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Ultra short pain effect during ovariectomy

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1. Introduction

Pet overpopulation is a serious problem worldwide. It threatens the lives of companion animals more than any infectious disease.

It encompasses two primary factors: allowing cats and dogs to reproduce and have offspring with little chance to find them homes and the abandonment of pets by their owners.

Animal shelters have become overcrowded and are unable to care for the millions of the homeless pets. Every year approx. 8-10 million dogs and cats only in the US, enter shelters and 5 million of these animals are euthanized due to lack of homes and crowded shelters.

Short pregnancies and large litter of both dog and cats enable that one individual female, along with her reproducing female offspring can be the source of over 4000 new lives within 7 years (Quisenberry & Clapp, 1983).

Also, unwanted dogs and cats can act as reservoirs or vectors of transmissible diseases (Kutzler & Wood, 2006).

The overpopulation cycle continues if the animals are allowed to breed so spaying and castration are the most important solution for stopping this problem.

Spaying and castration are both reliable, affordable, safe procedures, relatively painless and can be produced intensively.

The benefits of spaying are numerous; prevention of unwanted litters, reduction of stray and feral animals, reduce spreading of genetic disorder and inferior genetic traits or congenital deformities, prevention of uterine diseases such as ovarian cancer, pyometra, uterine prolapse, ectopic uterus and many more.

It also reduces or prevents diseases which can be caused by hormones such as vaginal hyperplasia, mammary neoplasia, cystic endometrial hyperplasia and also estrogen- inducing dermatosis, diabetes mellitus and Type 2 diabetes which can be caused by high progesterone level.

Behavioral problems can also be prevented by spaying. It eliminates stress and discomfort that females endure during heat periods and prevents males from trying to fight with the owners due to pheromones secreted by the female in heat.

Ovariectomy (OVE) is a common procedure for the sterilization of female dogs. Removing both ovaries eliminates the source of sexual steroids, this results in reproductive sterility and the elimination of estrous cycles. OVE has been accepted as a standard procedure in Europe

and an alternative to Ovariohysterectomy (OVH) which is common in the USA and mean removal of ovaries and the uterus.

Technically, OVH is more complicated (more tissue is ligated and transected), time consuming (larger celiotomy is needed to expose the entire uterus) and therefore expected to be associated with a greater short-term morbidity when compared with OVE. (Shariati, 2014), (Schaefers-Okkens et al., 2006)

The purpose of our study was to compare surgical stress and understand the ultrashort pain effects during the OVE procedure by measuring the levels of cortisol and insulin during the surgery.

We hypothesis that cortisol, a hormone released during stressful conditions via the hypothalamic-pituitary- adrenal axis will increase from the beginning of the operation as part of the defense system of the body and will cause a reduction in insulin sensitivity, and the sympathetic activity will reduce insulin secretion.

Also, we wanted to understand if the different drugs used for anesthesia have different effects on those changes and as we found that there are different results between the two groups.

In order to make sure time and experience has no effect on our results we used a control group which were operated by an expert surgeon.

2. Physiology of Pain

Pain can be defined as unpleasant sensory and emotional experience associated with an injury, and essential for survival.

Pain management is very important and very challenging in non-verbal patients because of the narrow ability to diagnose effectively, and how subjective pain is (Mckune et al., 2013).

Pain receptors (nociceptors) are sensory structures located at the end of axons and activate by noxious stimuli (Messlinger, 1997). They are found in many tissues of the body, including the skin, periosteum, walls of arteries, surfaces of joints, lining tissues, the cornea and tissues around the eye, the meninges of the brain and the spinal cord.

Nociceptors are called "free nerve endings" because they are not encapsulated or associated with traditional receptor structures but are branched forming tree like structure.

Cutaneous nociceptors are the primary components of peripheral mechanisms of pain. They are extremely heterogeneous group of neurons which transduce the external stimuli in the skin up to the CNS (Dubin & Patapotian, 2010). There are two groups of fibers which affect the speed of transmission: A-delta nociceptors and C-polymodal nociceptors. the cell body of both type of neurons found in the spinal or cranial nerve ganglia (Kuchinka & Riedesel, 1994).

They can be classified into various types based on their functional properties;

A-delta nociceptors: heat mechanoreceptors. Their corresponding A delta fibers are myelinated fibers, fast pain, responsible for sharp, well localized superficial pain. These nociceptors allow the animal to be rapidly warned about impending damage to some area of the body, and allow them to react quickly.

C-polymodal nociceptors: respond to strong mechanical stimulation, heat, and to irritant chemicals and endogenously occurring chemicals as H+ ions, serotonin, histamine, bradykinin, prostaglandins, and leukotrienes. Their corresponding fibers are un-myelinated, slow pain responsible for delayed, dull, burning, poorly localized, superficial pain. These nociceptors allow the pain sensation to continue and provide a mechanism for chronic pain to develop.

The pain process: includes 5 steps; The first step is called *transduction:* the creation of action potential when nociceptors are activated by adequate noxious stimuli (Dubin & Patapotian, 2010). The second step is *conduction*: the action potential generated in a nociceptor terminal conducted to the central process where it depolarized the presynaptic terminal which interfaces with interneurons and 2nd neurons in the dorsal horn. The third step is *transmission*: the action potential causes the presynaptic terminal of the two groups of fibers to release pro-nociceptive substance into the synaptic cleft and activate the postsynaptic receptors. Activation of the postsynaptic receptors results in depolarization of interneuron and 2nd order neurons, which causes the generation of action potential through the dorsal horn of the spinal cord to the CNS. The fourth step is, *modulation*: which both excitatory and inhibitory mechanisms occur and involve mediators such as GABA, or enkephalin that transmit the information through the thalamus to the cerebral cortex, in which there it processes. The sixth step is *pain perception* (Garimella & Cellini , 2013), The perception of pain can occur only in a conscious animal as the cerebral cortex must be active for pain sensation, but pain reflex can occur without pain recognition as a result of nociceptor stimulation.

Peripheral sensitization: the incision of the surgery cause injury to the tissues, which causes the release of chemicals that make the C-polymodal nociceptors sensitive and evoke stronger responses than in the non-sensitized state.

Central sensitization: conditioning stimulus to C-polymodal pathways can also cause changes in spinal processing of the sensory signals by increasing membrane excitability and synaptic efficacy as well as reduced inhibition (Latremoliere & Woolf, 2009).

Those changes can be increased responses to noxious stimulate applied to inflamed or surround non-inflamed tissue and lowering the threshold of nociceptive specific spinal cord neurons.

Induction and maintenance of central sensitization is dependent on N-methyl-D-aspartate (NMDA) subclass of receptors for the excitatory activation of nociceptive spinal neurons. Blocking this change in spinal processing, induced by surgery and increased C-polymodal input, is referred to as preemptive analgesia.

Surgery causes tissue damage. The larger the incision is, the greater the pain it will provoke. The type and duration of surgical procedure or injury and the expected postoperative level of

pain should be considered when assessing the potential for pain and when developing a management protocol.

It is important to remember that the spinal cord receives a massive barrage of nerve impulses also in the unconscious patient under general anesthesia, from the surgical site. These afferent impulses are exacerbated when peripheral nerves are cut.

Post-operative pain is a form of acute pain and its qualities are related to the site of origin of the pain, where somatic and visceral pain form the two major divisions of pain.

In general, fear, sleep deprivation and anxiety tend to contribute to stress and decrease tolerance to pain and in order to reduce the acute postoperative pain, it is important to minimize the stress using premedication, which reduces anxiety, inhibits peripheral and central sensitization. It is also important to give postoperative opioid analgesics, along with the use of local anesthetic or epidural opioids when needed (Kuchinka & Riedesel, 1994).

OVE and OHE both are common and standard procedures causing a moderate level of pain which make it suitable for clinical studies of analgesia (Hansen, 2003).

3. The Hypothalamic-Pituitary-Adrenal Axis

In order to defend the body functions in stressful situations, the endocrine system, together with the nervous and circulatory systems, integrate to regulate homeostasis in the body which are essential for life.

The stress response is mediated by the interconnection between these systems and are based on cellular molecular physiological and behavioral levels (Tsigos et al., 2016).

3.1. Corticotropin releasing hormone (CRH):

CRH are produced and secreted from the hypothalamus in response to a stressful stimulus. It has central role in regulating the hypothalamic pituitary-adrenal-axis (HPA) and by that, it is a very important hormone in stress response (Smith & Vale, 2006). In reaction to neural inputs, synthesis and release of CRH is made in the small nucleus of the hypothalamus by regulation of glucocorticoids feedback. The Amino acid peptide is released into the hypophyseal portal blood and carried to the anterior part of the pituitary gland where it binds to its receptors. The binding of the CRH to its receptors induces the release of adrenocorticotropic hormone (ACTH) into the

systemic circulation and increasing the production of pro opio melanocortin (POMC) as well (Seasholtz, 2000).

3.2. Adrenocorticotropic hormone (ACTH):

As a result of the CRH- receptors binding, ACTH, which is produced in the anterior part of the pituitary gland, is released into the blood to bind the ACTH receptors.

The ACTH receptors are cell surface proteins coupled to adenylyl cyclase and found in the adrenal cortex. As a result of the ACTH-receptors binding, cAMP levels increase, and this stimulates the synthesis and secretion of glucocorticoid from the zona fasciculata of the adrenal glands. ACTH also can be regulated by other hypothalamic compounds such as vasopressin, oxytocin, and norepinephrine but at much lower potencies (Seasholtz, 2000).

3.3. The adrenal glands:

Paired endocrine glands are found craniomedial to the corresponding kidney. One of the major functions of the adrenal gland is to respond to stress, (Munck et al., 1984) and it the most important steroidogenic tissue in the body.

Each gland is comprised of two distinct parts that are important in synthesis the secretion of hormones to the circulatory system; outer cortex and inner medulla which are surrounded by a capsule compose of connective tissue that help to maintain its structure (Miller, 2007) (Figure 1.).

The adrenal cortex composes of three concentric zones which produce steroid hormones from cholesterol. Each of the zones are responsible for the synthesis of a specific type of hormones which are secreted into the blood in circadian rhythm.

- Zona glomerulosa; the outermost layer, is the site of mineralocorticoids synthesis, as aldosterone.
- Zona fasciculate; the largest layer where glucocorticoids synthesis take place.
- Zona reticularis which secretes androgens.

The adrenal medulla is composed of chromaffin cells with granules within them containing the adrenal hormones as; dopamine, epinephrine and norepinephrine.

The blood flow in the adrenal is centripetal; causing increase levels of steroid hormones at the medulla which are necessary for epinephrine biosynthesis.

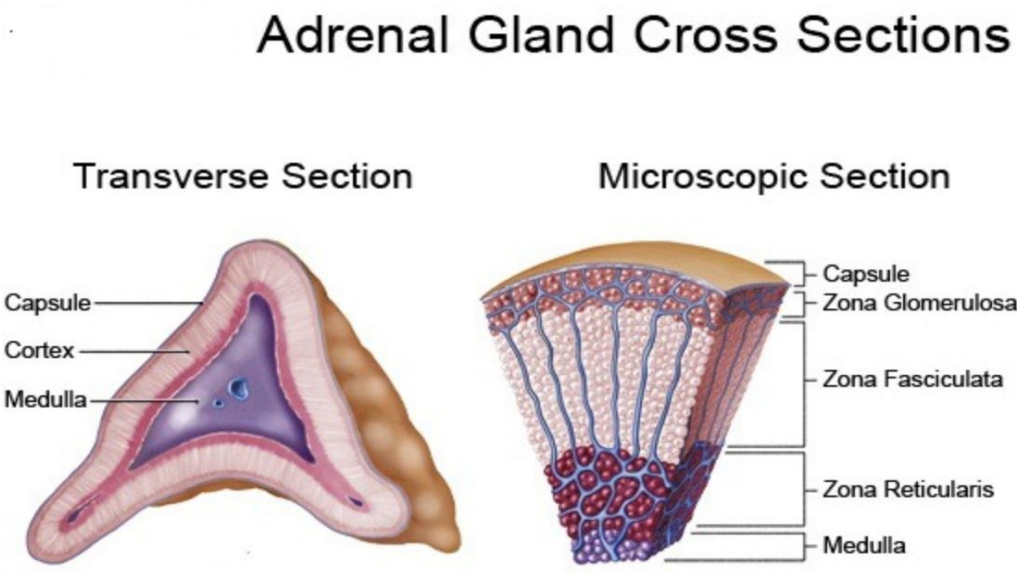


Figure 1. transverse and microscopic sections of the adrenal glands (Mehendiratta, 2017).

4. Stress Response

The stress response is part of a systemic reaction to injury or trauma including multitude of hormonal and metabolic changes. The overall effect is increases catabolism to provide energy sources, maintain homeostasis, retain salt and water and maintain fluid volume.

The stress response is activated by afferent neuronal impulses from the site of injury, through the dorsal root of spinal cord up to the medulla to activate the hypothalamus and result in increase of hormone secretion from the pituitary glands.

A stressful experience triggers the acute release of catecholamine from the sympathetic nervous system which evoke rapid physical responses and also activate the HPA axis by secretion of CRH and ACTH and finally trigger glucocorticoids from the adrenal cortex into the blood circulation. Epinephrine, glucagon and cortisol increase in stressful situations to maintain the blood sugar level. Under continued stress, the constant simulation of the adrenal cortex can enhance its capacity to secrete glucocorticoids by increasing the levels of key steroidogenic enzymes.

Additionally, when experiencing acute illness, ACTH stimulation on the adrenal cortex increases and cause enhanced steroidogenic capacity (Hankolgu, 1992).

Once the stress ends, hormonal levels return to homeostasis by the negative feedback action of glucocorticoids on the HPA axis.

Endocrine gland	Hormones	Change in secretion
Anterior pituitary	ACTH	Increases
	Growth hormone	Increases
	TSH	May increase or decrease
	FSH and LH	May increase or decrease
Posterior pituitary	AVP	Increases
Adrenal cortex	Cortisol	Increases
	Aldosterone	Increases
Pancreas	Insulin	Often decreases
	Glucagon	Usually small increases
Thyroid	Thyroxine, tri-iodothyronine	Decrease

Figure 2: principal hormonal change to stress (AVP-arginine vasopressin, TSH-thyroid stimulating hormone, FSH-follicle stimulating hormone, LH-luteinizing hormone) (Desborough, 2000)

5. Steroids Hormones Biosynthesis

Steroid hormones are groups of hormones secreted from the adrenal cortex, testes, ovaries and the placenta.

The adult adrenal cortex produces three classes of steroids hormones; glucocorticoids, mineralocorticoids and adrenal androgens from the precursor *cholesterol*.

The steroid hormones play a significant role in carbohydrates metabolism, the control of inflammation, the second sexual characteristics, sodium and fluid homeostasis and reproductions (Sewer & Li, 2008).

The conversion of *cholesterol* into other steroid hormones and intermediates has rate limiting step, the transport of cholesterol from outer mitochondrial membrane to the inner mitochondrial membrane, by regulation of the Steroidogenic Acute Regulatory Protein (StAR).

The *cholesterol* converts to a common precursor steroid called *pregnenolone* by the cytochrome P450scc (side chain cleavage enzyme) which are found in the mitochondria of this cells. *Pregnenolone* can be converted into three different pathways, by activity of

different enzymes found in each zone of the adrenal. At the zona fasciculata, the pregnenolone convert to *progesterone* by the 3fl-hydroxysteroid dehydrogenase, then it hydroxylated to *17α-hydroxyprogesterone,* metabolized to *deoxycorticosterone* and convert to cortisol by P450c11 at the mitochondria (Hu. Jie et al., 2010).

The overall rate of steroidogenesis is controlled by tropic hormones (LH, FSH or ACTH). These hormones bind their respective G protein-coupled receptors and lead to stimulation of cAMP-PKA signaling cascade that exerts both acute and chronic effects on the steroid hormone production;

- *The acute phase* occurs within minutes followed by rapid synthesis of new steroids. It is characterized by the rapid mobilization of lipid droplet and increased delivery of cholesterol into the mitochondria to be cleavage by cytochrome P450 enzyme.
- *The chronic phase* occurs at the level of the transcription of the genes coding for steroidogenic proteins, including the StAR and other enzymes that mediate the stepwise conversion of cholesterol to cortisol (Hankolgu, 1992).

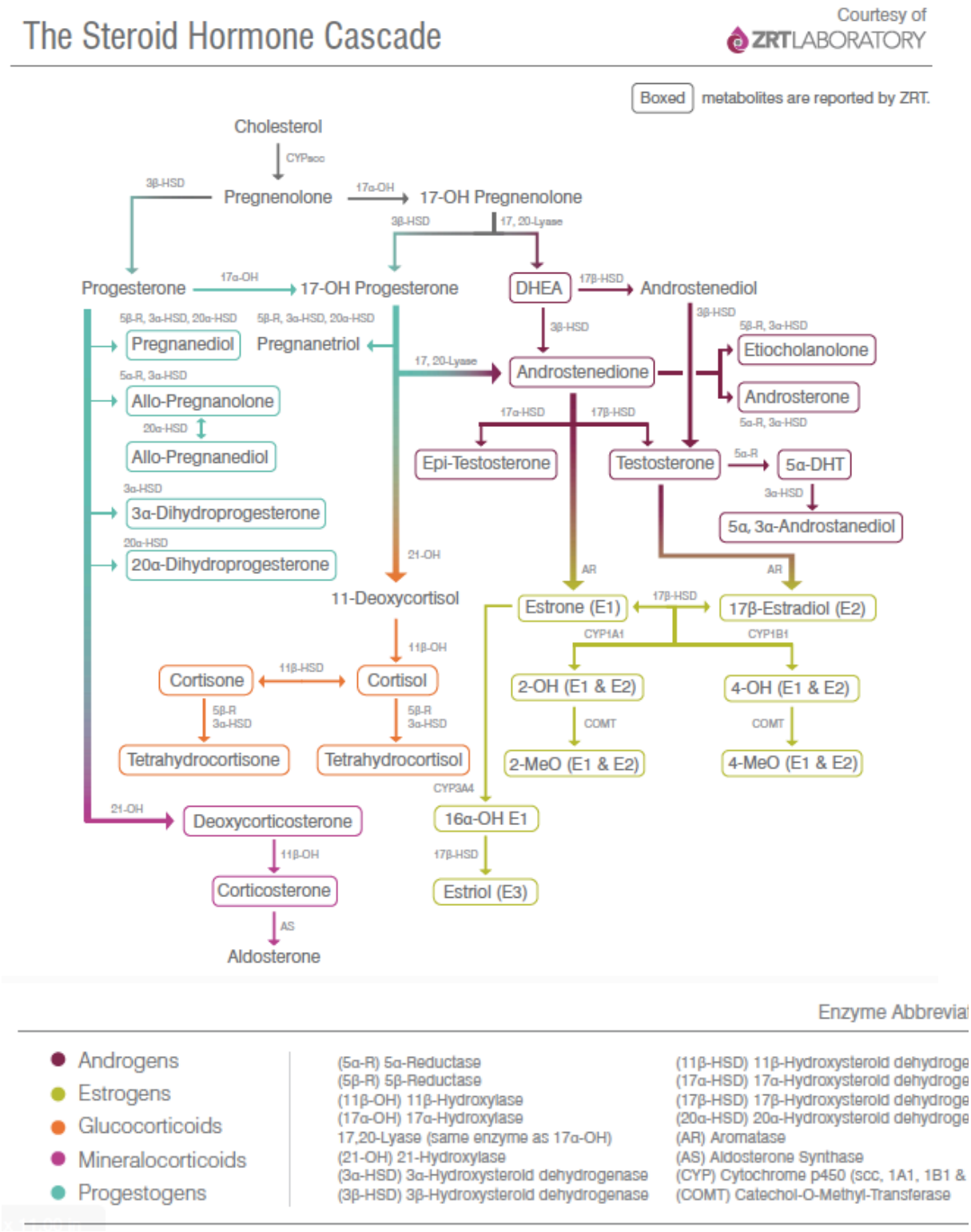


figure 3: steroid biosynthesis pathways (ZRTlabratory, 2016).

6. Glucocorticoids

Glucocorticoids are a type of steroid hormone produced at the zona fasciculata of the adrenal glands. They play an essential role in maintaining basal and stress-related homeostasis under the control of the HPA axis (Oconnor et al., 2000).

They help in stimulation of gluconeogenesis- the production of glucose from released amino acids and lipids. They conserve the glucose level by inhibiting glucose uptake into muscle and fat cells, they mobilize the fats and stimulate the fat breakdown in adipose tissues and have anti-inflammatory and anti-allergic effects.

Glucocorticoids have a negative feedback on hypothalamus and anterior pituitary gland; increase in glucocorticoids, causing inhibition of production and secretion of CRH and ACTH that lead to decrease in production and secretion of glucocorticoids from the adrenal glands (Oakley & Cidlowski, 2013).

7. Glucocorticoid receptors (GR)

Glucocorticoids receptors (GR) are nuclear receptors with modular structure. They are found in extensive distribution in most mammalian tissues, usually numbering from 1000-2000 per cell and are responsible for mediating the physiological and pharmacological actions of the glucocorticoids. They function as transcription factors that regulate cell function even following the termination of acute stress.

The GR are composed of three major domains; N-terminal transactivation domain (NTD), a central DNA-binding domain (DBD), and a C-terminal ligand binding domain (LBD). (Oakley & Cidlowski, 2013)

In the absence of hormones, GR resides in the cytoplasm of cells as part of a large multi-protein complex. These proteins maintain the receptor in a conformation that is transcriptionally inactive but favors high affinity ligand binding.

The binding of ligand to the GR causes a conformational change which results in the dissociation of its associated proteins.

The ligand-receptor complex translocates into the nucleus via the nucleus pores where it regulates gene expression by inducing or repressing the transcription of target genes by three

pathways:

- directly activate transcription by binding to the GREs in the promoter of target genes
- tethering itself to other DNA-bound transcription factors, such as activator protein 1 (AP1), nuclear factor κB (NF-κB), transcription factor IID (TFIID), signal transducer and activator of transcription 5 (STAT5), and CREB (Finsterwald & Alberini, 2014)
- binding directly to DNA and interacting with neighboring DNA-bound transcription factors

GR can also signal in a non-genomic pathway by elicit rapid cellular responses that occur within a few seconds to minutes through alterations in the activity of various kinase such as PI3K, AKT, and MA.

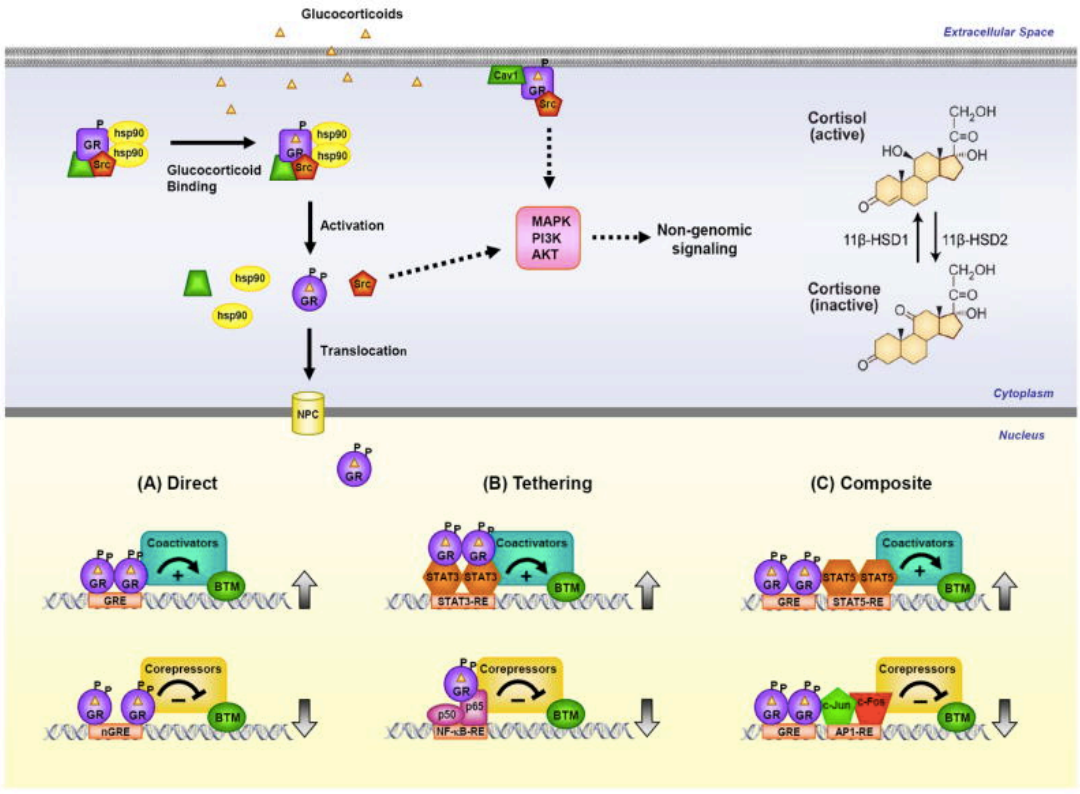


figure 3: GR regulation of the expression of target genes by three pathways; (a) by direct binding to DNA components, (b) by tethering to other DNA bound transcription factors, (c) by binding directly to DNA, and interacting with neighboring DNA-bound transcription factors (Oakley & Cidlowski, 2013).

8. Cortisol

Principle glucocorticoid, plays a role in front line endocrine mechanism in order to defend the organism in a stressful condition.

Its secretion increases rapidly following the beginning of the surgery but is subjected upon the anesthetic intervention of the surgery (Möstl & Palme, 2001).

The cortisol is essential for life, its regulates or supports a variety of function such as: metabolic, immunologic, cardiovascular and homeostasis. It has a crucial role in regulating the blood glucose level- it counters insulin by stimulating gluconeogenesis by triggering the expression of enzymes of the gluconeogenesis and increases glucose blood level. In contrast, it also stimulates glycogen synthesis in the liver, which decreases the blood glucose level.

Additionally, it maintains a role in ion regulation by preventing cells from losing sodium and accelerates the rate of potassium excretion (Randall, 2011).

It has anti-inflammatory activity; it inhibits the accumulation of macrophages and neutrophils into area of inflammation and interferes with the synthesis of inflammatory mediators.

9. Insulin

Insulin is a peptide hormone which plays a major role in metabolism regulation. Its produced by beta cells of pancreatic islets of Langerhans cells.

After feed intake when glucose blood and amino acids concentration increases, insulin release is increase in turn to maintain glucose level in the blood by facilitate the glucose uptakes of the cells into insulin- dependent tissues as muscles and adipose tissues.

When blood glucose level drop, the alpha cells of the pancreas release glucagon which cause the liver to release glucose to the blood.

Insulin furthermore stimulates the formation of glycogen from glucose in the liver, inhibit protein catabolism and also inhibit lipolysis.

The insulin mediates its action through its receptor which is a large protein, consisting of heterotetramer (2 alpha ,2 beta) glycoprotein subunits which are located on cell membranes (Wilcox, 2005). Hyperglycemia after stress is a very common clinical phenomenon. Insulin concentrations may decrease after the induction of anesthesia, since both, anesthesia and

surgery stress response effect the glycemic homeostasis by increasing anti-insulin hormones such as catecholamine, glucagon and cortisol. These hormone causes reduction of insulin secretion which may lead to a failure of insulin to match the catabolic hyperglycemic response. (Desborough, 2000) Additionally, during surgery, unexplained defects of the pancreatic beta cells can also impair the insulin secretion in response to glucose.

These hormonal and metabolic changes promote occurrence of hyperglycemia and insulin resistance in all surgical patients with or without diabetes (Jovanovski-Srceva et al., 2015).

10. Sympathetic Nervous System

The sympathetic nervous system is part of the autonomic nervous system (ANS) which acts as a control system, maintaining homeostasis in the body.

In response to stimuli, the hypothalamus activates the adrenal medulla to secrete hormones as adrenaline to the blood stream and prepare the body for a 'fight or flight' response.

Activation of the sympathetic nervous system results in tachycardia and hypertension. Its directly modifying the functions of liver, kidney and pancreas causing retention of water through the renin-angiotensin system, glucagon release from pancreas to maintain glucose level and responsible for mobilization of free fatty acids from lipid stores (Burton et al., 2004).

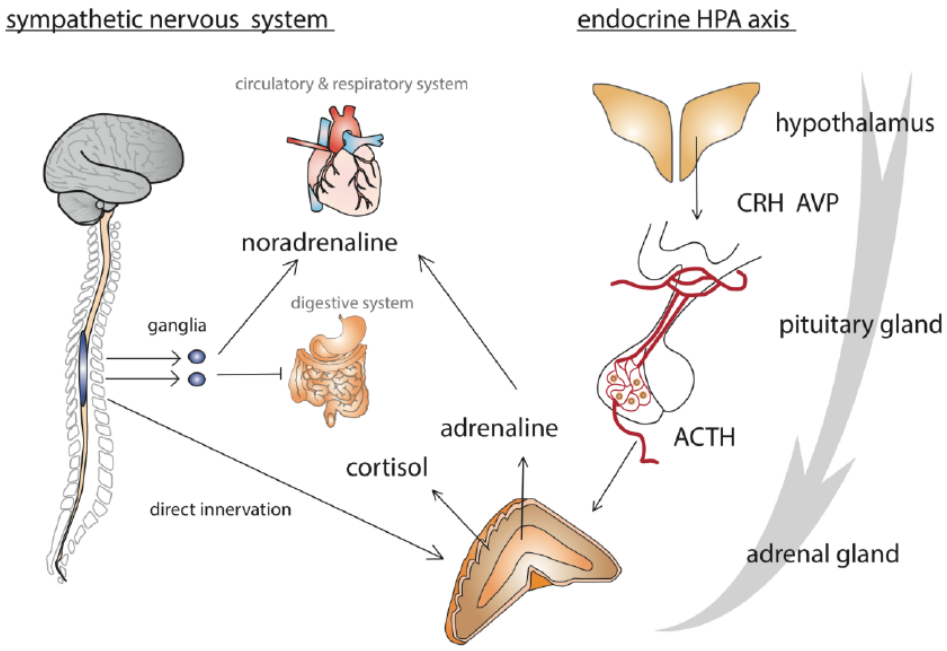


Figure 4: stress response through sympathetic and endocrine systems (Barron et al., 2012).

11. General Anesthesia

Multi-modal analgesia, promotes the use of two or more classes of analgesic drugs with different mechanisms of actions combined with different administration techniques. This technique is safer and more effective, resulting in pain control across several body systems. Each of the drugs we used in the combination act on different pain receptors along the pathway or in the brain in slightly different ways, thus ensuring that pain is reduced as much as possible during each stage.

11.1. Ketamine

ketamine plays a crucial role in the modulation of central sensitization. It the most commonly used NMDA-antagonist in veterinary practice used mainly for starting and maintaining anesthesia, or as an induction agent followed by muscle relaxant and tracheal intubation (Fu et al., 1997). Ketamine improves analgesia and decreases postoperative opioid consumption in surgical patients (Kaka et al., 2016). In order to prevent pathologic pain, ketamine is applied throughout the operation in an attempt to reduce the sensitization of central and peripheral pain pathways.

11.2. Xylazine

Xylazine is agonist which acts on central and peripheral Alpha-2 receptors class of adrenergic receptor. It is used for sedation, anesthesia, muscle relaxation, and analgesia and is often used in combination with ketamine. It appears to reduce sensitivity to insulin and glucose uptake in humans (Sinclair, 2003).

11.3. Diazepam

Diazepam is a benzodiazepine derivative tranquilizer. It provides anxiolytic, sedative, anticonvulsant, and central muscle relaxant effect. It increases the inhibitory effect of GABA in the CNS and appears to act on parts of the limbic system, the thalamus and hypothalamus, and induces calming effects. This medication may cause anticholinergic effects (anderson, 2014).

11.4. Fentanyl

Fentanyl is an opioid which acts on opioid receptors that are found primarily in the spinal cord and brain, but are also distributed in muscle, and the gastrointestinal tract. It acts as a pain relief which often used for anesthesia and analgesia along with a hypnotic agent, and in combination with a benzodiazepine to produce sedation for procedures. The use of opioid analgesics should be based on the expected postoperative pain response.

11.5. Midazolam

Midazolam is a benzodiazepine which effects the central nervous system by enhancing GABA at their receptors leading to the decrease of the excitability of neurons and causing a reduction of communication between neurons. It reduces anxiety and promotes muscle relaxation.

11.6. Propofol:

Propofol is an IV anesthetic agent which induces unconsciousness. It increases GABA-mediated inhibitory tone in the CNS, decreasing the rate of dissociation of the GABA from the receptor and increasing the duration of the GABA-activated opening of the chloride channel resulting in the hyperpolarization of cell membranes. It is a short-acting medication, resulting in a decreased level of consciousness within 15-30 seconds, and lack of memory for events. Propofol anesthesia is generally rapid, short duration and lacks excitatory effects on induction and during maintenance and recovery (Anandmay et al., 2016).

12. The Effect of Anesthesia and Analgesia on Stress Response

The hypothalamus reacts to noxious stimuli even in deep planes of anesthesia therefore general anesthesia may limit the perception of sensation to pain, but does not eliminate the response completely.

12.1. Opioids

Chronic use of exogenous opioids suppresses the hypothalamic and pituitary hormone secretions. In contracts, opioids also can affect the circadian rhythms of cortisol secretion,

which can result in persistently raised levels of ACTH and cortisol and eventually blunting the stress response (Seyfried & Hester, 2012).

12.2. Benzodiazepine

Benzodiazepine blocks the production of steroids by the adrenal cortex (both aldosterone and cortisol) on the hypothalamic pituitary level by reversible inhibition of the enzyme 11b-hydroxylase (Desborough, 2000).

12.3. Xylazine

The alpha 2 agonist inhibit the stress responses by mediating the sympathetic nervous system. (Desborough, 2000). Maintaining blood glucose homeostasis involves complex neurohumoral regulation. Stress can increase blood glucose by changing several hormones including insulin, glucagon, GLP-1, and catecholamine. Several studies demonstrate that the administration of xylazine increases blood glucose in various animal species. (Xiao et al., 2013)

13. Female genital tract

13.1. Ovaries:

Ovaries are important organs of the female reproductive system, responsible for ovum production. They are located within the ovarian bursa, which is made by the mesosalpinx, mesovarium, and the ovary itself. The ovaries create a suitable environment for the ovum, provided by follicles which are composed of different types of cells according their maturation. Two main classes of the female sex hormones are produced mostly by the ovaries, both produced from cholesterol;

- **Estrogen:** primary female sex hormone which is responsible for the development and regulation of the female reproductive system. The main production site for the estrogen are the egg follicle and the interstitial cells of the ovaries.
- **Progesterone**: produced by the corpus luteum and plays a very important role for the endometrium; primarily in the implantation and development of the fertilized ovum. It is involved in the proliferative to secretory transition of the endometrium, in synthesis and triggering synthesis of proteins which are essential

for pregnancy. Also, it is involved in the stimulation of prostaglandin E2 production and the suppression of the matrix metalloproteinases MMP3 and MMP9 (Regidor, 2014).

13.2. Uterine tube:

The uterine tube of the female dog is 5 to 9cm long. It exits laterally from the uterine horn, extends cranially and terminates medial to the ovary in a funnel-shaped infundibulum that has fimbriae. It opens into the uterine horn allowing the ovum to pass through.

13.3. Uterus: The uterus consists of cervix, body and uterine horns.

Cervix: short, thick muscular wall & narrow canal

Body: relatively short in carnivores

Uterine horns: paired, relatively long

13.4. Vagina: Extends from the vestibule to a fornix and displays longitudinal folds when not expanded.

13.5. Vestibule: Extends from vulvar cleft to the transverse fold.

13.6. Ligaments associated with the female genital tract:

- **Suspensory ligament**: originates from the last rib and attached to the ovaries from its craniodorsal aspects.
- **Proper ligament** of the ovary passes between the ovary and the cranial end of the uterine horn.
- **Round ligament** passes from the cranial end of the uterine horn within a fold of peritoneum laterally from the broad ligament toward the deep inguinal ring.
- **Broad ligament:** bilateral sheets of connective tissue positioning of the reproductive tract.

  It is arranged into three functional regions:

  - **Mesovarium** - attaches to the Ovaries.
  - **Mesosalpinx** - attaches to the Oviduct.
  - **Mesometrium** - attaches to the Uterus, Cervix and Cranial Vagina.

The uterine tube passes within the mesosalpinx from its origin adjacent to the ovary to join the tip of the uterine horn. The ovarian artery and vein supply the ovary, the uterine tube, the mesosalpinx, and a part of the suspensory ligament.
The arteries are parallels to the uterine horn and the veins are roughly parallel the arteries but may anastomose. (Forsberg, 2001)

14. Material and Method

14.1. Methods

The OVE operations took place at University of Veterinary Medicine, Budapest. For this research, 34 female dogs were obtained from a local shelter in Budapest.
We divided the dogs into two groups;

Control group: were operated with an expert surgeon(n=22)

- Premedication and induction:
  - Ketamine + Xylazine + Diazepam (S) (n=10)
  - Fentanyl + Midazolam + Ketamine + Propofol (n=12)

Study group: were operated by non-expert students (n=12)

- Premedication and induction:
  - Ketamine + Xylazine + Diazepam (S) (n=6)
  - Fentanyl + Midazolam + Ketamine + Propofol (n=6)

Blood samples were collected into vacuum tube without coagulant at four different time points during the surgery:

0- During the insertion of the vena catheter (T0)
1- Prior to premedication and induction (T1)
2- Post removal of both ovaries (T2)
3- Upon completion of the procedure (T3)

14.2. OVE Procedure

An incision was made extending from the umbilicus to about halfway between umbilicus and Os pubis. The surgeon located one ovary by palpation of the uterine horn until reaching the ovary, then pulled it out via the excision and teared the suspensory ligament. The surgeon then made a hole in the broad ligament (between the blood vessels) and passed through it an absorbable suture material. The surgeon then placed one ligature proximal to the mesosalpinx and tightened it around the ovarian pedicle and second ligature at the tip of the uterine horn caudal to the proper ligament. Transection was made between the ligatures, and repeated in the same manner for the second ovary.

14.3. Analgesia and Anesthesia

The *control* group and the *study* group were divided into two groups, each and treated with different premedication and induction:

- Premedication and Induction with:
  Ketamine (10 mg/kg bw.) + Xylazine + Diazepam (1 mg/kg bw.)
  Maintenance with Isoflurane

- Premedication and Induction with:
  Fentanyl (5µg/kg bw) + Midazolam (0.25mg/kg bw) + Ketamine (0.5 mg/kg bw) + Propofol (10 mg/kg bw)
  Maintenance with Fentanyl (0.25mg/5ml)/Ketamine (100mg/ml) infusion and Isoflurane.

14.4. Hormone Measurement

Blood insulin concentration was measured with Mercodia Canine Insulin ELISA 10-1203-01, and blood cortisol concentration was measured with Cortisol ELISA test: DRG EIA-1887 at the laboratory of Department of Obstetrics and Gynecology.

14.5. Data Analysis:

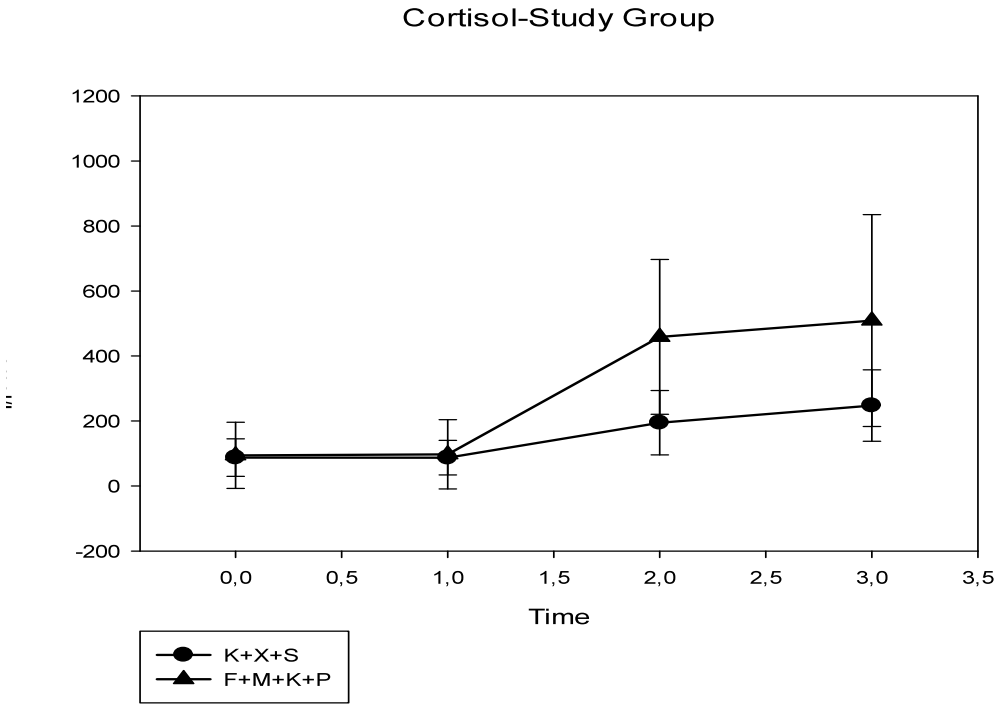
Data was analyzed with SigmaPlot statistic program, Student-t probe and Pearson Moment correlation analysis, level of significance is $p<0.05$.

15. Results

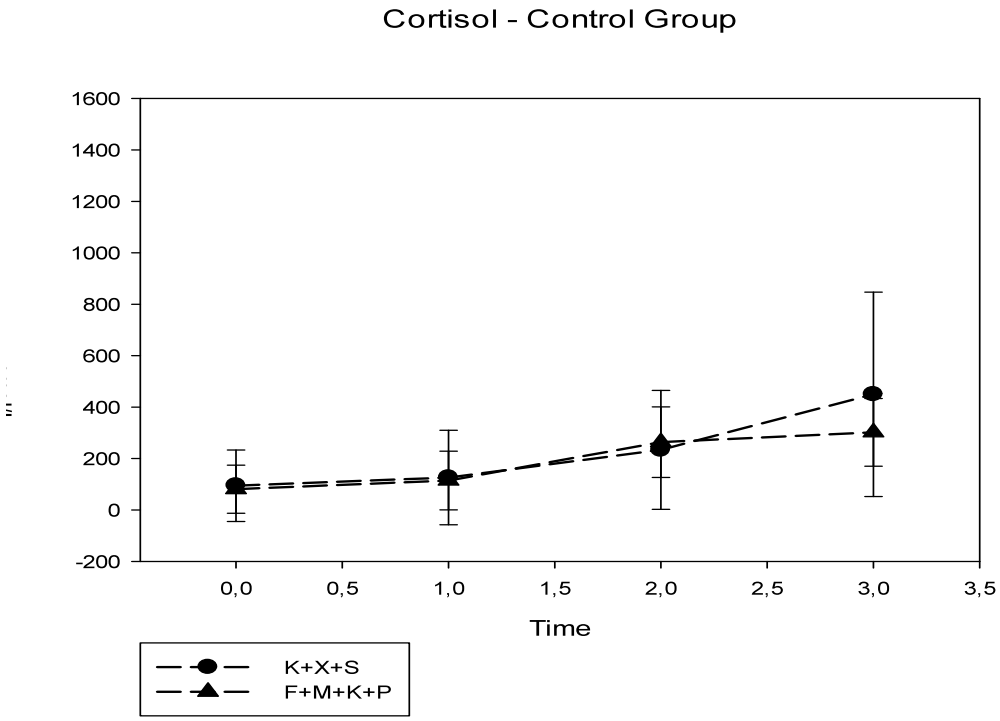
Control group: average operation time: 16,3 minutes
Study group: average operation time: 30,5 minutes
Cortisol: as we can see at graph 1-4, the cortisol concentration increased at all time points T0-T3, in both control and study groups. In the study group, comparison between the anesthetic types, the cortisol had higher increase in the group that treated F+M+K+P for premedication and induction. In the control group, comparison between the anesthetic types, the cortisol had higher increase in the group that treated K+X+S for premedication and induction. We can see that the highest cortisol measurement was at T3, at the end of the operation made by the study group that were treated with F+M+K+P (Graph 1).

Insulin: during the operation, the overall insulin concentration decreased as we can see in our results in graphs 5-8, basal average level of insulin in both control and study groups was 0.168 (T0). At the end of the operation, the insulin level average of both, control and study group were 0.088 (T3). The larger decrease of insulin level happened between T1 to T2, from right after induction until both ovaries were removed.
At Graphs 9-12, the correlation of both, control and study groups, anaesthetized with the two different combinations, show us that when the higher the cortisol is, the lower the insulin.

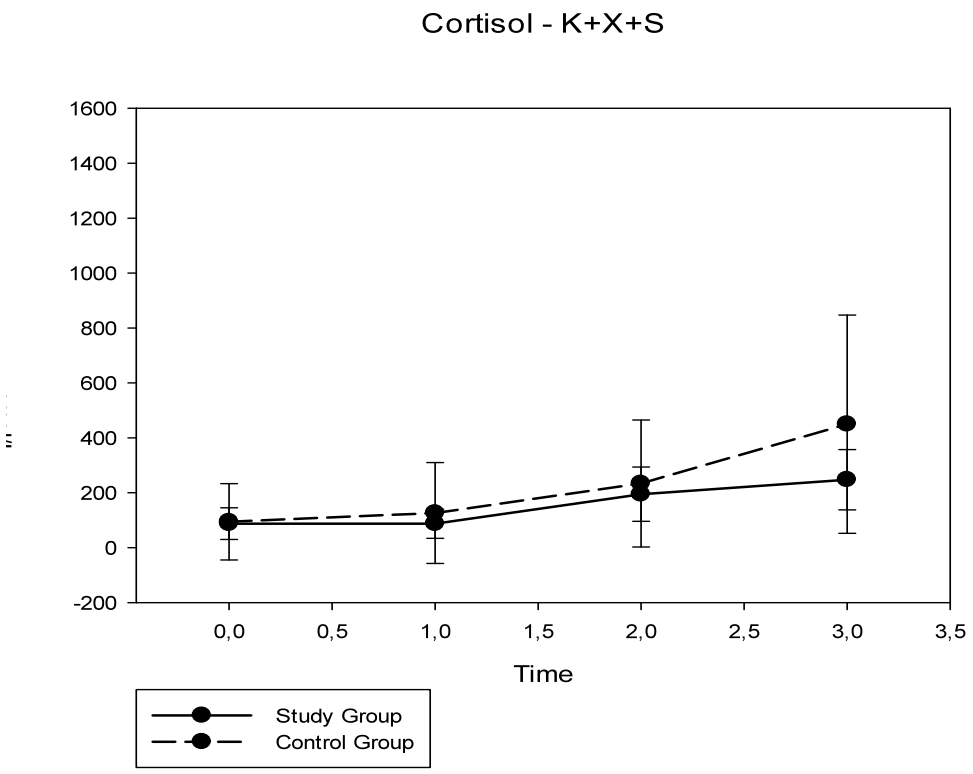
15.1. Cortisol measurements



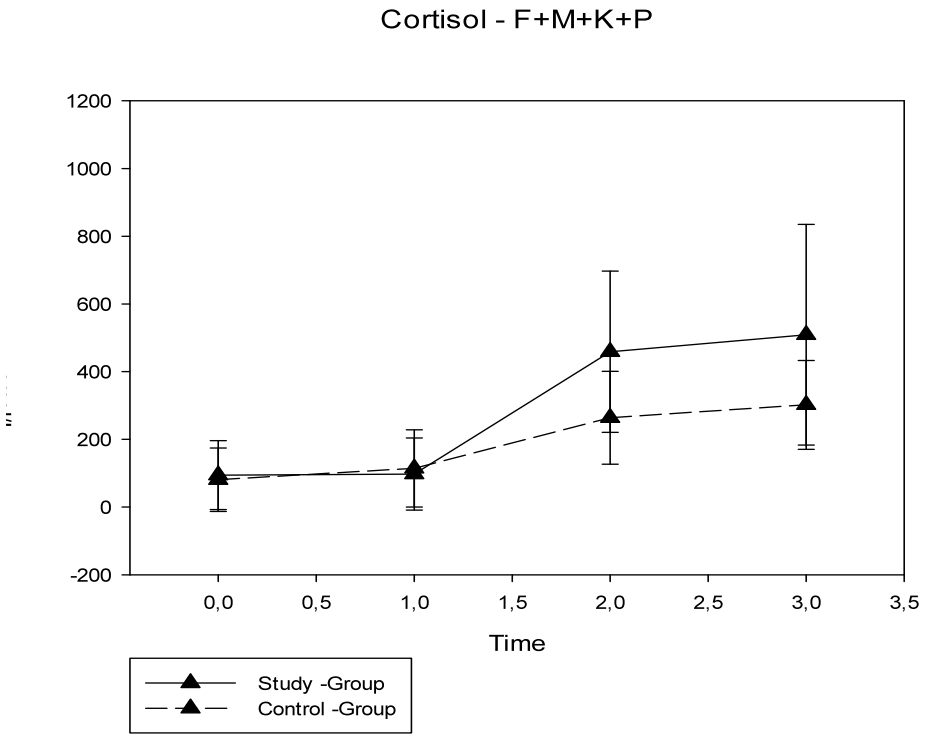
Graph 1: cortisol measurements of study group anaesthetized with two different combination.



Graph 2: cortisol measurements of control group anaesthetized with two different combinations

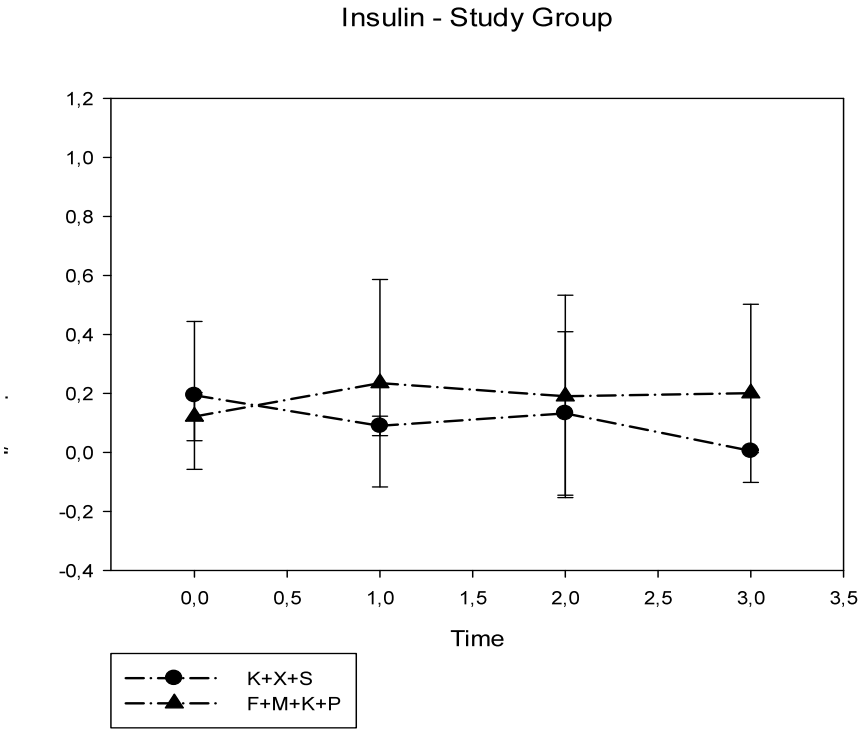


Graph 3: comparison between control to study groups, treated with K+X+S anesthesia.

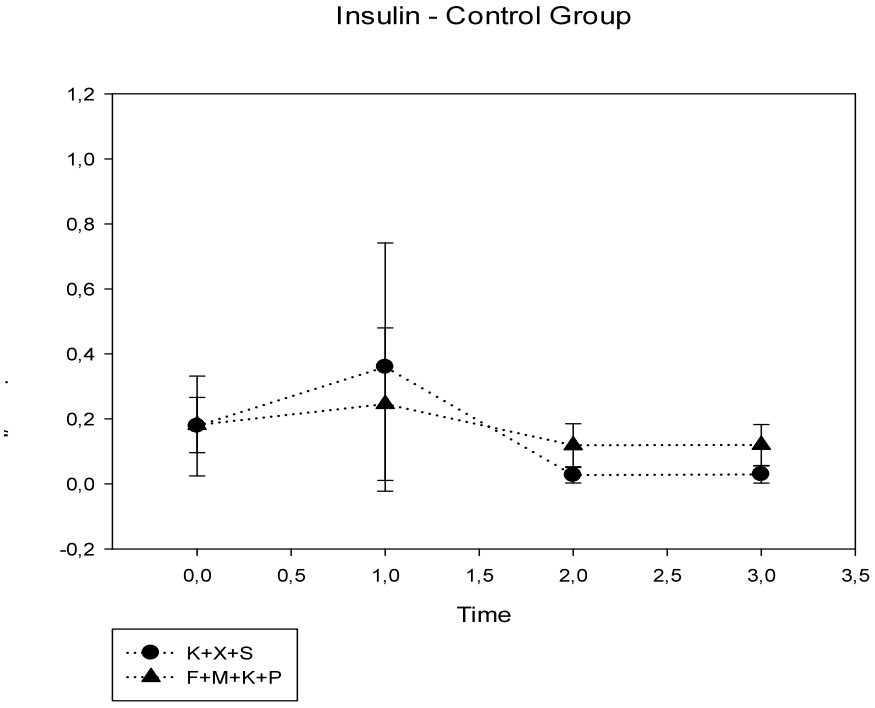


Graph 4: comparison between control to study groups, treated with F+M+K+P anesthesia.

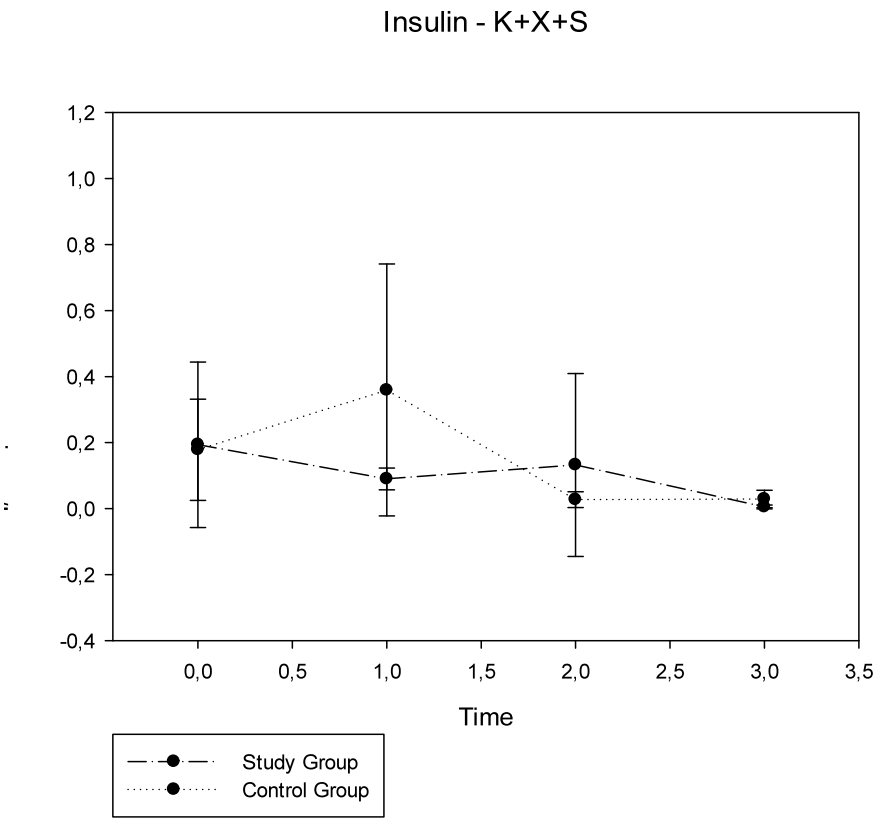
15.2. Insulin measurements



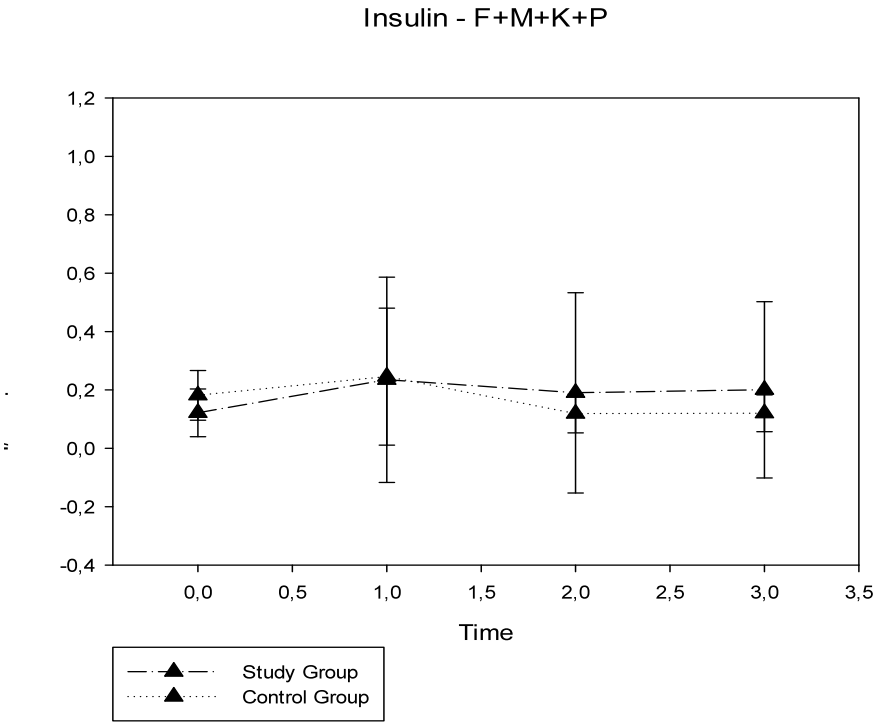
Graph 5: insulin measurements of study group anaesthetized with two different combination.



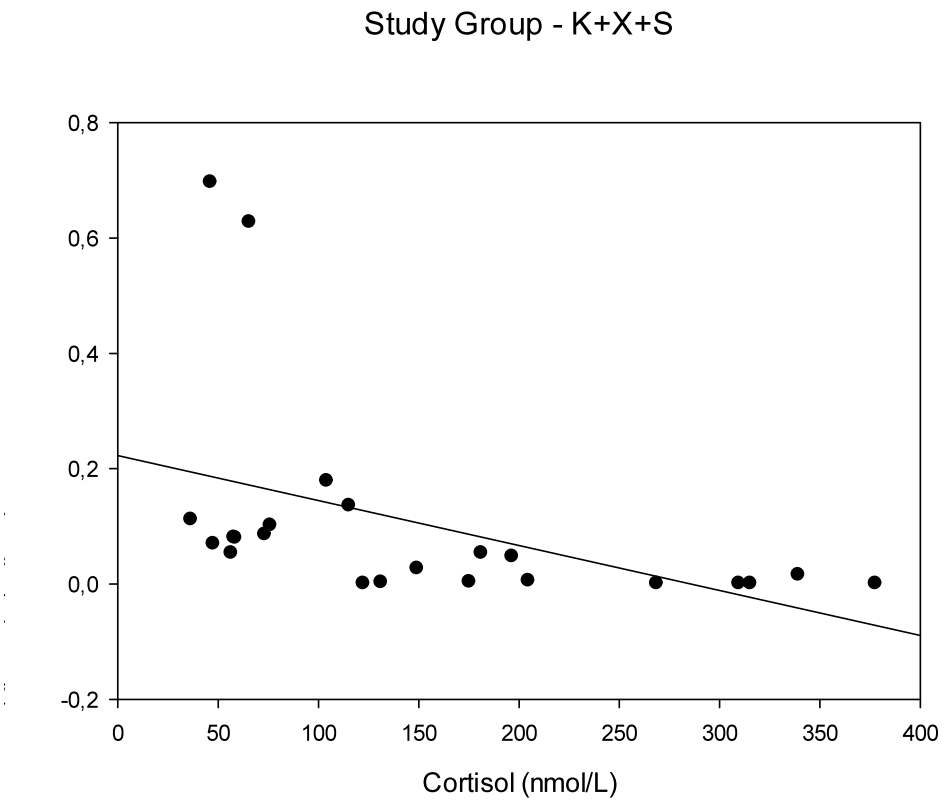
Graph 6: insulin measurements of control group anaesthetized with two different combination



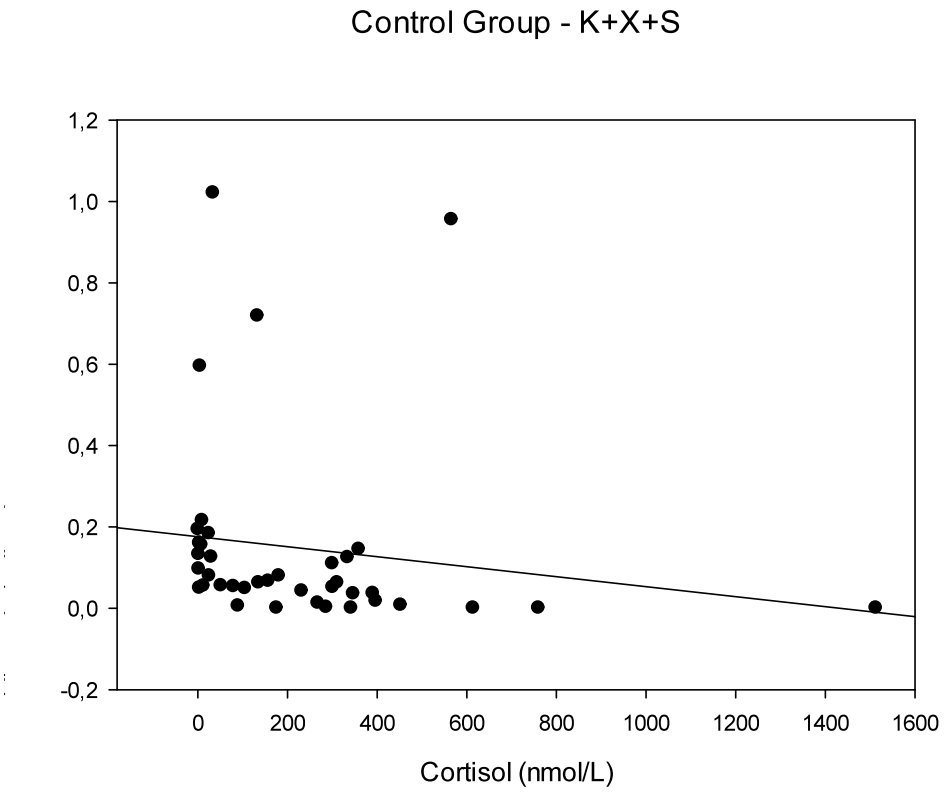
Graph 7: comparison between control to study groups, treated with K+X+S anesthesia



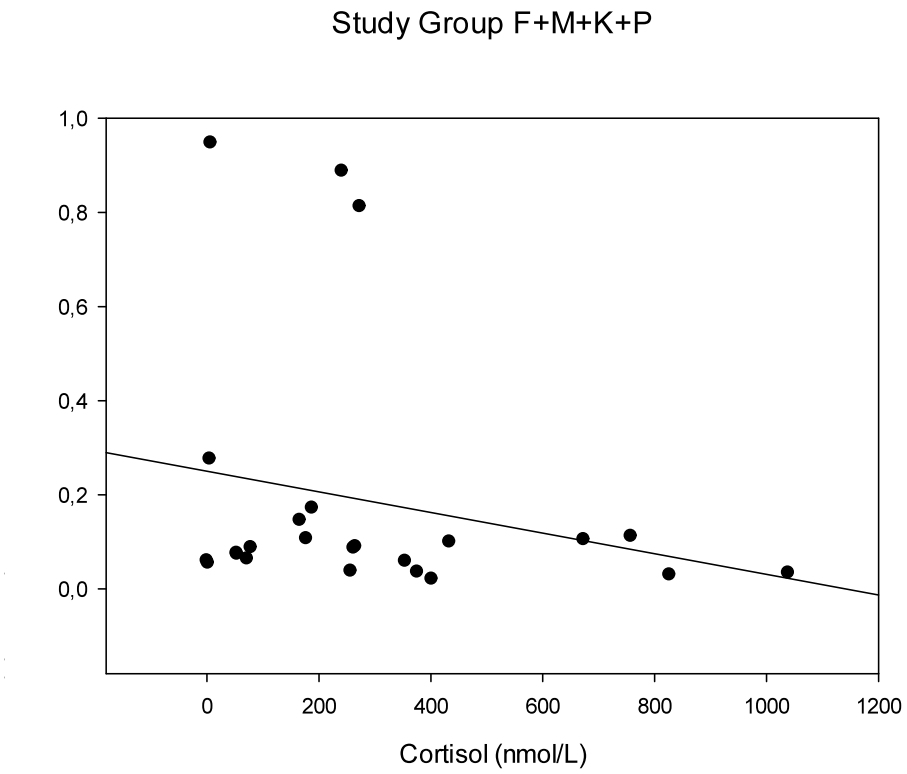
Graph 8: comparison between control to study groups, treated with F+M+K+P anesthesia



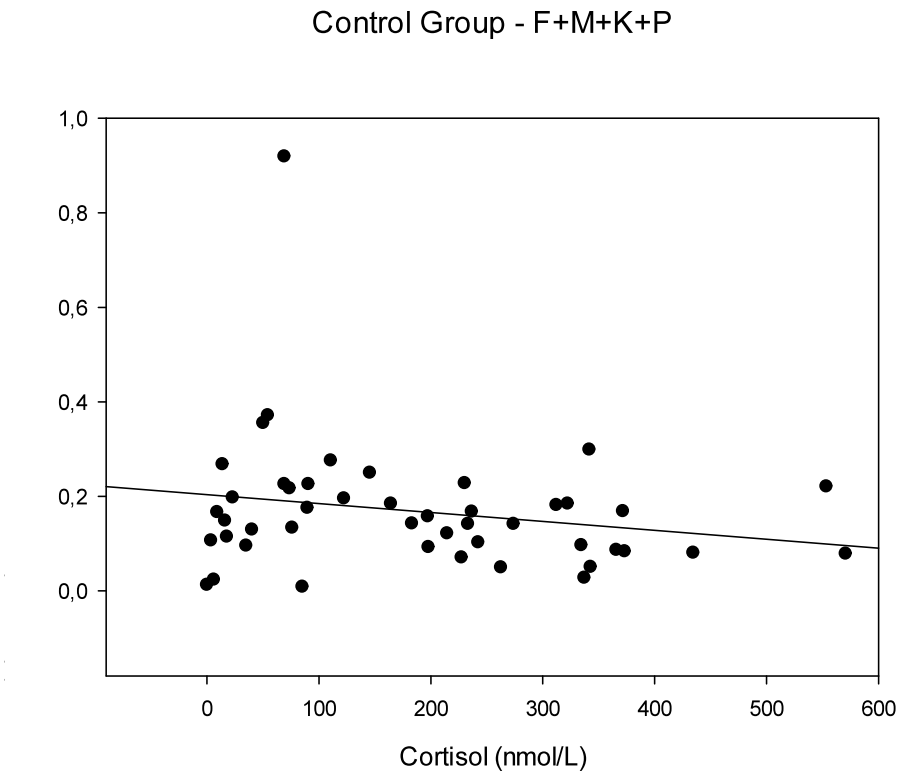
Graph 9: Cortisol and insulin correlation of study group treated with K+X+S



Graph 10: Cortisol and insulin correlation of control group treated with K+X+S



Graph 11: Cortisol and insulin correlation of study group treated with F+M+K+P



Graph 12: Cortisol and insulin correlation of control group treated with F+M+K+P

16. Discussion

As we assumed, cortisol as a final product of the HPA axis, secreted to the blood in response to stress from T0-T3 in both study and cortisol groups in each point time during the procedure. The first peak of cortisol level was at T2, immediate after both ovaries were removed. this suggest that the ovaries removal provoke higher response of pain.
The cortisol secretion to the circulation continued to increase until the procedure has ended and the incision was sutured completely
Comparing the control and study groups, we know that the average time of the procedure in the control group was 16,3 minutes while in the study group it was 30,5 minutes. The measuring T2 and T3, in the control group were closer, compare to the study group, mean that both ovaries were removed faster and in adjacent to the end of the surgery T3 in the control group. That explain us the results of the cortisol increase in the control group.
Comparing the two types of anesthetic combinations, showing no definite results since in the control group there is higher increase when using K+X+S and in the study group, cortisol is higher when using F+M+K+P as premedication and induction. But since the average time of the procedure in the control group was shorter, the cortisol secretion did not decrease until the next sample collection occurred.

The overall degree of insulin level showed a decrease and we can confirm our hypothesis regarding insulin response to stress. Anesthesia and surgery stress response can cause hyperglycemia by increasing anti-insulin hormones that reduce insulin secretion, such as catecholamine, glucagon and cortisol. Comparing the anesthetic types groups and the control and study groups, did not show significant conclusive differences.

17.Conclusions

As we expected, the secretion of cortisol which produces by the adrenal gland in response to stress, increased from (T0) until the end of the operation (T3) drastically in both control and study groups. The insulin blood level showed decrease mainly after induction with no significant difference between the control and study groups.

Beside the increase cortisol and decrease insulin levels, we can see from our results that there is lack of significant differences between our two type anesthetic combinations.

18.Summary

In order to measure ultrashort pain effects during a surgery, 34 female dogs undergo OVE procedure, which consider causing a moderate level of pain. In our research, we focused on the stress system composed of the nervous, endocrine and circulatory system and its response to the surgery by measuring the cortisol and insulin level at four time point during the OVE.

Two types of anesthetic drugs combinations have been used in our research to compare their effect on the stress response during the procedure; xylazine, ketamine and diazepam combination, and fentanyl, ketamine, midazolam and propofol combination.

Moreover, additionally to our research group, a control group operated by expert surgeon was used to discover the effects of time and experience of the operators on our results.

Cortisol concentration level, as we expected, started to increase after induction (T1) and continued to be secreted to the blood reach to highest concentration at the end of the surgery (T3), and insulin concentration level showed an overall decline from time of induction (T1) until the end of the procedure in most of the cases.

19. Bibliography

Anandmay, A.K.,Dass L.L, Sharma A.K. Gutpa M.K.singh K.K Roy B.K (2016) Clinico-anesthetic changes following administration of propofol alone and in combination of meperidine and pentazocine lactate in dogs, *Veterinaty World,*9(11),pp.1178-83.

Even, N., Devaud, J.-M., & Barron, A. B. (2012). General Stress Responses in Honey Bee. *Insects* 2012, *3*, 1271-1298

Burton, D., Nicholson, G. & Hall, G. (2004) Endocrine and metabolic response to surgery. *Continuing Education in Anaesthesia Critical Care & Pain*, 4(5), pp.144-47.

Desborough, J.P. (2000) The stress response to trauma and surgery. *British Journal of Anesthesia, 85(1)*, pp.109-17.

Dubin, A.E. & Patapotian, A. (2010) Nociceptors: the sensors of the pain pathway. *The Journal of Clinical Investigation*,120(11), pp.3760-72.

Finsterwald, C. & Alberini, C.M. (2014) Stress and glucocorticoid receptor-dependent mechanisms in long-term memory: from adaptive responses to psychopathologies. *Neurobiology of Learning and memo*ry, pp.17-29.

Forsberg, Cathrina L. (2001), Biology of reproduction of the dog and modern reproductive technology.
Available from:
https://www.researchgate.net/profile/Catharina_Linde_Forsberg/publication/255573604_Biology_of_Reproduction_of_the_Dog_and_Modern_Reproductive_Technology/links/0deec53b1b42f7d446000000.pdf

Fu, E.S., Miguel, R. & Scharf, J.E. (1997) Preemptive Ketamin Decreases Postoperative Narcotic Requirements in Patients Undergoing Abdominal Surgery. *Anesthesia and Anelgesia,* 84(5), pp.1084-90.

Garimella, V. & Cellini , C. (2013) Postoperative Pain Control. *Clinics in Colon and Rectal Surgery*, 26(3),pp.191-6.

Hankolgu, I. (1992) Steroidogenic enzymes: structure, function, and role in regulation of steroid hormone biosynthesis. *J. Steroid Biochem. Molec. Biol.*, 43(8), pp.779-804.

Hansen, B.D. (2003) Assessment of Pain in Dogs. *Veterinary Clinical Studies, ILAR Journal*, 44(3), pp.197-205.

Hu. Jie, Zhang. Zhonghua, Shen. Wen-Jun & Azhar. Salman. (2010) Cellular cholesterol delivery, intracellular processing and utilization for biosynthesis of steroid hormones. *Nutrition & Metabolism,* 7,47.

Jovanovski-Srceva, M. Kuzmanovska, B. Mojsova, M. & Spirovska, T. (2015) Insulin Resistance, Glycemia and Cortisol Levels in Surgical Patients who Had Preoperative Caloric Load with Amino Acids. *DE GRUYTER,* 36(3), pp.61-69.

Kaka, U., Saifullah, B. & Abubaka, A. (2016) Serum concentration of ketamine and antinociceptive effects of ketamine and ketamine-lidocaine infusions in conscious dogs. *BMC Veterinary Research*, 12(1), pp.198.

Kuchinka, G.D. & Riedesel, D.H. (1994) managment of acute postoperative pain in dogs and cats. *Digital Repository*, 56(2), Article 9, pp. 71-77.

Kutzler, M. & Wood, A. (2006) Non-surgical methods of contraception and sterilization. *Theriogenology,*66(3), pp.514-25.

Latremoliere, A. & Woolf, C.T. (2009) Central Sensitization: A Generator of Pain Hypersensitivity by Central Neural Plasticity. The journal of pain : official journal of the American Pain Society. *The Journal of Pain : Official Journal of the American Pain Society.*, 10(9) pp.895-926.

Mckune, C.M., Pascoe , P.J., Duncan X., B. & Kass, P.H. (2013) The challenge of evaluating pain and a pre-incisional local anesthetic block. *PeerJ*, 2:e341, pp.1-17.

Mehendiratta, O. (2017) *Medicalworld* [Online]. Available from: http://www.medicalworld.co.in/2017/07/physiology-of-adrenal-gland-flash-cards.html

Messlinger, K. (1997) What is nociceptor? *Anaesthesist.*, 46(2), pp.142-53.

Miller, W. (2007) The adernal cortex and its disorders, *In Clinical Pediatric Endocrinology*. 5th ed. Oxford UK: Blackwell publishing Ltd. pp.555-711.

Möstl, E. & Palme, R. (2001) Hormones as indicators of stress. *Domestic Animal Endocrinology*, 23(1-2), pp.67-74.

Munck, A., Guyre, P. & Holbrook, N.J. (1984) Physiological functions of glucocorticoids in stress and their relation to pharmacological action. *Endocrine Rev. 5*, pp.25-44.

Oakley, R.H. & Cidlowski, J.A. (2013) The Biology of the Glucocorticoid Receptor: New Signaling Mechanisms in Health and Disease. *The Journal of Allergy and Clinical Immunology*,125(5), pp.1033–44.

Oconnor, T.M., Ohalloran, D.J. & Shanahan., F. (2000) The stress response and the hypothalamic pituitary adrenal axis: from molecule to melancholia. *QJ Med*, 93(6), pp.323-33.

Quisenberry, D. & Clapp, M.E. (1983) Selecting a Spay/Neuter Program for Animal. *The Humane Society Institute for Science and Policy*, 4(3), 214-225

Randall, M. (2011). The Physiology of Stress: Cortisol and the Hypothalamic-Pituitary-Adrenal Axis
http://dujs.dartmouth.edu/ [Online]. Available from: http://dujs.dartmouth.edu/2011/02/the-physiology-of-stress-cortisol-and-the-hypothalamic-pituitary-adrenal-axis/#.WU-6VhN946V [Accessed 12 fall 2010].

Regidor, P.-A. (2014) “Progesterone in Peri- and Postmenopause: A Review.”. *Geburtshilfe und Frauenheilkunde 74.(11)*, pp.995–1002.

Schaefers-Okkens, A.C., van Goethem, B. & Kirpensteijn, J. (2006) Making a rational choice between ovariectomy and ovariohysterectomy in the dog: A discussion of the benefits of either technique. *Veterinary Surgery*, 35(2), pp.135-43.

Seasholtz, A. (2000) Regulation of adrenocorticotropic hormone secretion: lessons from mice deficient in corticotropin-releasing hormone. *Journal of Clinical Investigation*, 105.(9), pp.1187-88.

Sewer. M.B & Li. D, (2008) Regulation of steroid hormone biosynthesis by the cytoskeleton. *Lipids*,.43(12), pp.1109-15.

Seyfried, O. & Hester, J.H. (2012) Opioids and endocrine dysfunction. *British Journal of Pain*, 6(1):17-24.

Shariati, E., Bakhtiari, J. & Niasari-Naslaji, A.,(2014) Comparison between two portal laparoscopy and open surgery for ovariectomy in dogs. *Veterinary Research Forum*, 5(3), pp.219-23.

Sinclair, Melissa D. (2003). A review of the physiological effects of α_2-agonists related to the clinical use of medetomidine in small animal practice. *The Canadian Veterinary Journal*, *44*(11), 885–897.

Smith, S.M & Vale,W.W,(2006) The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in Clinical Neuroscience*. vol. 8, no. 4,pp. 383-395.

Tsigos, C., Kyrou, I. Kassi, E. & Chrousos, G.P. (2016) Stress, Endocrine Physiology and Pathophysiology. Endotext [Internet] *South Dartmouth (MA):* MDText.com, p.2000.

Wilcox, G. (2005) "insulin and insulin resistance". *Clinical Biochemist Reviews 26.2*, pp.19-39.

Xiao, Y.F., Wang, B., Wang, X., Du, F. Benzinou, M. & Wang Y.X.J (2013) Xylazine-induced reduction of tissue sensitivity to insulin leads to acute hyperglycemia in diabetic and normoglycemic monkeys. *BMC Anesthesiology*, vol.10, pp.13:33.

ZRTlabratory, c.o. (2016) *hormone cascade* [Online]. Available at: https://www.zrtlab.com/images/documents/Steroid_Hormone_Cascade.pdf [Accessed 30 October 2017].

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21. Appendix

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