Faculty of Veterinary Science, Szent István University

DEPARTMENT AND CLINIC OF REPRODUCTION

Endocrine function of fallopian tube in cat

Miltiades Ioannou

SUPERVISOR: DR. THURÓCZY JULIANNA PH.D.,
Department and Clinic of Reproduction

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Summary

A great deal is known about the internal functions of the oviduct for example how the oviduct can assist the oocyte with maturation in order to be ready for the spermatozoon or it can alter the morphology of the spermatozoa in order to prepare them for fertilization. Despite that, research has just scratched the surface on finding out how the oviduct can work as an endocrine organ and secrete hormones it produces in the blood rather that in its lumen. The hormones it produces can provide assistance in the reproductive system function but they can also give rise to many endocrinological diseases such as pyometra. The oviduct is able to produce many substances such as glycoproteins, glucose, growth factors, prostaglandins, progesterone, estrogen and much more. In order to find out if these substances are excreted in the blood as well as finding out the concentration of progesterone and oestrogen in the serum before and after ovariohysterectomy ELISA was carried out. The progesterone and oestrogen in the oviduct as well as the ovaries was measured in the tissues homogenate using ELISA kits. 37 sexually mature, clinically healthy cats were chosen at different stages of their estrus cycle and different ages. The values of progesterone in the serum were significantly higher before the operation than after ($p<0.005$). Progesterone and oestrogen concentrations were higher in the oviduct compared to the serum. Marked differences were seen between our results when the cats sampled were pregnant or where sampled during estrus. The results of this experiment indicate that the concentrations of feline progesterone assayed with ELISA are correlated strongly in the serum but they are weak when investigating their correlation between the oviduct and serum. Furthermore, the oviduct besides its roles in capacitation, maturation of the oocyte, fertilization, and transport of the zygote does not have a significant endocrine function compared to the ovaries and the adrenal glands, when hormones are concerned it acts mostly in an exocrine way.

1. Introduction

Gabriele Fallopius published the first anatomical description of the mammalian oviduct in 1561 (Johnson & Foley, 1974). It is the tubular organ which connects the uterus with the ovaries. The oviduct has two major segments which are the ampulla and the isthmus which vary both in structure and in function (Croxatto, 2002). The main function of the oviduct is to receive the oocytes from the ovary and to provide an optimal environment for the maturation and transport of oocytes, sperm capacitation, fertilization and early embryonic development. After all this it will deliver the embryo to the endometrium at the right time for implantation (Croxatto, 2002).

The female reproductive tract is a target of many hormones. Steroid hormones such as oestrogen and progesterone have a role in the development of secondary sexual characteristics in both male and female animals, regulating the oestrus cycle in the female as well as stimulating the production and release of other hormones such as follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Mooney, 2004).

Here we focus partially on the role these steroid hormones have on controlling the functions and morphology of the oviduct. There has been extensive research on how the oviduct reacts to the stimulation of these hormones and the localization of the hormonal receptors.

Oestrogen in females can be produced mainly by the ovaries and in fewer amounts by the adrenal glands and liver. Progesterone is also majorly produced by the ovaries and in a smaller amount by the adrenal glands. Oestrogen and progesterone can also be produced by other organs or organ systems which do not act as endocrine glands but might contain endocrine cells. Some of these are the nerves, fat cells and in our case the oviduct. These cells will receive stimuli and start producing and secreting hormones in the blood (Mooney, 2004).

Only a small amount of research is dedicated to finding out if the oviduct has similar endocrine properties with the ovary and if it is also responsible for the production of oestrogen and progesterone. It was found that different sections of the oviduct have a high activity of sterol dehydrogenase, an enzyme found in tissues which have the ability to produce steroid hormones (Ogunranti, 1992).

The aim of this study is to see if the oviduct of the cat can act as an endocrine organ and produce progesterone and oestrogen with its own cells and consequently secrete these hormones in the circulation. By doing this we aim to discover if the oviduct is capable of

producing enough of these above mentioned hormones to alter the functions of certain organs and lead to evident changes in the metabolism of cats.

Complications can arise after ovariectomy or ovariohysterectomy if a residue of the ovarian tissue remains in the queen after the operation. This ovarian tissue is mostly a part of the ovary which has extended into the proper ligament of the ovary (Miller, 1995). This ovarian tissue revascularizes and proliferates and thus become functional. The revascularized ovarian tissue can form connections with the actual circulation (Bojrab, 1975).

In order to prove if the oviduct can produce enough hormones to actually play a role in the remnant syndrome we will use the ELISA method to measure progesterone and oestrogen levels in the serum of the cat before and after ovariohysterectomy. The level of hormones in the oviduct and ovaries removed during ovariohysterectomy will be also be measured with ELISA.

By proving that by synthesis of oestrogen and progesterone the oviduct can significantly affect the physiological levels of these steroids in the blood both pre and post ovariohysterectomy we can arrive to the conclusion that this organ plays a significant role in the development of different endocrine diseases. Some of these diseases might be pyometra, reproductive tract tumours (cervical, vaginal, and endometrial) and many others (Okkens *et al*., 1981).

The use of assays in order to find the concentration of hormones always presents certain difficulties because these substances circulate in the blood in low amounts. An additional difficulty is the fact that many different types of hormones are found in the blood.

In our experiment we will discover the concentrations of certain hormones by using specific antibodies that discriminate between hormones and attach to them with high affinity. Nowadays ‘kits’ have been designed and used by endocrine laboratories which are for diagnosing specific hormones (Mooney, 2004).

This study may be used as a reference point for discovering the further endocrine functions of the oviduct as well as diagnosing and or excluding the source of many reproductive tract diseases.

2. Literature review

The uterus and oviducts derive from the Mullerian or paramesonephric ducts. The cranial segments of the bilateral embryonic ducts differentiate into right and left oviducts,

The broad ligaments support the oviduct as well with a section called the mesosalpinx which is a double peritoneal layer which contains blood vessels and nerves (Johnson & Foley, 1974).

The oviduct is divided into four segments: 1) the uterotubal junction is the area of transition between the uterus and the oviduct, 2) the tubal segment of the isthmus which is distal to the uterotubal segment, 3) the ampulla which forms the anterior two-thirds of the oviduct and 4) the isthmus. The last part of the oviduct is the infundibulum which is a dilation of the ampulla and its funnel shaped containing fimbriae (Croxatto, 2002).

The oviduct comprises of three layers: 1) An outer connective tissue coat which is continuous with the peritoneum (tunica serosa), 2) a middle muscular coat which has circular and longitudinal muscles (tunica muscularis) and 3) an inner mucosal coat which forms longitudinal folds and is divided into two layers (tunica mucosa) (Croxatto, 2002).

The two layers of the mucosa are 1) the lamina propria which is highly vascularised, has loose connective tissue, is free from glands and has many lymph vessels. 2) Lamina epithelialis is made of columnar epithelial cells which are divided into two main cell types which are the ciliated and the non-ciliated. Half of the total number of cells throughout the oviduct are ciliated cells and are found mostly at the anterior of the tube. Non-ciliated secretory cells are found most often in the oviduct and are mostly seen in its posterior part (Johnson & Foley, 1974).

The oviduct receives its blood supply from ovarian and uterine arteries. The uterine artery gives blood to the isthmus. The ovarian artery supplies the ampulla. Venous plexuses are located in all 3 layers of the oviduct (Croxatto, 2002).

Lymphatic vessels are found in bigger numbers in the isthmic region rather than the ampullary region. There are three lymphatic networks each draining one layer of the oviduct, they all drain into a main vessel found in the mesosalpinx and ultimately drain in the para-aortic nodes (Johnson & Foley, 1974).

The reproductive processes which take place in the oviduct span for 1-10 days in most mammals. Studies have shown that spermatozoa incubated in ampullary fluid gain higher velocities and their movement to the oocyte is much more linear (Grippo *et al*., 1995).

The isthmus is believed to have a role in capacitation, the acrosome reaction and acting as a reservoir, this has been proved by studies which resulted in finding higher calcium concentrations and other proteins in isthmic luteal fluid which are required for the acrosome reaction and capacitation (Grippo *et al*., 1995). Research done on bovine oviducts has shown that the capacity of the oviductal epithelium to bind to spermatozoa can be induced by oestrogen and progesterone (Croxatto, 2002).

Transport of the oocyte in the ampulla is helped by the beating action of the cilia and by the contraction of the muscles in the isthmus. Transport to the ampulla takes only a few minutes, whilst the transport forward to the isthmus can take hours or even days. Transport through these structures is under hormonal and nervous control (Croxatto, 2002).

There are receptors found in the myosalpinx for oestrogen and progesterone which are not responsible to cause contractions or relaxations but just mediate the response of the muscular cells to myotropic agents. An example of a myotropic agent can be noradrenalin which causes relaxation or contraction according to the hormone prevailing at the time. Prostaglandins, endothelins, platelet activating factors and steroid hormones can affect the beating of the cilia which are mostly located in the ampulla (Croxatto, 2002).

A hormone is an agent released into the bloodstream that stimulates activity in a different part of the body. The word ‘endocrine’ is to indicate the substances secreted internally into the bloodstream whilst those substances secreted into ducts such as the lumen of the oviduct are called exocrine (Rijnberk & Kooistra, 2010).

Endocrinology deals with the glands that produce hormones and the concentration of these hormones required to elicit action on cells expressing receptors for these hormones. Hormones are not only produced by endocrine glands, they can be also produced by organs whose primary functions are not endocrine but have specialized endocrine cells scattered in them (Rijnberk & Kooistra, 2010).

Hormones may also be activated outside the endocrine organs by enzymatic cleavage. Chemically, hormones can be proteins (including glycoproteins), peptides, amino acid analogues, cholesterol derivatives (steroid hormones are derived from this), or lipids (e.g. prostaglandins). Water soluble hormones such as proteins and peptides can move freely in the

blood whilst insoluble hormones need to be bound to transport proteins (e.g. steroid hormones) (Rijnberk & Kooistra, 2010).

In order for a hormone to initiate its action on a cell it needs to react with the cells receptor. This process begins with the hormone binding on the receptor. Receptors usually have an intracellular and extracellular domain. The intracellular domains are protein structures and consist of: 1) an amino terminal domain that mediates the transcription, 2) the DNA-binding domain and 3) the carboxyl terminal domain that mediates ligand interaction (Rijnberk & Kooistra, 2010).

Steroid hormones need to be transported inside the cell by carrier proteins and then bind to the receptor to form a complex; this regulates transcription and the concurrent increase or decrease in production of mRNA which will lead to regulation of protein production by the cell (Rijnberk & Kooistra, 2010).

The mammalian oviduct is not only a conducting tube. Through its secretions it creates an environment which is optimal for fertilization and early embryonic development by providing nutrition for the gametes, zygote and the blastocyst. The fluids formed in the oviduct can either originate by selective transudation from the blood capillaries, secretion after synthesis from the secretory cells lining the lumen of the oviductal epithelium and they can also originate from follicular fluid (Lauschova, 2003).

Porcine oocytes treated with oviductal fluid had higher cleavage and blastocyst developmental rates compared to untreated oocytes. Due to the results of this study we can somehow hypothesize that the oviductal fluid can protect the embryos from unwanted apoptosis and transcription/replication of its DNA (Aviles *et al*., 2010).

The quality and quantity of this fluid is influenced by circulating steroid hormones which either have a direct effect on the epithelial cells or act on the vascular bed. Oestrogen has a stimulatory action whilst progesterone an inhibitory one, this can also be stimulated by exogenous hormone administration. Additionally this fluid has been found to increase during oestrus and decrease during dioestrus and pregnancy. The ampulla of the cow usually forms around 70% of the secretions whilst the isthmus produces the rest (Aguilar & Reyley, 2005).

Secretory cells have protrusions on their apical part. In mice secretory granules accumulate in these protrusions which eventually are released in the lumen of the oviduct (Lauschova, 2003).

As mentioned above the oviduct can also function as an exocrine organ. The rate, volume and products of secretions greatly vary according to the oestrous cycle and the pre-implantation stages (Beier, 1974). Secretory rates found in the sheep where 0.8mL in 24 hours during oestrus and 0.3mL in 24 hours during the luteal phase (Iritani *et al*., 1969). The secretory rate is 0.8mL per day and it diminishes to 0.14mL per day after ovariectomy indicating that oestrogen affects these secretions (Gott *et al*., 1988).

In sows the oviduct secretion rates start increasing two days before the onset of oestrus and have a peak at the 2nd day of the oestrus with a rate of 15.5mL per hour which lasts longer than ewes (Iritani *et al*., 1974). The same research indicated that the maximum volume collected from the oviduct of sows was 5mL.

Secretory cells in the oviduct of mice produce a transparent, colourless and slightly alkaline (pH 7.7-8.2) fluid (Lauschova, 2003). Compared to the serum the oviduct in humans has higher concentrations of chloride and potassium (Aguilar & Reyley, 2005).

Calcium concentrations are much higher in the isthmus rather than the ampulla and they increase over plasma levels at ovulation (Aitken, 1979). The ratio of potassium and sodium in the oviduct fluid is very important for capacitation (Iritani *et al*., 1974). Bicarbonate ions found in the tubal fluid of the rhesus monkeys are thought to initiate the dispersal of the corona radiata from around the oocyte, its concentration increases after ovulation (Aitken, 1979).

Albumin and transferrin concentrations are similar to those found in the serum. Studies have also shown that a binding protein called uteroglobulin is present in the oviduct and binds to progesterone (Beier, 1974). IgG is also found in the tubal fluid and together with the albumin they make up 95% of the protein content of the tubal fluid (Aguilar & Reyley, 2005).

Certain oviduct specific glycoproteins have been found in the tubal fluid. The secretion of these glycoproteins is initiated by oestrogen and they are predominant during the pre-ovulatory period only in the ampulla assisting in the fertilization (Murray, 1992).

The production of these glycoproteins by the ampulla of the ewe is decreased during the luteal phase due to a surge of progesterone (Murray, 1992). Finally a more recent study done to find the function of these glycoproteins in the oviducts of baboons has proved that they also have a function to promote binding of the spermatozoa on the *zona pellucida* (Jaffe *et al*., 1996).

The zona maturation is a process originally believed to occur only in the ovary during follicular growth and involves maturation of the *zona pellucida*. A recent study has shown that oviduct specific glycoproteins and more specifically OVGP1 are involved in the modification of this extracellular oocyte coat after ovulation (Aviles *et al*., 2010).

Aviles, Gutierrez and Coy (2010) also state that OVGP1 is involved in changing the chemical and biological properties of the *zona pellucida* when it enters the oviduct. Two of these properties are the avoidance of polyspermy and the resistance to protease enzymes.

Passage of the proteins mentioned above in the lumen of the oviduct is via a process of selective transudation as well as endocytosis from the plasma. The majority of these proteins come from the serum transudate of the cow (Roberts *et al*., 1975). In addition proteins can be secreted from the cells in the sheep oviductal epithelium (Gandolfi *et al*., 1989).

Some other proteins secreted by the oviduct are ostopontin, oviductin and atrial natriuretic peptide- A (ANP A). These proteins are secreted in the oviductal fluid and can increase the in-vitro fertilization (IVF) rates and improve early embryonic development in human, porcine and bovine.

Osteopontin and ANP-A has also been confirmed in the oviduct of equines. Osteopontin has the ability to increase cleavage rates and the percentage of blastocysts whilst ANP A can improve the acrosome reaction of spermatozoa (Mugnier *et al*., 2009).

There are certain proteins which are secreted continuously throughout the cycle whilst other proteins are secreted only in certain phases. This can only mean that each protein fraction has a different role in oviductal function and embryonic development (Iritani *et al*., 1974). The usual protein concentration in the oviductal fluid of the ewe is 15-30 mg/mL (Gandolfi *et al*., 1989) which is 10-15% compared to the serum (Aguilar & Reyley, 2005). Protein secretion per day increases significantly at the time of oestrus (Iritani *et al*., 1969).

Co-culture of equine gametes with epithelial cells of oviductal origin can increase the rate of in vitro fertilization (IVF). Oocytes collected from the oviduct have higher chances to succeed in IVF rather than pre-ovulatory oocytes. This is proven by studies that have shown that co-culturing oocytes with oviduct cells before gamete co-incubation increases the IVF rates and also improves the connection of oviductal proteins with the *zona pellucida* (Mugnier *et al*., 2009).

The oviductal fluid also contains glucose, pyruvate and lactate which provide energy and nourishment to the spermatozoa and early embryo. Glucose and pyruvate derive mostly from the blood but 75% of the lactate is produced by the tubal epithelial cells of the oviduct (Aitken, 1979).

These energy providing molecules are found in greater amounts in the ampulla rather than the isthmus. This can be because the ampulla is where the fertilization occurs and these molecules provide energy to the spermatozoa and sustenance to the embryo. Furthermore the glucose concentration in sows decreased dramatically after ovulation (Aguilar & Reyley, 2005).

An experiment carried out by collecting oviductal fluid daily from pseudopregnant rabbits as well as rabbits in oestrus came to the conclusion that estradiol concentrations in rabbit fluid during oestrous and pseudopregnancy were similar to those in serum (Libersky & Boatman, 1995) but progesterone concentrations were similar to those in serum during oestrus but lower during pseudopregnancy (Richardson & Oliphant, 1991).

These steroids might be coming from three sources 1) embryo, 2) serum, 3) oviduct secretory cells. As mentioned with the proteins the steroid concentrations in the oviduct vary according to the stage of the oestrous cycle. As far as estradiol and progesterone receptors in the uterine tube are concerned they increase during oestrus and decrease thereafter, their localization also varies between regions (Aguilar & Reyley, 2005).

Growth factors are also secreted in the tubal fluid and are regulators of cell proliferation and differentiation. They are mostly insulin-like growth factor (IGF)-1 and IGF-2 and are reported to have a role in embryogenesis, growth of the trophoblast and regulating steroidogenesis (Aguilar & Reyley, 2005).

In the sow these growth factors tend to increase in oestrus, increasing sharply at the follicular phase (Aguilar & Reyley, 2005). IGF-1 and IGF-2 receptors have been expressed in the oviduct and are under the influence of steroid hormones especially ovarian oestrogen (Ciftci, 2011).

As mentioned above, growth factors tend to have a role in streroidogenesis. It has been proven that IGF-1 stimulates growth hormone synthesis during the oestrous cycle and pregnancy. Growth hormone in turn will stimulate the corpus luteum to produce progesterone (Ryu *et al*., 2003).

The same study by Ryu *et al* (2003) states that IGF-1 concentrations in goats tend to start increasing two days before behavioural oestrus, reach a peak at oestrus and start decreasing to reach basal values four to five days after oestrus.

In contrast to what is mentioned, the same study goes on to state that despite IGF-1 having a role in the maintainance of pregnancy it also has a luteolytic role. IGF-1 also has the capability of stimulating the synthesis of PGF_{2a}, resulting in the functional regression of the corpus luteum (Ryu *et al*., 2003).

Culturing embryos in vitro with oviductal epithelial cells is a proven method to develop ovine and bovine zygotes to blastocysts. IGF also has receptors in the muscular layer of the bovine oviduct indicating that it might be involved with its contractions (Pushpakumara *et al*., 2002).

The oviductal monolayer secretory cells contain mRNA coding for the production of certain binding proteins which function to transport the IGF, increase their half-life and affect the distribution of these hormones. These proteins tend to increase in concentration during the 18^{th} day of the oestrous cycle and during early pregnancy in the cow oviduct (Winger *et al*., 1997).

Proteins can also be in the form of enzymes. An example of an enzyme produced and secreted by oviduct epithelial cells is glycosidase. These enzymes were initially found in the epididymal fluid and are responsible for sperm maturation. Recent studies have found that this enzyme is also found in the oviductal fluid and is responsible for modifying the glycoproteins found on the *zona pellucida* and the membrane of the sperm cells. Due to this modification the glycosidases can affect the binding of the sperm on the *zona pellucida* (Aviles *et al*., 2010).

The concentrations of enzymes in the oviductal fluid also vary according to the phase of the oestrous cycle. The oviductal fluid in ewes can contain alkaline phosphatase, amylase and glycerophosphoryl choline (GPC)-diesterase which can be involved in sperm metabolism (Iritani *et al*., 1969). GPC-diesterase activity increase by three times during oestrus which shows the vital role of utilization of GPC by spermatozoa (Iritani *et al*., 1974).

Protease inhibitors found in the tubal fluid are thought to be of serum origin, they can inhibit fertilization at all stages of the cycle except the immediate post ovulatory stage and in this way act as contraceptive agents. It is believed that oestrogen is responsible for making this enzyme disappear and make other essential compounds such as calcium or pyruvate appear (Aitken, 1979).

The oviduct of cows is also a site of nitric oxide production. It is produced by the endothelial cells of the vascular bed and induces a continuous contraction and relaxation of the smooth muscle lining the oviduct and in this way it regulates the muscular tone and thus contributes to the reproductive processes of the oviduct (Rosselli *et al*.,1996).

It is believed that tubal fluid formation is modulated by adrenergic agonists and cAMP via trans-epithelial tubal transport. This is coupled by chloride transport as well as calcium transport. This process occurs by different ways such as the activation of sodium-potassium ATPase by chloride ions or the exchange of chloride with bicarbonate ions (Gott *et al*., 1988).

An example of the endocrine function of the oviduct is seen in Johnson & Foley (1974). It describes an experiment on the amount of glycogen in the *biceps femoris* of rats and how it is affected by the reproductive organs. The rats were divided into three groups (sham operation, ovariectomy, ovariectomy and salpingectomy). The results of the experiment show that the oviduct tends to maintain the levels of muscle glycogen at a constant and normal level by maintaining a balance between glycogenesis and glycogenolysis.

An additional experiment is also described in Johnson & Foley (1974). Ten rats are sham operated and ten others have their oviducts disconnected from their ovaries. The result was that the ones which were sham operated had an expected number of corpora lutea on their ovaries whilst the salpingectomized did not have any structures beyond the ones of near-matured Graafian follicles. This shows that without a doubt the oviduct has an influence on ovarian function.

This is proven by a steroid extracted in a similar study which had similar properties to those of progesterone.

The endocrine system affects the oviduct in many ways. The oviduct itself responds to these endocrine stimuli and in its own way it is responsible for synthesizing many different types of hormones. One of these hormones is the luteinizing hormone; studies have shown that the bovine oviduct has LH receptors which are also situated in the blood vessels and smooth muscles of the oviduct (Wijayagunawardane *et al*., 1999). These receptors are expressed by the stimulation of oestradiol and progesterone together.

Progesterone by itself cannot provide a stimulatory effect. Luteinizing hormone is believed to promote the relaxation of the oviduct musculature during the periovulatory stage of the oestrous cycle. The receptors are mostly situated in the ampulla of the oviduct.

With all the information stated above, we can conclude that in order for LH to promote oviduct contractions it needs oestrogen to be present (Gawronska *et al*., 2000). Furthermore LH can increase the activity of cyclooxygenase (COX) -1 and COX-2 activity in the oviduct as well as prostaglandin secretion in endothelial cells and the uterine vein.

Different studies have shown that the contractile pattern of the oviduct is strictly dependent on the phase of the oestrous cycle and more specifically by certain local mechanisms which are in the majority hormones secreted by the oviductal epithelium (Wijayagunawardane *et al*., 2001).

Wijayagunawardane *et al* (2001) then goes on to say that during the luteal phase there is a low amplitude and frequency of contractions of the oviduct musculature. The contractions will increase eventually when the progesterone levels drop in the serum prior to oestrus and will reach a maximum during oestrus. Contractions can be arrested when there will be retention of the ova in the ampullary-isthmic junction and they can be anti-peristaltic in order to facilitate sperm transport to the ampulla.

Embryonic prostaglandin E_2 in equine embryos is known to affect the oviduct by stimulating the oviductal transport. Continuous application of PGE_2 in the equine oviduct results in the oviduct starting to move the oocytes towards the uterus (Weber *et al*.,1991). It is also proved that LH as well as other ovarian steroids stimulate PGE_2 production by the oviductal mucosa by acting on phospholipase A2 especially during the periovulatory period (Wijayagunawardane *et al*., 1999).

It goes without saying that progesterone acts as an antagonist to these processes by reducing the phospholipase A2 activity (Wijayagunawardane *et al*., 1998). PGE_2 also controls the ciliary beat frequency. Prostaglandins are mostly produced during the luteal phase rather than the follicular phase with highest concentrations found in the fimbria and lowest in the uterotubal junction (Aguilar & Reyley, 2005).

LH is not only responsible for production of PGE_2 of PGF_{2a} and ET-1 (endothelin-1). LH can also increase the amplitude of oviductal contractions during the follicular and postovulatory periods (Wijayagunawardane *et al*., 2001). The same study goes on to say that during the periods mentioned earlier prostaglandin and endothelin secretions were the highest.

Oxytocin is another hormone involved in the oviduct and more specifically in its smooth muscles where receptors have been found and they were observed mostly during oestrus in

cows (Wijayagunawardane *et al.*, 1999). Oxytocin affecting oviduct receptors is mostly produced by the cyclic corpus luteum.

Oxytocin will increase oviduct contractions in order to facilitate the transport of the male and female gametes (Wijayagunawardane *et al.*, 1998). Oxytocin remains low in the luteal phase. Another study from the same author suggests that oxytocin in bovine does not really stimulate the oviductal production of PGs and endothelins (Wijayagunawardane *et al*., 1999).

In another study done by the same author (Wijayagunawardane *et al*., 2001) oxytocin was found to block prostaglandin and endothelin production but at the same time increase the amplitude of contractions of the oviduct smooth musculature. This study also states that oviduct musculature in the ewe has increased sensitivity to oxytocin during oestrus and that the ovine oviduct contains increased amounts of oxytocin receptors at the postovulatory phase rather than the luteal phase. Finally it is worth mentioning that we can arrive to the conclusion that luteal oxytocin can block the contractions stimulated by LH by blocking prostaglandin and endothelin secretion.

Another hormone the oviduct is known to produce is gonadotrophin releasing hormone (GnRH). Oestrogen mediates the production of GnRH by increasing the concentration of mRNA coding for this hormone as well as increasing the calcium influx in the cells responsible for its production. In turn progesterone increases the response of the spermatozoa towards GnRH. GnRH has an effect in humans to increase the binding effect of spermatozoa on the *zona pellucida* by working with progesterone to increase calcium influx in the spermatozoa (Morales *et al*., 2002).

Endothelins (END) have a primary function in regulating vascular tone and blood pressure. They are divided into three isoforms: END1, 2 and 3. They are formed by endothelin converting enzymes (Bridges *et al*., 2011). In the oviduct, luminal epithelial cells have been found to be involved with endothelin production mostly in the contralateral oviduct during the oestrous cycle (Rosselli *et al*., 1994).

Endothelin production is mostly stimulated by oestradiol (Jeoung *et al*., 2010), maximal number of endothelin secretory cells in the mouse oviduct were found during the time of ovulation. Endothelin receptors have been localized in the muscle layer of the human oviduct as well as its epithelium (Bridges *et al*., 2011). Their function in the oviduct has to do mostly with affecting smooth muscle tone, oviductal contractility, and transport of gametes. They are also crucial for embryonic development, steroidogenesis and apoptosis (Jeoung *et al*., 2010).

Findings suggest that the levels of hormones found in the bovine oviduct are higher in the oviduct which is found ipsilateral to the ovary containing the developing follicle and will subsequently ovulate (Wijayagunawardane *et al.*, 1998).

Ovariectomized cats can undergo oviductal atrophy (Verhage & Brenner, 1975). If these cats are treated with exogenous oestradiol this can restore the oviductal epithelium fully. Additionally, in another experiment, Verhage & Brenner (1976) had found that the oviductal epithelium undergoes a similar differentiation when the queen is pregnant.

This helps us reach to the conclusion that the atrophy and deciliation is mostly due to a lack of oestrogen and elevated progesterone (West *et al*., 1977). Additionally if exogenous progesterone is administered then the same atrophy and deciliation of the epithelium occurs (Brenner *et al*., 1983).

A study showed that when rats where injected with progesterone in the form of depo-medroxy progesterone acetate (DMPA) the activity of the glands, stromal cells and endothelium was decreased whilst the activity of progesterone receptors in the epithelium and stromal cells was increased. This reduces ciliary activity which means that progesterone can be used as a contraceptive (Hegazy & Hegazy, 2015).

If a cat is administered progesterone and oestrogen together there is first an oviductal growth, the effect of progesterone antagonism is delayed until about three days later when the atrophy will commence. Finally it is suspected that in spayed cats progesterone receptor numbers fall dramatically after spaying (Lessey *et al*., 1981).

Increase in oestradiol concentration increases the oviduct oestradiol receptors, besides that, oestradiol will increase progesterone receptor concentration as well but with a delay. This delay can be up to 3 days (West *et al*., 1977).

Progesterone will decrease the availability of its own receptors and those of oestrogen as well (West *et al*., 1977). This is easily explained when you think that progesterone signals the end of the cycle so if pregnancy does not occur then high receptor concentrations are no longer needed.

According to a study, the main function of oestrogens in the cow is modifying the composition of the oviduct fluid in order to have a greater protein secretion during the follicular phase (Ulbrich *et al*., 2003). It does that by regulating the type of secretory cells in the oviductal epithelium. Progesterone always antagonizes the actions mentioned above.

Oestrogen receptors (ER) are divided into two types α and β while progesterone receptors have two types, PR-A and PR-B. ERα is considered to be the predominant receptor (Saruhan *et al*., 2011). Ulbrich, Kettler and Einspanier (2003) also state that the highest mRNA expression of ERα in the oviduct is in the ampulla.

ERα are found in all regions and layers of the oviduct in both luteal and follicular phases. Another interesting finding in this research is the fact that ERβ receptors tend to increase during the early luteal phase after progesterone stimulation rather than with oestradiol. The ERβ receptors are located mostly in the isthmus (Saruhan *et al*., 2011).

When referring to PR these receptors are mostly found in the oviduct epithelium. PR concentrations in bitches are low from the pre-LH period to 7 days after ovulation (Tahir *et al*., 2012).

Progesterone is one of the most important hormones involved in reproduction. This means that it has a crucial role in the functions of the oviduct. Progesterone levels in the bovine oviduct are higher in the luteal phase and early pregnancy and the ipsilateral oviduct produces larger amounts rather than the contralateral (Wijayagunawardane *et al*., 1996).

Progesterone during the oestrous cycle does not only arise from the ovary but from other sources as well such as the back fat of pigs (Hillbrand & Elsaesser, 1983). Their experiment reached to the conclusion that progesterone concentrations in the middle of the luteal phase where two hundred times higher in the back fat rather than the blood.

Progesterone has a greater effect than oestradiol in the transport of the ovum or zygote through the oviduct. This is regulated by the oviduct by increasing its blood supply which increases local counter current transfer of P_4 to the oviduct. Progesterone also has the ability to induce acrosomal exocytocis in human spermatozoa and increases sperm binding to the *zona pellucida* (Morales *et al*., 2002).

It is already known that the oestrous period in cats lasts for three to four days if coitus occurs and six to ten days if it does not. Oestradiol levels will drop one to two days after mating to 20-30 pg/mL. It is also known that progesterone levels tend to rise during pregnancy and pseudopregnancy with higher levels seen during the former with concentrations up to 30-40 ng/mL (Morales *et al*., 2002).

A study found that special cells called amine precursor uptake and decarboxylation cells (APUD) are found between the *lamina propria* and muscularis of the rat oviduct. Beside their

function of making the *lamina propria* oedematous and dilate the subserosal plexus they are also believed to secrete gonadotrophins, oxytocin, opioids as well as other neuropeptides (Ogunranti, 1994).

The APUD cell theory is coupled with another publication from the same author stating that steroid production in the ewe oviduct can be proved especially in the ampulla, and more specifically in the ampulla-isthmic junction, by locating sterol dehydrogenase activity in these cells as well as the substrate pregnenolone (Ogunranti, 1992). This author also theorises that progesterone is the main product of these cells found in the epithelium and stroma of the oviduct.

3. MATERIALS AND METHODS

3.1. Animals, collection of samples

Our experiment involves 37 sexually mature, clinically healthy cats. These cats are of different breed at different stages of their oestrous cycle and different ages. Clinical history of cats was recorded. Out of the cats which were used in this research 6 of them were examined more specifically and the following results were obtained: 3 of them were pregnant, one of them had a corpus haemorrhagicum in the ovary, one was at the onset of the prooestrus and one was just after the ovulation.

The cats were ovariohysterectomized under anaesthesia with a combination of Butorphanol-Dexdomitor-Ketamin for induction and isoflurane for the maintenance. The oviducts and ovaries where weighed and stored at - 20 °C.

Blood samples were collected from each cat before induction of the anaesthesia as well as after the ovariohysterectomy from the cephalic vein into evacuated tubes without anticoagulants. Each of the oviduct and blood samples was given a code each representing one cat. The blood samples were centrifuged at 1500/minute for 12 minutes at 4 °C. The supernatant was separated and serum was stored at - 20 °C until measurement.

3.2. ELISA

The frozen oviduct and ovary samples were decapsulated and extra connective tissue and fat were removed. The samples were measured with analytical scales and were homogenized with buffered PBS solution (pH=7.2) in room temperature by a tissue homogenizator.

The result of homogenization was 0.1 g parenchyma tissue/mL solution. The homogenates were centrifuged at 4000/minute for 20 minutes. The supernatant was stored at - 20 °C until process. The samples were diluted tenfold and were prepared in four dilutions (1:10, 1:20, 1:40, and 1:80).

The homogenate and serum progesterone and oestrogen levels were measured with ELISA kits. As far as the ovaries are concerned we only measured progesterone levels.

The kit used in our experiment for progesterone measurement is developed and manufactured by the Faculty of Veterinary Science of the Szent István University in Budapest, Hungary. It is called Quanticheck® and it is a reagent kit for quantitative determination of the intra-assay coefficients of variation for the samples were 1.68 %, 0.98% and 1.15% for means of 4.33, 3.44 and 5.25 ng/mL, respectively.

The specificity varies depending on the type of steroid it reacts with. For example it has a cross reaction of 100% with progesterone and 95 % with 11α-OH progesterone-HS.

The kit used for estrogen measurement is called DRG Estradiol sensitive ELISA, an enzyme immunoassay for the quantitative in vitro diagnostic measurement of oestradiol in serum and plasma.

The principle of this kit is that the microtiter wells are coated with a polyclonal antibody directed towards an antigenic site on the oestradiol molecule. The oestradiol of the cat serum will compete with an oestradiol horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off.

The analytical sensitivity was calculated from the mean minus two standard deviations of twenty (20) replicate analyses of Standard 0 and was found to be < 1.399 pg/mL. Its specificity varies depending on the type of steroid it reacts with. For example it has a cross reaction of 100% with oestradiol, 0.2 % with oestron and 0.05 % with oestriol.

3.3 Statistical Analysis

Data were analysed by Student-t test and Pearson's product moment correlation. The level of significance was $p<0.005$.

4. Results

In our tests we discovered that higher amounts of progesterone in our serum samples before ovariohysterectomy were correlated with higher oviduct progesterone concentrations because $r= 0.35$, this can be considered a medium effect, the correlation is not very strong.

Furthermore we have seen that P4 concentrations in the serum post-op and oviduct P_4 concentrations have a correlation coefficient of 0.26, with this we can arrive to the conclusion that the correlation between oviduct P_4 concentrations and serum P_4 concentrations post-op does not exist.

In order to express our results in a clearer way the values we found from our experiment were expressed in Table 1. The table consists of the mean of all values taken for each parameter, their standard deviation which explains how spread out our values are and the percentage difference between serum progesterone concentrations before and after ovariohysterectomy. Our table also consist of the median value for each of our parameters and their minimums and maximums.

Mean (±SD) progesterone values (Table 1) were significantly higher (5.76±8.70) in the serum before ovariohysterectomy compared to the progesterone values of the serum after the operation (3.66±4.81).

A paired samples t-test was performed to check the difference between progesterone values before and after ovariohysterectomy, p value was 0.001, thus there is an actual change between these two parameters, we are rejecting the null hypothesis. Figure 1 shows the magnitude of decrease of serum progesterone concentrations before compared to P4 concentrations in the serum after ovariohysterectomy in the same queens.

The degree of change in P4 concentrations in our results before and after the operation varies and can range from 0.10 ng/mL to 20 ng/mL. We took blood samples from cats that were pregnant and we have observed that serum progesterone concentration in those queens tends to be higher, the same can be seen in queens who have a corpus haemorrhagicum.

Finally towards the end of pregnancy and more specifically in the case of a cat at the D52 of pregnancy we have observed a lower progesterone value. Furthermore we have also carried out a correlation coefficient test and found an r-value of 0.94 when we compared serum progesterone concentrations pre- and post- spaying. Our results have shown us that since the r-value is 0.94 and that our regression line (Figure 1) is very linear that there is a strong relationship between these two types of samples.

Mean (±SD) progesterone concentrations (Table 1) were pointedly higher in the oviduct (178.84±266.60) compared with the serum (3.66±4.81). Figure 2 shows the relationship between the P_4 concentrations in the oviduct and serum in the same queens.

We can see an evident difference between the two parameters with the oviduct showing significantly higher values of progesterone. This difference in our values varies from as small as 4 ng/mL to as big as 900 ng/mL.

According to our samples oviductal progesterone concentrations tend to be higher when the cat is pregnant or has a corpus haemorrhagicum and is relatively low when the queen enters the oestrus phase of the her cycle.

We carried out correlation coefficient testing to measure the strength of the relationship between the hormone concentrations in the oviduct with their counterparts in the serum.

Firstly, the r-value between oviductal and serum progesterone concentration was 0.35 and the regression line in Figure 2 has an upward slope but was less steep than the one when we were comparing serum progesterone concentrations before and after spaying. We also observed that the points on the graph were much more scattered

Mean (±SD) oestrogen concentrations (Table 1) in the oviduct (22.46±28.80) were much higher than those in the serum (820.15±1221.25). Figure 3 shows the relationship of oestrogen concentrations between the oviduct and serum in the same queens. It is evident that the oviduct has a much greater concentration of oestrogen than the serum. The difference in our values can vary from 100 pg/mL to 3000 pg/mL.

With these results we can arrive to the conclusion that the greatest hormone difference between the oviduct and serum is that of oestrogen. Oestrogen concentrations in the oviduct were non-existent in queens were the corpus haemorrhagicum was observed. The same low values of oestrogen were seen in the serum during pregnancy. When comparing oestrogen

concentrations between the oviduct and serum the r-value was – 0.15 and the regression curve had a slightly downward slope (Figure 3).

Additionally ovarian progesterone concentrations were recorded and compared to oviductal progesterone values in the same queens. The mean (±SD) progesterone concentrations (Table 1) in the ovary (3817.48±6707.64) were higher than the oviductal progesterone concentrations (178.83±266.60).

It is apparent from these results that the ovary is no doubt the major organ for progesterone production in the organism. The difference in values between oviductal and ovarian progesterone varied from as small as 2ng/mL to as great as 6000 ng/mL.

Queens which exhibited only small differences between serum and oviduct progesterone where those which were close to ovulation or where ovulating.

High progesterone value differences were observed especially in queens that were pregnant or had a corpus haemorrhagicum, it is known that during these phases in the reproductive cycle progesterone concentrations can increase dramatically. There was also a case of a cat having greater concentrations of progesterone in the oviduct rather than the ovary; this cat was at the beginning of her oestrus cycle.

Mean (±SD) progesterone concentrations were pointedly higher in the ovary (3817.48±6707.64) compared with the serum before the operation (5.76±8.70). Figure 4 shows the relationship between the P_4 concentrations in the ovary and serum in the same queens.

We can see an evident difference between the two parameters with the ovaries showing significantly higher values of progesterone. This difference in our values varies from as small as 16 ng/mL to as big as 22100 ng/mL.

According to our samples ovarian progesterone concentrations tend to be higher when the cat has a corpus haemorrhagicum and is relatively low when the queen enters the oestrus phase of her cycle. The values vary when the queen is pregnant with progesterone being relatively high during the end of pregnancy in this case.

We carried out correlation coefficient testing to measure the strength of the relationship between the hormone concentrations in the ovary with their counterparts in the serum. The graph in figure 4 shows that the relationship is linear

Furthermore we have seen that P_4 concentrations in the serum pre-op and ovarian P_4 concentrations have a correlation coefficient of 0.49, with this we can arrive to the conclusion that the correlation between those two parameters has a moderate uphill (positive) relationship (Figure 4).

Due to the fact that the weight of the oviduct and the ovarian samples was known the results showed that the heavier our sample the higher the progesterone and oestrogen value in both organs.

5. Discussion

This research has a primary goal on identifying if the oviduct has enough endocrine cells and endocrine properties that it can produce and secrete such a high amount of progesterone and oestrogen. By studying this we can either prove or exclude the role of the oviduct in altering the levels of these hormones in the blood in such a way as to create certain complications of gynecological nature in the queen. An example of these complications is pyometra.

It is very important to state the fact that in case of ovarian remnant syndrome the ovarian tissue will start synthesizing and releasing progesterone and oestrogen in the bloodstream. In this case cats can experience recurrent oestrus, behavioural changes and diseases such as pyometra (Okkens *et al*., 1981).

Neither the age nor the breed of animal seems to be significant (Miller, 1995). A study reported that out of 109 dogs with complications after ovariohysterectomy, 47 of them had residual ovarian tissue (Okkens *et al*., 1981). These complications may arise from 2 weeks to 3 years after the operation (Wallace, 1991).

Ovariohysterectomy has higher chances of causing this ovarian remnant syndrome than ovariectomy. This is because the celiotomy performed in ovariohysterectomy is more caudal making it more difficult to visualize the right ovary (ovarian remnant syndrome most commonly involves the right ovary because it is located deeper and more cranial (Berzon, 1979).

Another very important complication that might arise from an ovarian remnant or in our case an oviductal remnant is the formation of pyometra. It is a disease which is not as common in cats as it is in bitches. Pyometra or cyctic endometrial hyperplasia is a disease induced mainly by progesterone and leads to cystic dilation of the endometrial glands and uterine inflammation which consequently leads to purulent content in the lumen (Agudelo, 2005).

There hasn't been any real research about how the oviduct can influence the hormonal levels in the organism by itself. In order to be able to produce hormones the oviduct needs to receive first a stimulus. When this stimulus is received there is a change in the cytoplasmic hormone receptor concentrations in different sections of the oviduct (Lessey *et al*., 1981). By doing this, the hormones might not only provide increased sensitivity of the oviductal epithelial cells towards this hormone but it may also lead to the production and secretion of hormones synthesized in the oviduct itself.

A research done on rats showed that in the ampullary-isthmic junction where the egg is arrested for a period of time the oviductal epithelial and stromal cells have a high activity of sterol dehydrogenase (Ogunranti, 1992). The same was seen in a research that involved rabbit oviducts (Puri & Roy, 1981).

This stimulus is in the form of a hormone which is mostly either progesterone or oestrogen coming from the ovaries. The type of hormone that will stimulate the oviduct is greatly governed by the stage at which the oestrous cycle is. For example oestrogen will stimulate the oviduct mostly during proestrus and oestrus with its effect decreasing significantly 8-40 days post-coitus. On the other hand progesterone will stimulate the oviduct mostly during pregnancy (West *et al*., 1977).

As mentioned above progesterone will greatly affect the expression of oestrogen receptors in the oviduct by decreasing their concentration the reason for this is that if pregnancy does not occur progesterone increases to signal the end of the cycle as well as decreasing the concentration of receptors because they are no longer needed in such a high amount (Lessey *et al*., 1981).

Hormone assays carried out in different mammals showed that progesterone which was assayable in plasma and ovarian blood was secreted prior to luteinisation of the follicle. This statement allows us to reach the conclusion that the oviduct amongst other organs is responsible for the periovulatory production of progesterone (Ogunranti, 1992).

As far as oestrogen is concerned (Richardson & Oliphant, 1991) came to the conclusion that oestrogen is also produced by the oviduct but its production is minimal when comparing it to other organs.

A study reported that progesterone concentrations in blood before and after ovariectomy are different with a decrease of the hormone after the operation (Alagwu & Nneli, 2005).This

coincides with our result that showed a significant difference in means of 5.76 pre-op and 3.66 post-op.

Furthermore serum samples taken from a cat in early pregnancy and a cat with a corpus haemorrhagicum were found to have a higher progesterone concentration both pre- and post-op when comparing them with the serum progesterone values of other cats.

What is mentioned above can be confirmed by another research done on cats which showed that indeed during pregnancy because of the formation of corpora lutea progesterone levels will rise, reaching a peak at around 10-12 days post-coitum. This research goes on to state that progesterone values will gradually decline after implantation until they reach 1-2 ng/mL at the 60th day post-coitum (Verhage *et al*., 1976).

The findings above coincide with our sampling results which show a cat sampled at the D30 of pregnancy had progesterone values of 5.4ng/mL whilst another queen sampled at the D52 of pregnancy had a smaller progesterone value of 2.47ng/mL.

Since we have rejected the null hypothesis and proven that there is an actual change in progesterone concentrations in the serum we can now move on to finding out the origin of the remaining progesterone in the serum, if in fact the oviduct contributes to the amount of progesterone in the serum of queens.

Contrary to our studies research has shown that especially after ovariectomy due to the stress of the surgery and the pain post-op progesterone levels may be high due to the release of pituitary adrenocorticotrop hormone (ACTH). The ACTH may stimulate the synthesis and release of progesterone by the adrenal glands which could show a false-positive value (Hillbrand & Elsaesser, 1983).

According to researches we know for a fact that progesterone is indeed produced in the oviduct. The main question that arises with our research is if the oviductal epithelial and stromal cells have the capability of releasing the progesterone they produce in the serum and not only in the oviductal lumen.

Progesterone receptor distribution in the different tissue layers depends on the periovulatory stage. A research has shown that progesterone receptors around ovulation are increased in the stroma and muscle layer but are found in the highest concentrations in the epithelium (Tahir *et al*., 2012).

Tahir *et al*, (2012) goes on to say that the number of these receptors will start decreasing from the pre-luteal phase until the D7 post-ovulation in the stromal and muscle layer (time of oocyte meiosis and fertilization), this decrease can also be seen in the ampulla of the oviduct from proestrus to late metestrus.

Progesterone and oestrogen are responsible for the number of progesterone receptors especially in the muscle layer and might provide a positive feedback for the oviduct endocrine cells to produce their own hormones (Vermeirsch *et al*., 2002).

Previous researches on the amount of progesterone found in the oviduct have shown that progesterone is the highest during the luteal phase and early pregnancy than at the follicular phase and post-ovulation (Wijayagunawardane *et al*., 1996).

Research carried on hamsters has also proven that despite oviductal progesterone concentrations being low at the post-ovulatory stage progesterone concentrations in the follicular fluid are high (Libersky & Boatman, 1995). Due to this it is fair enough to say that Libersky & Boatman (1995) have shown us that follicular fluid does not contribute in a big amount to the oviductal progesterone concentrations during ovulation and thus progesterone must arrive from somewhere else.

Furthermore in the case of sows only 0.5 % of the available follicular fluid is present in the oviducts after ovulation (Hansen *et al*., 1991).

Wijayagunawardane *et al* (1996) have also shown that due to the higher concentrations of progesterone in the oviduct contralateral to the ovary bearing the corpus luteum the oviductal musculature contract at higher amplitude.

Another experiment carried out using cow oviducts has shown that between D14-18 of pregnancy the blood flow to the ipsilateral oviduct as well as the diameter of the uterine artery will increase in the ipsilateral side thus accommodating a higher flow of progesterone (Ford *et al*., 1979). This was seen with ovine oviducts between D14-16 (Greiss JR & Miller, 1974).

The above findings coincide with our results that show progesterone concentrations being higher in the oviduct of queens that were spayed during pregnancy, 580 ng/mL at the D30 of pregnancy compared to those that were spayed at other phases of the oestrous cycle e.g. at oestrous: 14.5 ng/mL.

When carrying out a correlation analysis to find out the strength of the relationship between serum progesterone and oestrogen with the same hormones in the oviduct we found out that both had a weak correlation and thus a weak relationship with the oestrogen having the weakest. Additionally whilst serum and oviduct progesterone had a positive correlation the oestrogen had a negative relationship.

From these results we can conclude that the connection between the serum and oviduct is not very strong. The hormones found in the oviduct might be those excreted by the oviductal cells in the oviduct lumen which are responsible mostly for the capacitation, fertilization and movement of the zygote in the oviduct through muscle contractions.

The oviduct might be able to contribute to a small amount of the serum hormone concentration but as it seems that is mostly the function of the ovaries and to a smaller extent the adrenal glands.

In conclusion, this report demonstrates that serum progesterone concentrations are greatly affected by spaying the animal, the concentrations of this hormone can be halved by this operation and there is a strong relationship between the two parameters.

Additionally we have not found a strong relationship between the oviduct and the serum, the oviduct is mostly programmed to deal with processes going on within the lumen so as to ensure an uneventful fertilization and transport to the uterus.

With the above being said, research for this topic has been minimal and this allows me to draw two conclusions: 1) it is an area which is yet to be researched and has a lot of potential or 2) it might be hypothesised that there is no strong relationship between serum and oviduct so researchers do not want to explore the topic further. Finally diseases arising from hormonal imbalances such as pyometra are more likely to arise from remnants of the ovary rather than the oviduct.

Acknowledgments

I'm grateful to the Veterinary Faculty of Szent István for allowing me to carry my research in their facilities. I would also like to thank my supervisor: Professor Julianna Thuróczy for her guidance and patience. Lastly I want to thank my family and friends for all their support and time they gave me for completing this work.

6. APPENDICES

Table 1. Results of progesterone in serum samples pre-, post-op, oviduct and ovary

	Serum samples P4, pre-op	Serum samples P4, post-op	Serum samples E2, pre-op	Oviduct P4	Ovarian P4	Oviduct E2
Number (N)=	37	26	32	37	37	37
Mean	5.76 ng/mL	3.66 ng/mL	22.46 pg/mL	178.84 ng/mL	3817.48 ng/mL	820.15 pg/mL
Standard Deviation=	8.70	4.81	28.80	266.60	6707.64	1221.55
Percentage Δ		-58%				
Median	1.93	2.29	7.78	74.5	292	295.5
Minimum	0	0	0	0	0	0
Maximum	40	23.48	108.89	1168	22100	5221.5

The table consists of the mean of all values taken for each parameter, their standard deviation which explains how spread out our values are and the percentage difference between serum progesterone concentrations before and after ovariohysterectomy. Our table also consist of the median value for each of our parameters and their minimums and maximums.

Figure 1 Correlation between serum Progesterone levels before and after ovariohysterectomy

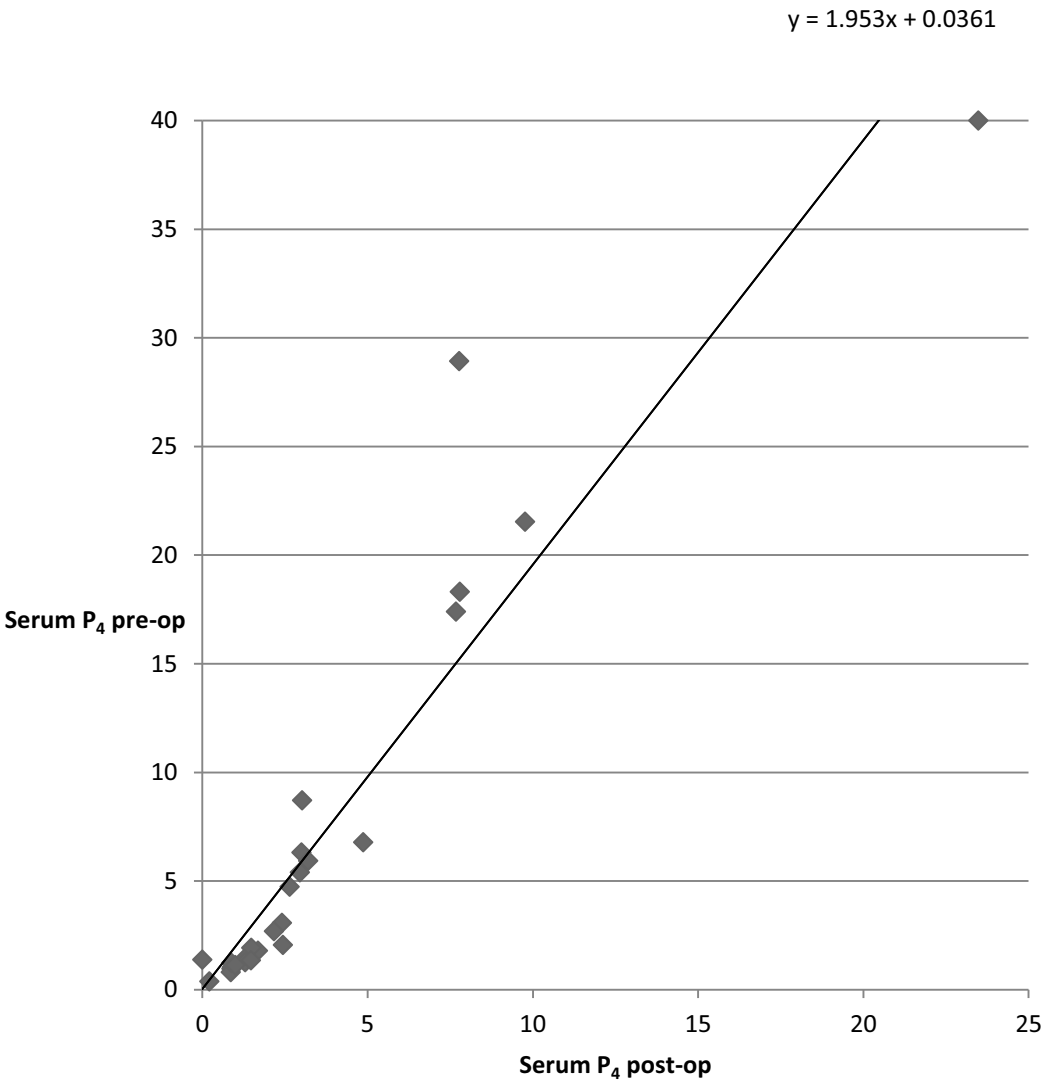

Figure 1 shows the magnitude of decrease of serum progesterone concentrations before compared to P4 concentrations in the serum after ovariohysterectomy in the same queens. The regression line is linear with an r-value of 0.94 which shows a correlation of strong, positive, uphill relationship between the two parameters.

Figure 2 Correlation between oviductal and serum progesterone concentrations

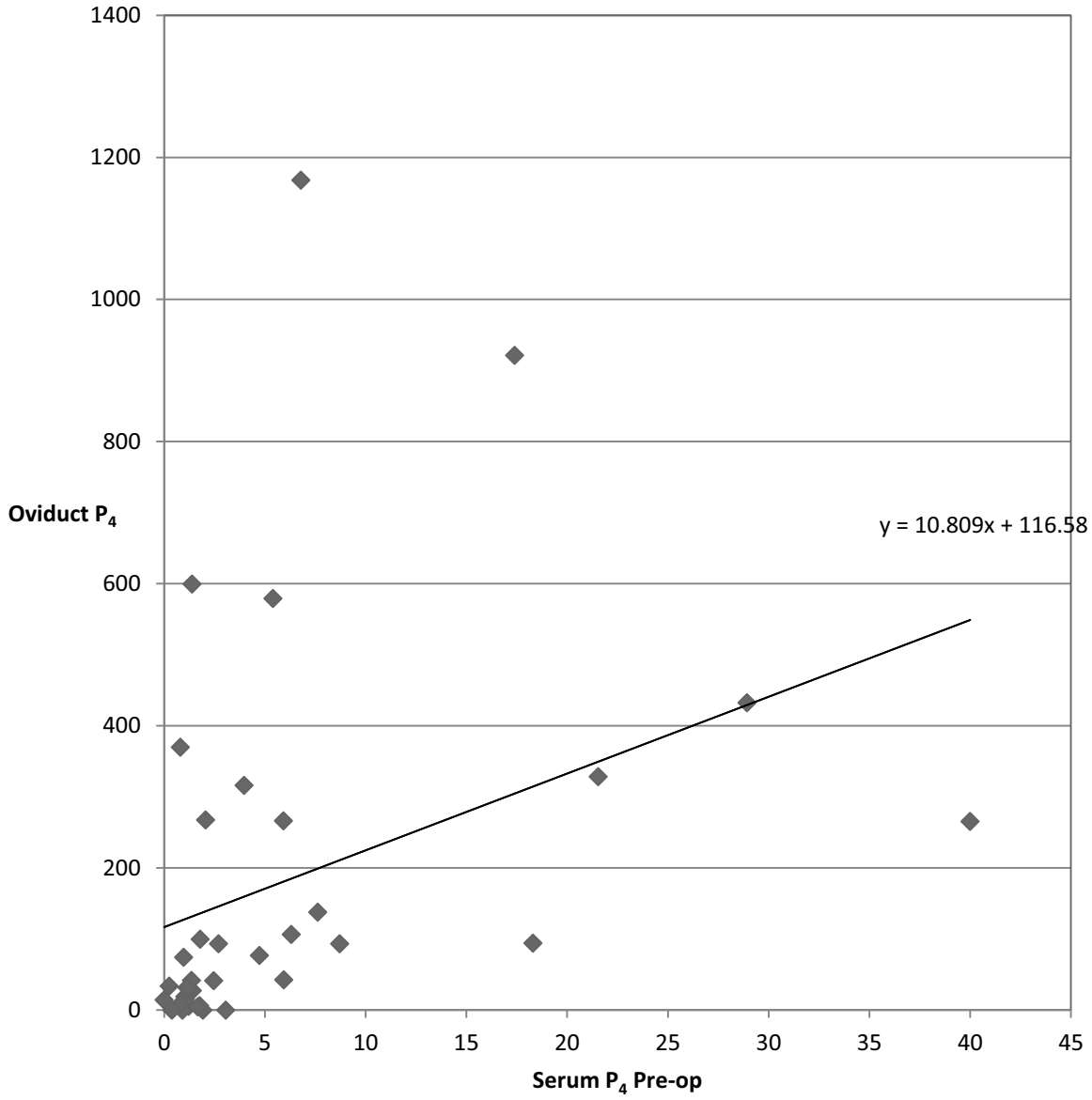

Figure 2 shows the difference of serum progesterone concentrations compared to progesterone concentrations in the oviduct of the same queens. The regression line is linear with an r-value of 0.35 which shows a correlation of medium, positive, uphill relationship between the two parameters.

Figure 3 Correlation between oviductal and serum oestrogen levels

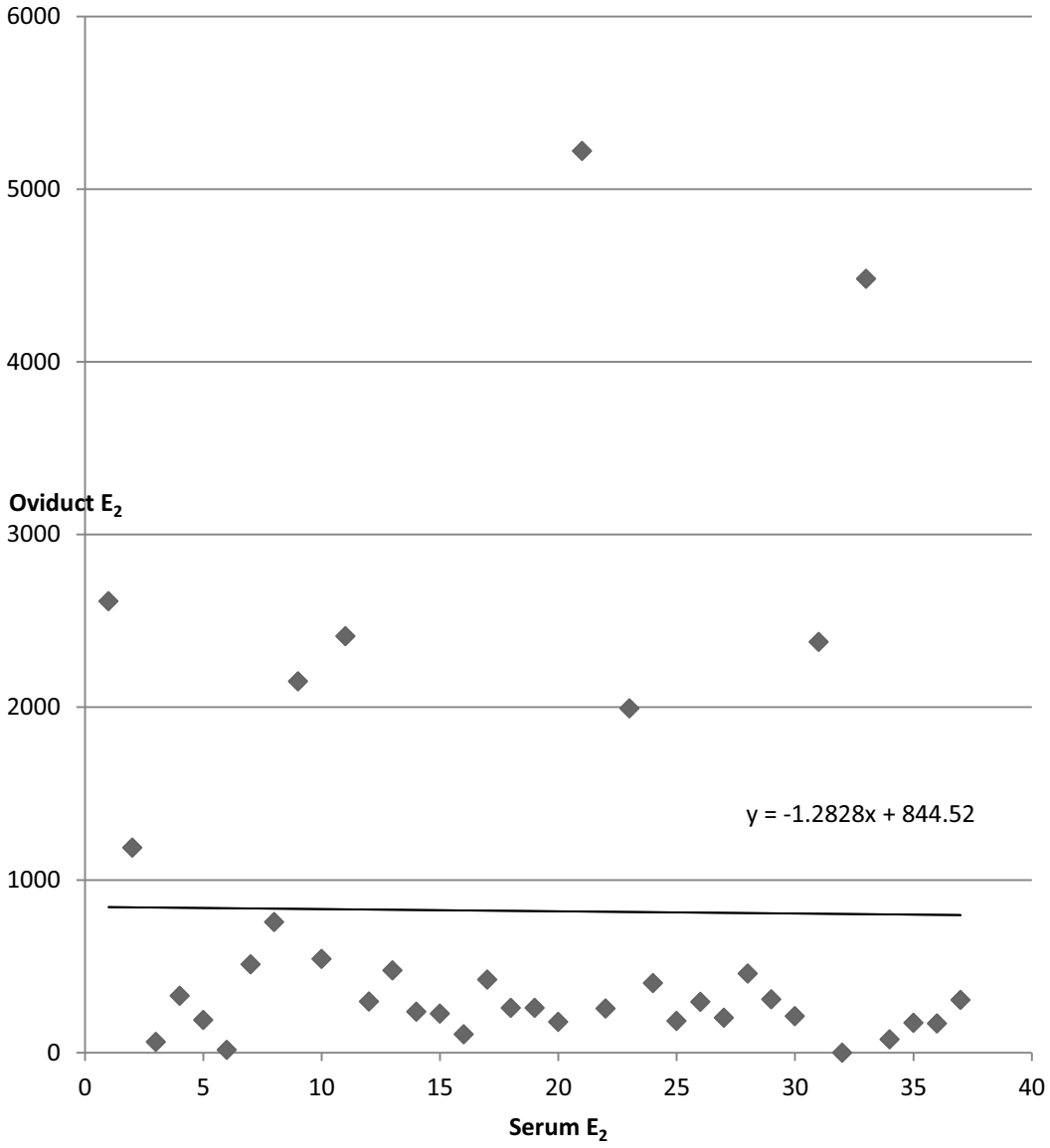

Figure 3 shows the difference between serum oestrogen concentrations compared to oestrogen concentrations in the oviduct of the same queens. The regression line is linear with an r-value of - 0.15 which shows a correlation of weak, negative, downhill relationship between the two parameters.

Figure 4 Correlation between ovarian and serum progesterone levels

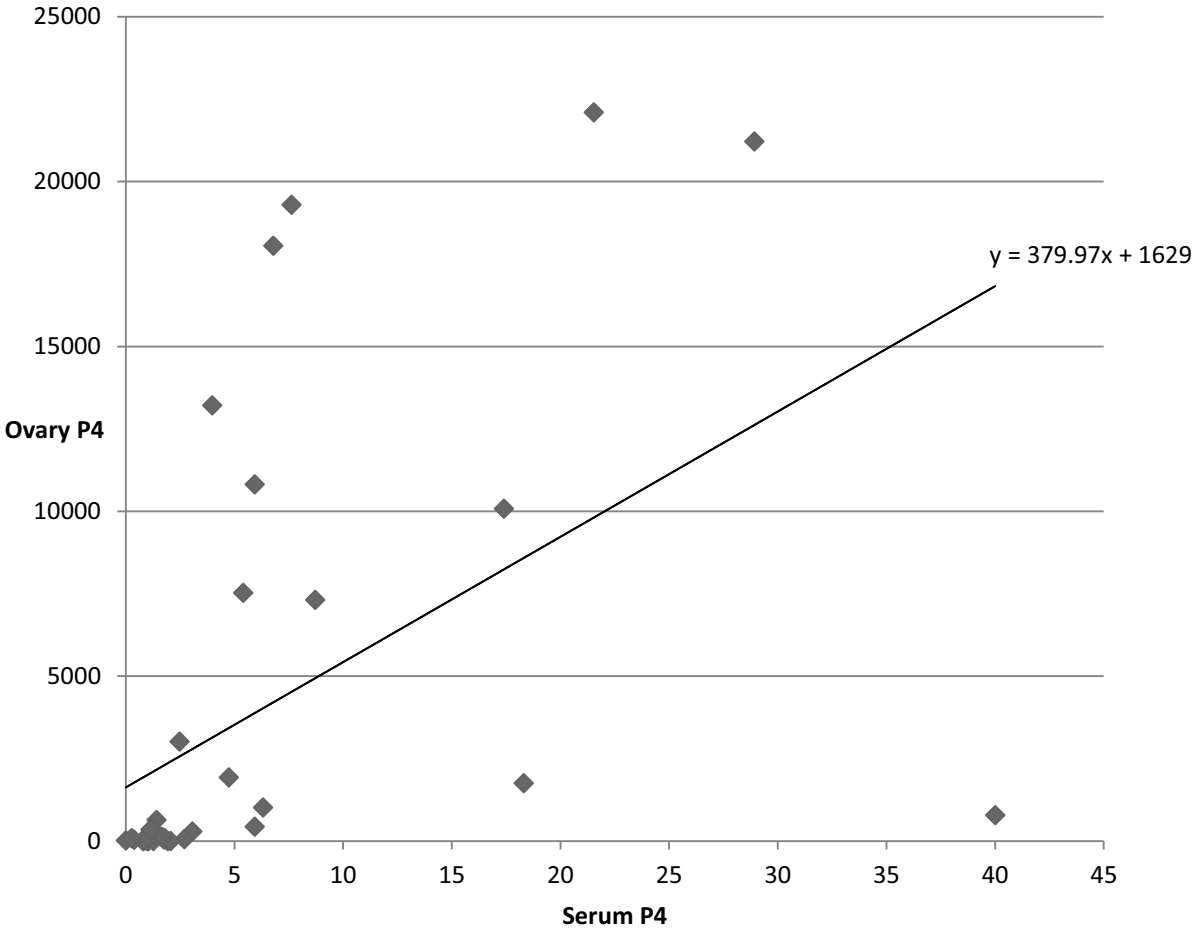

Figure 4 shows the difference between serum progesterone concentrations compared to progesterone concentrations in the ovary of the same queens. The regression line is linear with an r-value of 0.49 which shows a correlation of medium, positive, uphill relationship between the two parameters.

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