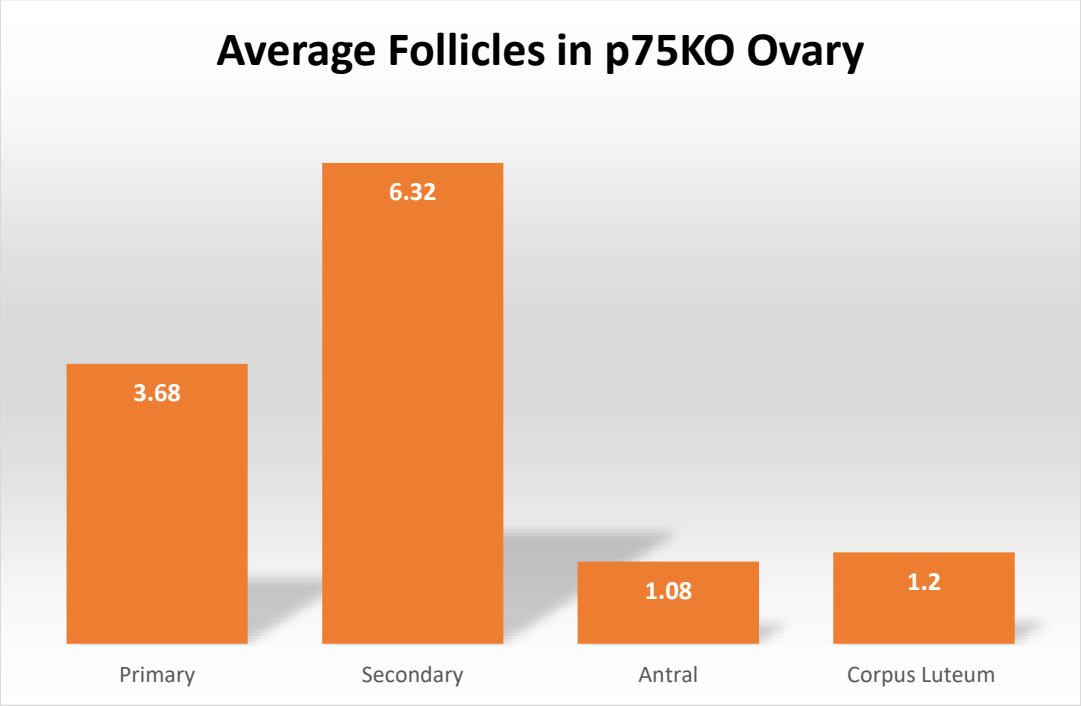


Figure 18. Example of a corpus luteum identified in the knock out mouse ovary.

3.4 Average Follicles in p75KO Ovary

The data collected indicated that on average, there were 3.68 primary follicles, 6.32 secondary follicles, 1.08 antral follicles, and 1.2 corpus luteum in the p75 knock out mouse ovary sectionings (Table 2).



3.5 Average follicles in WT Ovary compared to KO Ovary

The relationship between the average number of follicles in the wild type ovaries compared to the p75 knock out ovaries is visualized in Table 3.

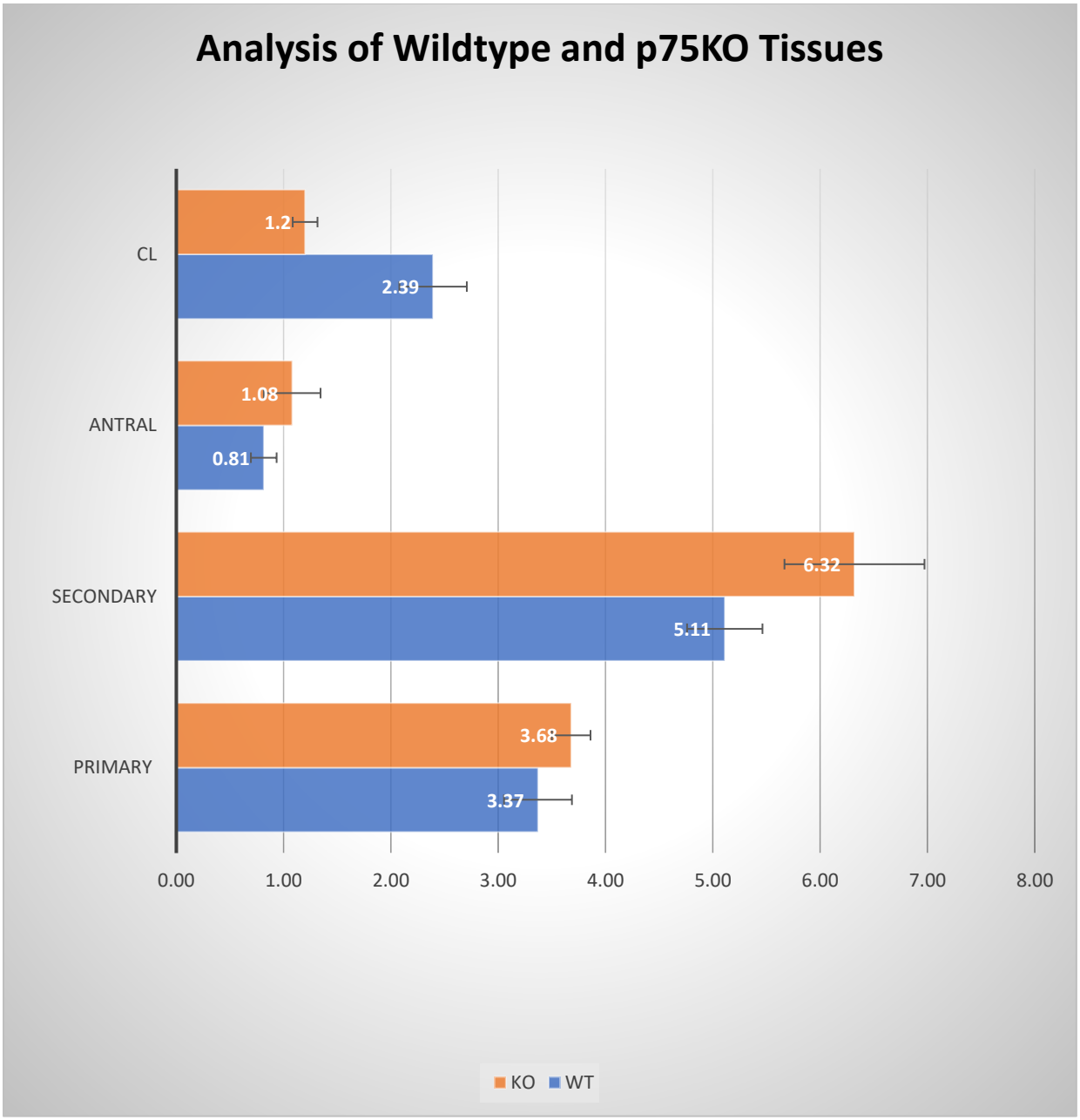


Table 3. Visualization of the relationship between the average number of follicles in the wild type tissues compared to the average number of follicles in the p75KO ovaries.

3.6 Table of Wild Type and KO Data Analysis

The standard deviation, standard error, and mean for each follicle type of the WT ovary is reflected in Table 1.

Wild Type Ovarian Tissue			
Follicle Type	Mean	Standard Deviation	Standard Error
Primary	3.37	1.644	0.316
Secondary	5.11	1.826	0.351
Antral	0.81	0.622	0.119
Corpus Luteum	2.39	1.663	0.314

Table 1. Standard deviation, standard error, and mean of wild type ovarian follicles.

The standard deviation, standard error, and mean for each follicle type of the knockout ovary is reflected in Table 2.

P75KO Ovarian Tissue			
Follicle Type	**Mean**	**Standard Deviation**	**Standard Error**
Primary	3.682	0.900	0.180
Secondary	6.322	3.262	0.652
Antral	1.080	1.320	0.264
Corpus Luteum	1.202	0.577	0.115

Table 2. Standard deviation, standard error, and mean of p75 knock out ovarian follicles.

3.5 Tables of significance

Utilizing the data analysis software tool on excel, a t-test was performed on the data collected and the results are reflected in Table 3.

Significance	
p-value (one-tail)	0.393
p-value (two-tail)	0.786

Table 3. Significance levels of the wild type ovary data compared to p75 knock out ovary data.

Utilizing the data analysis software tool on excel, a t-test was performed on the data collected and the results are reflected in Table 4.

Significance	**P-value**
Primary Follicle	0.456
Secondary Follicle	0.093
Antral Follicle	0.462
Corpus Luteum	0.006

Table 4. Significant levels of the wild type ovarian data compared to the p75 knock out ovarian data when each follicle type is analyzed.

Discussion

4.1 Discussion

The objective of this study was to determine the role that p75NTR plays in ovulation by analyzing the histology of wild type mouse ovaries and comparing that to the histology of p75KO mouse ovaries. As previously mentioned, structure is paramount to function, so the objective was to determine the function based upon structural similarities or anomalies between the p75 knock out mice and wild type mice. Prior to conducting the experiment, it was expected that a larger number of mature follicles would be observed in the wild type mice compared to the p75 knock out mice. However, this was not fully reflected in the data collected. As the data suggests, there were similar averages of mature follicles in the wild type mice compared to the p75KO mice.

On average there were 3.37 primary follicles, 5.11 secondary follicles, 0.81 antral follicles, and 2.39 corpus luteum in the wild type ovary sectionings (Table 1). The data collected indicated that on average, there were 3.68 primary follicles, 6.32 secondary follicles, 1.08 antral follicles, and 1.2 corpus luteum in the p75 knock out ovary sectionings (Table 2). This information suggests that p75KO mice are producing similar number of primary and antral follicles compared to the wild type mice. Interestingly, the p75KO mice had a higher average of secondary follicles compard to the wild type mice. Secondary follicles are considered pre-antral, however the secondary follicles identified in the p75KO ovaries were abundant and mature. Also interestingly, a higher average of corpus luteum were identified in the wild type ovaries compared to the p75KO ovaries. Corpus Luteum are produced only after ovulation has occurred; this information indicates that the wild type mice are in fact ovulating more than p75KO mice.

Utilizing data analysis software from excel, the p-values of the data collected were determined. The significance of the data is portrayed by the p-value. This information suggests if the data collected and the relationship analyzed holds significance. In order for a numerical value to be considered significant it must be found to be less than or equal to 0.05. As Table 4 shows, the only data that portrayed significance was the amount of corpus luteum present in the wild type ovary compared to the corpus luteum present in the p75 knock out ovary. The p-value for this relationship of corpus luteum was determined to be 0.006. This significant relationship indicates that the wild type mice were ovulating at higher rates compared to the p75KO Mice. The p-values determined for primary, secondary, and antral follicles were determined to be greater than the significance level. Interestingly, the data suggests that the p75KO mouse follicles seem to be making it to a mature stage, as they had very high numbers of secondary follicles. However, the p75KO had low numbers of antral follicles and corpus luteum formed which indicates that these deficient mice are failing to make it to the pre-ovulatory, antral stage. This means that the p75KO mice are struggling to make it to ovulation. Data suggests that this struggle may be due to the lack of the p75 gene.

The mice in this study were subjected to natural ovulation, meaning that they were allowed to naturally ovulate for eight weeks. Most studies of follicular development subject the mice utilized in the experiment to a super ovulation technique. This means that the mice are injected with specific human gonadotrophin hormones to stimulate folliculogenesis and ovulation. This technique was not utilized in this study, which could have impacted the results. However, some suggest that this super ovulation technique

overstimulates the ovary causing inaccurate results of follicular development (Van der Auwera, 2001).

The results of this study are parallel to a study conducted by Huang and collaborators. In this study, the lack of neurotrophins present in the mice studied caused a defect in luteal development (Huang, 2001). This supports that this signaling pathway is therefore crucial to the determination of female reproductive lifespan (Spears, et al 2003). Also pertaining to $p75^{NTR}$, a study conducted found that TrkB plays an important role in survival of germ cells in mouse and human ovaries during follicle formation. $p75^{NTR}$ modulates the cellular response to neurotrophins by enhancing sensitivity of the trk receptors (Hantzopoulos 1994).

It has been identified that reduced levels of $p75^{NTR}$ have major effects in the nervous system and cause myelin thickness and thermal sensitivity (Nikliaon-Chirou, 2013). $p75^{NTR}$ is expressed strongly in the peripheral nerve, the most important for nerve development. Similarly, neurotropins and p75 are expressed strongly in the ovary (Meeker, 2015) So, in the absence of $p75^{NTR}$ it makes sense to see reduction in ovulation in the p75KO mice. This relationship impacts follicular development negatively.

This research could have an important impact on the study of fertility in women. Infertility is an issue that affects millions of women each year. Treatments are available for infertility; however, none of these treatments are actively targeting neurotrophins or neurotrophin receptors. The results of my study and the implications of this type of research is important as it broadens fertility research and reveals the role that $p75^{NTR}$ has on follicular development and ovulation. Fertility drugs used to treat infertility generally work like natural hormones, follicle stimulating hormone (FSH) and luteinizing hormone

(LH) to trigger ovulation. Fertility drugs have negative effects including side effects such as ovarian hyperstimulation syndrome (OHSS). OHSS causes the ovaries to become overstimulated which causes swollen, painful ovaries and other painful symptoms. Fertility drugs increase the risk of ovarian tumors and in severe cases, cancers. Targeting $p75^{NTR}$ or p75 gene rather than overstimulating the ovary with hormones could offer a safer and more effective fertility treatment in the future.

The results of my study were not completely parallel to what I expected to observe. There were some limitations to this study that could have affected the hypothesized outcome. Firstly, three sequential, five micrometer sections of the ovarian tissues were taken from each ovary. For a more complete profile of the ovary and the follicles within, the entire ovary could be sectioned. Sectioning the entire ovary would allow for more accurate follicle profile for each ovary. Further, the oocytes produced in the mice were not analyzed in the realm of this study. If this research were to be repeated, the number of oocytes produced in wild type mice compared to p75KO mice could be analyzed. This relationship would provide more insight on if the p75KO mice are developing to maturity and ovulation. Additionally, is important to consider that I have not been trained professionally in these laboratory techniques that were performed in this study, so human error as well as uncontrolled variables could have altered the results of this study as well.

Conclusion

The $p75^{NTR}$ is playing an active role in ovulation; however, the specific function was unable to be determined in the realm of this particular study. This study provided interesting insight into the role that $p75^{NTR}$ is playing in folliculogenesis. The wild type mice in this study had more corpus luteum present in their histology indicating a

significant relationship (p=0.006). This relationship indicates that the wild type mice ovulated more compared to the p75KO mice. The fields of biology, histology, and embryology continue to grow and reveal interesting relationships within the human body every day. Studying the $p75^{NTR's}$ role in ovulation could provide more insight into function and biology of the body and perhaps could provide more effective fertility treatments in the future. Based upon the results of my study and support from scientific literature, the $p75^{NTR}$ could help unlock the mysteries of infertility.

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