UNIVERSITÀ DEGLI STUDI DI PADOVA

UNIVERSITÁ DEGLI STUDI DI PADOVA
Dipartimento di Medicina Animale, Produzione e Salute

Corso di laurea magistrale a ciclo unico in
MEDICINA VETERINARIA

CLINICAL USE OF LOW DOSE MEGESTROL ACETATE TREATMENT IN THE QUEEN FOR REPRODUCTION CONTROL: GENERAL HEALTH, EFFICACY AND INTERVAL FROM TREATMENT END TO RESUMPTION OF CYCLICITY

Relatore:
Prof: Stefano Romagnoli
Correlatore:
Dott.ssa: Maria Carlos Pereira
Dott.ssa. Zakošek Pipan, Maja

Laureanda:
Anna Grassi
Matricola n° 1192911

ANNO ACCADEMICO 2023/2024

ABSTRACT

Megestrol acetate (MA) is a synthetic analog of progesterone. Originally formulated as an oral contraceptive for human use, its application has expanded to veterinary medicine. In bitches, it is used for estrus suppression, deferring the onset of heat. In queens, its administration comprises both short-term and extended suppression of estrus.
Estropill™ (MSD, Italy) is a syrup MA formulation commercialized with the indication of heat prevention in queens. Our aim was to investigate the efficacy and safety of a low-dose MA protocol in suppressing cyclicity in queens and mating behavior and fertility of tomcats. We further set to estimate the interval between the end of treatment and the resumption of ovarian activity resumption in treated females.
A total of 21 post-pubertal, healthy, privately-owned intact cats (18 queens and 3 tomcats) from 2 different facilities (Veterinary Teaching Hospitals of the universities of Padova, Italy and Ljubljana, Slovenia) were enrolled in the study and treated daily with an oral formulation of 11.5 μg/MA/kg (approximately 5 drops/kg/day PO). Animals were categorized according to treatment duration, which was decided based on owners' request: 4 months (G4 4 females and 1 male), 5 months (G5 – 3 females and 2 males) and 6 months (G6 – 11 queens).
A complete clinical and reproductive examination, hematology, biochemistry, urinalysis, and reproductive ultrasound were performed on all subjects before and after treatment. In addition, queens underwent vaginal cytology and progesterone assay while tomcats were checked for the presence of penile spikes before and after MA therapy. Subjects were monitored monthly repeating physical examination, reproductive ultrasound, and vaginal smears. Queens were reexamined upon demonstrating signs of heat after treatment. Estrus was confirmed cytologically.
Time until ovarian activity resumption post-treatment, for queens, and weight gain, for all subjects, were statistically analyzed.
Seventeen/18 queens exhibited consistent behavioral and cytological patterns of anestrus during treatment. The time until the resumption of ovarian activity post-treatment had an overall mean of 50.12 ± 17.08 days and was not significantly different between treatment groups (G4: 42.33 ± 30.08, G5: 49.3 ± 10.21, and G6: 52.45 ± 15.5 days). One queen was considered an outlier due to a prolonged delay in heat return.
Two out of three tomcats arrested urinary marking and mating behaviors one month after treatment, with penile spikes disappearing within three months. The third male showed only a reduction in the size
of the spikes and continued displaying mounting behavior, successfully impregnating a queen during treatment.
Laboratory and ultrasonographic parameters during monthly checkups remained unchanged. However, all subjects exhibited increased appetite during treatment, resulting in weight gain. Weight gain was observed in all treatment groups, being statistically significant in G4 and G6. Most queens and all tomcats experienced non-significant weight loss following the end of treatment.
The queen experiencing signs of heat during treatment showed substantial weight gain, which might have resulted in underdosing. This suggests that the dosage employed is close to the minimum effective dose of the drug, thus contributing to a greater safety of treatment.
Conversely, this therapy does not seem to be fully effective for contraception in males. Further studies with an increasing number of tomcats and MA dosage could enhance outcomes, while monitoring the weight fluctuation could allow a better understanding of the impact of the drug in influencing appetite and weight gain.

RIASSUNTO

Il Megestrolo Acetato (MA) è un analogo sintetico del progesterone. Originariamente formulato come contraccettivo orale per uso umano, la sua applicazione si è estesa anche alla Medicina Veterinaria. Nelle cagne, viene utilizzato per sopprimere l'estro, ritardando l'inizio del calore. Nelle gatte, la sua somministrazione comprende sia la soppressione a breve termine che quella prolungata dell'estro.
Estropill^{TM} (MSD, Italia) è una formulazione di MA in sciroppo commercializzata con l'indicazione per la prevenzione del calore nelle gatte. L'obbiettivo di questa tesi era quello di investigare l'efficacia e la sicurezza di un protocollo a basso dosaggio di MA nella soppressione della ciclicità nelle gatte e nel comportamento di accoppiamento e nella fertilità dei gatti maschi. Inoltre, registrare l'intervallo tra la fine del trattamento e la ripresa dell'attività ovarica nelle femmine trattate.
Un totale di 21 gatti interi post-puberi, sani e di proprietà privata (18 femmine e 3 maschi) provenienti da 2 diverse strutture (Ospedali Veterinari Didattici delle università di Padova, Italia e Lubiana, Slovenia) sono stati arruolati nello studio e trattati giornalmente con una formulazione orale di 11,5 μg/MA/kg (approssimativamente 5 gocce/kg/giorno PO). Gli animali sono stati categorizzati in base alla durata del trattamento, decisa secondo richiesta dei proprietari: 4 mesi (G4 4 femmine e 1 maschio), 5 mesi (G5 - 3 femmine e 2 maschi) e 6 mesi (G6 - 11 femmine).
Un esame clinico completo ed esame riproduttivo, ematologia, biochimica, analisi delle urine e ecografia riproduttiva sono stati eseguiti su tutti i soggetti prima e dopo il trattamento. Inoltre, le femmine sono state sottoposte a citologia vaginale e dosaggio del progesterone mentre i maschi sono stati controllati per la presenza di spicole peniene prima e dopo la terapia con MA. I soggetti sono stati monitorati mensilmente ripetendo l'esame fisico, l'ecografia riproduttiva e gli strisci vaginali. Le femmine sono state riesaminate alla manifestazione dei segni di calore dopo il trattamento. L'estro è stato confermato citologicamente.
Il tempo fino alla ripresa dell'attività ovarica post-trattamento, per le femmine, e l'aumento di peso, per tutti i soggetti, sono stati analizzati statisticamente.
Diciassette su diciotto femmine hanno mostrato pattern comportamentali e citologici di anestro durante il trattamento. Il tempo fino alla ripresa dell'attività ovarica post-trattamento ha avuto una media complessiva di 50,12 ± 17,08 giorni e non è stato significativamente differente tra i gruppi di trattamento (G4: 42,33 ± 30,08, G5: 49,3 ± 10,21 e G6: 52,45 ± 15,5 giorni). Una gatta è stata considerata un outlier a causa di un prolungato ritardo nel ritorno del calore.
Due dei tre maschi hanno interrotto la marcatura urinario e i comportamenti di accoppiamento un mese dopo il trattamento, con la scomparsa delle punte peniene entro tre mesi. Il terzo maschio ha mostrato solo una riduzione delle dimensioni delle spicole e ha continuato a mostrare comportamenti di monta, ingravidando con successo una gatta durante il trattamento.
I parametri di laboratorio e ultrasonografici durante i controlli mensili sono rimasti invariati.
Tuttavia, quasi tutti i soggetti hanno mostrato un aumento dell'appetito durante il trattamento, con conseguente aumento di peso. L'aumento di peso è stato osservato in tutti i gruppi di trattamento, risultando statisticamente significativo in G4 e G6.
La maggior parte dei soggetti ha sperimentato una perdita di peso non significativa dopo la fine del trattamento.
La femmina che ha manifestato segni di calore durante il trattamento ha mostrato un aumento di peso sostanziale, che potrebbe essere risultato in sottodosaggio. Ciò suggerisce che il dosaggio impiegato sia vicino alla dose minima efficace del farmaco, contribuendo così a una maggiore sicurezza del trattamento.
Al contrario, questa terapia non sembra essere completamente efficace per la contraccezione nei maschi. Studi futuri con un numero crescente di gatti maschi e dosaggio di MA potrebbero migliorare gli esiti, mentre il monitoraggio delle fluttuazioni di peso potrebbe consentire una migliore comprensione dell'impatto del farmaco nell'influenzare l'appetito e l'aumento di peso.

INDEX

CHAPTER 1 – INTRODUCTION

Traditionally, surgical interventions were employed to control reproduction in pet queens while breeding queens have been managed for decades using hormones to comply with the Fédération *Internationale Féline* (FIFe) rule that "queens must not have more than three litters in twenty-four months except with the prior written approval of a veterinarian and/or a FIF member" (Romagnoli & Ferre-Dolcet, 2022) (Fife, 2022). Therefore, to comply with this requirement, breeders often need to resort to a temporary interruption of the queen's estrous cycle for several months during each year. Historically, the reversible control of feline reproduction has predominantly relied on synthetic derivatives of progesterone, commonly referred to as progestogens or progestins. Unfortunately, progestogens have been misused for a long time based on an inadequate assessment of the evidence published in the veterinary literature (Romagnoli, 2015). Recent years have witnessed the emergence of alternative drugs for reversible reproduction control in queens, including long-acting gonadotropin-releasing hormone (GnRH) agonists and melatonin, which have gained substantial interest and recognition as standard approaches. Notwithstanding these advancements, progestogens maintain their relevance in the feline practitioner's arsenal due to their established safety profile and reliability in terms of duration of action.

The following chapters explain the reproductive anatomy and physiology of the queen and present Megestrol Acetate, the progestin used in this study.

CHAPTER 2 – REPRODUCTIVE ANATOMY OF THE QUEEN

The reproductive anatomy of the queen consists of the vulva, vestibule, vagina, cervix, uterus, uterine tubes, ovaries, and mammary glands (Johnson A. & Kutzler, 2022).

2.1 – OVARIES

The ovary has an ovoid and relatively dense structure, serving as the primary site for the production of female gametes (ova) and the hormones estrogen (E2) and progesterone (P4). Additionally, the corpus luteum (CL) contributes to the production of P4, oxytocin, relaxin, inhibin, and activin. The ovary is composed of an outer connective tissue called tunica albuginea. Situated beneath the tunica albuginea there is a region known as the ovarian cortex, housing the population of oocytes, the functional CL, and the degenerating CLs, so-called *corpora albicans*.
The central part of the ovary is the ovarian medulla, where the vasculature, nerves, and lymphatic vessels are allocated. It is composed of relatively dense connective tissue (Senger, 2012).

The feline ovary has an elliptical shape with a length ranging between 8 and 9 mm and it is typically situated caudally to the caudal pole of the kidney, firmly anchored just beneath the third and fourth lumbar vertebrae.
The left ovary commonly resides lateral to the descending colon, while the right ovary tends to be adjacent to small intestinal loops. The ovarian bursa does not present adipose tissue.
The macroscopic presentation of the ovary undergoes alterations corresponding to different stages of the estrous cycle. During periods of seasonal or lactational anestrus, the ovarian surface appears smooth, and pre-antral follicles are recognizable solely through histological examination. As the follicular phase (estrus) approaches, three to seven follicles undergo enlargement, while the remainder undergo atresia.
Feline follicles measure 2–3 mm in diameter at ovulation and are observable through transabdominal ultrasonography (see Figure 1) (Johnson A. & Kutzler, 2022).
Histologically, cat ovaries can be distinguished from those of other domestic animal species by the abundance of primordial follicles beneath the tunica albuginea.

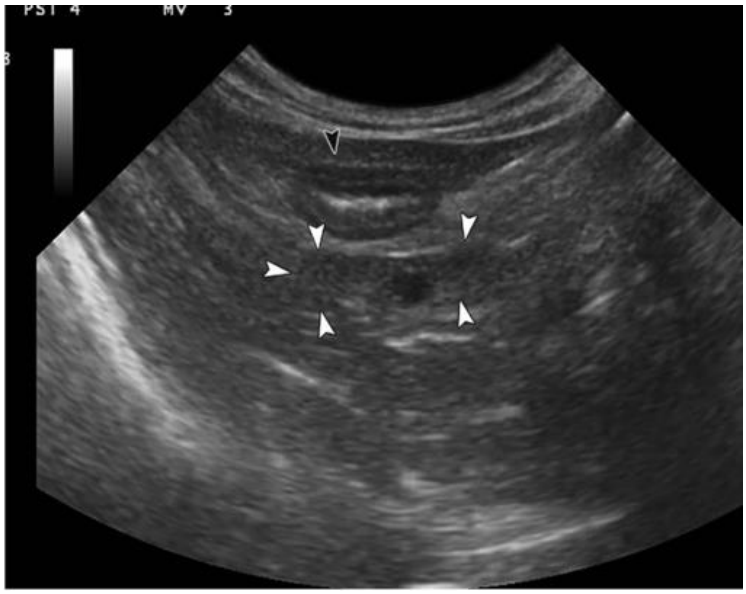

Figure 1: Ultrasound image of the right ovary of a queen (white arrowheads) with a mature follicle (2 mm anechoic structure) - black arrow is a loop of small intestine *(Johnson A. & Kutzler, 2022)*.

Primordial follicles are characterized by a single layer of flattened pre-granulosa cells surrounding an oocyte that is 20–30µm-long in diameter. Primary follicles, larger than primordial follicles, exhibit a zona pellucida with a single layer of cuboidal granulosa cells and a basement membrane separating the granulosa cells from the ovarian cells.
Secondary follicles feature more than one granulosa cell layer and a theca cell layer on the opposite side of the basement membrane (Johnson A. & Kutzler, 2022).
In prepubertal queens, there are over 300 primordial follicles, 25 primary follicles, and 9 secondary follicles per mm^2 of ovarian cortex, and this composition remains relatively unchanged until after puberty. Tertiary follicles, characterized by their large size ranging from 400 to 1000 µm in diameter, exhibit increased layers of theca cells. The primary feature of a tertiary (antral) follicle is the presence of a fluid-filled antrum (see Figures 2 and 3) (Johnson A. & Kutzler, 2022).

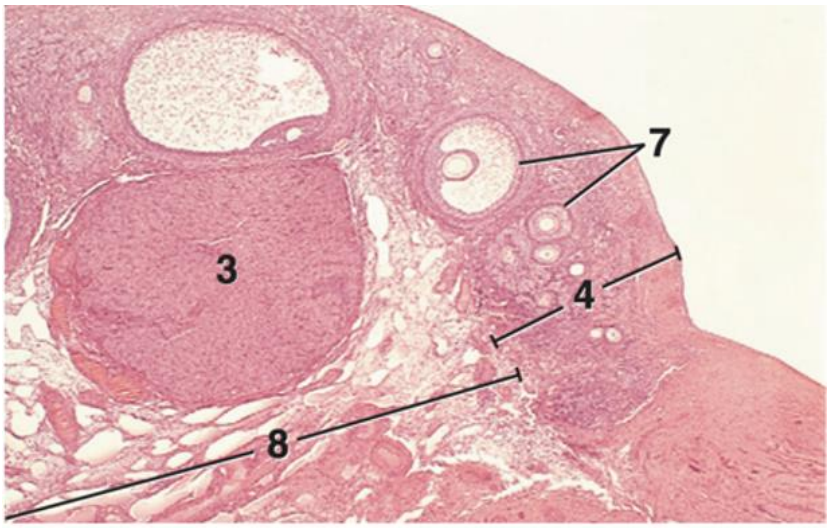

Figure 2: Ovary, queen. Follicles of various ages and CL can be seen in the cortex. A portion of the vascular medulla is present.
3 - CL, 4 - Cortex, 7 - Growing follicles, 8 - Medulla *(Bacha & Linda M., 2012)*.

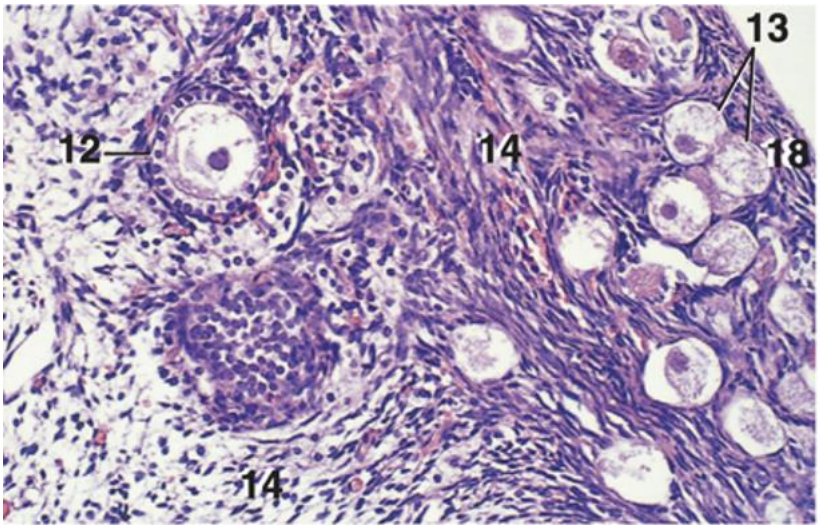

Figure 3: Early follicles in the outer portion of the cortex region of the ovary. 13 - Primordial follicles, 12 - Primary follicles, 14 - Stroma, 18 - Tunica albuginea *(Bacha & Linda M., 2012)*.

2.2 – UTERINE TUBE

The feline uterine tube has its opening into the uterus through a papilla that extends into the uterine lumen. This structure measures 4–9 cm in length and is divided into 4 portions: fimbriae, infundibulum, ampulla, and isthmus.
During ovulation, oocytes are captured by the fimbriae, and transported through the conical-shaped infundibulum into the ampulla. Fertilization occurs at the junction of the ampulla and isthmus in the presence of sperm. The uterine tubes' secretions create an environment that allows the gamete's survival, fertilization, and the initial days of embryonic life.
Unlike the dog, the feline mesosalpinx does not contain adipose tissue (Johnson A. & Kutzler, 2022).

2.3 – UTERUS

The uterus serves as a link connecting the oviducts to the cervix. The outer layer is made of serosa, known as the perimetrium, which is a component of the peritoneum.
Together, the outer longitudinal muscle layer and the inner circular muscle layer form the myometrium that fulfills several physiological functions.
The inner portion of the uterus is constituted by the mucosa and submucosa, collectively referred to as the endometrium. Within the mucosal epithelium, there are uterine glands responsible for secreting substances into the uterine lumen, enhancing embryo development, and promoting sperm viability (Senger, 2012).
The primary functions of the uterus are:

- Sperm transport.
- Luteolysis and control of cyclicity.
- Provision of an environment for the survival of the embryo.
- Maternal contribution to the placenta.
- Expulsion of the fetus and fetal placenta (Senger, 2012).

Queens possess a bipartite uterus characterized by a small uterine body (up to 2 cm in length), long uterine horns (up to 10 cm), and a weight of approximately 1.5 grams in the nonpregnant state. However, the dimensions of the feline uterus are dependent upon the queen's size, age, parity, and the stage of the estrous cycle or pregnancy (Johnson A. & Kutzler, 2022).
The feline myometrium undergoes thickening after puberty, with both the endometrium and myometrium exhibiting increased thickness during the follicular phase.
In the anestrus phase, the feline endometrial glands are straight, short, and narrow. Transitioning to proestrus and estrus, the endometrial glands dilate, and both the surface and glandular epithelium increase in thickness, accompanied by the development of mitotic figures within the cells of the epithelium. During the luteal phase, the endometrial glands enlarge and assume a tortuous configuration (Johnson A. & Kutzler, 2022).

Ultrasonographically, the uterine body can be identified between the urinary bladder and colon as a homogeneous, hypoechoic tubular structure encircled by a thin hyperechoic line.
In most examinations, a discernible thin hypoechoic line exists between the endometrium and myometrium (see Figure 3). The lumen of the nonpregnant uterus appears as a thin hyperechoic line at the center of the uterus (Johnson A. & Kutzler, 2022).

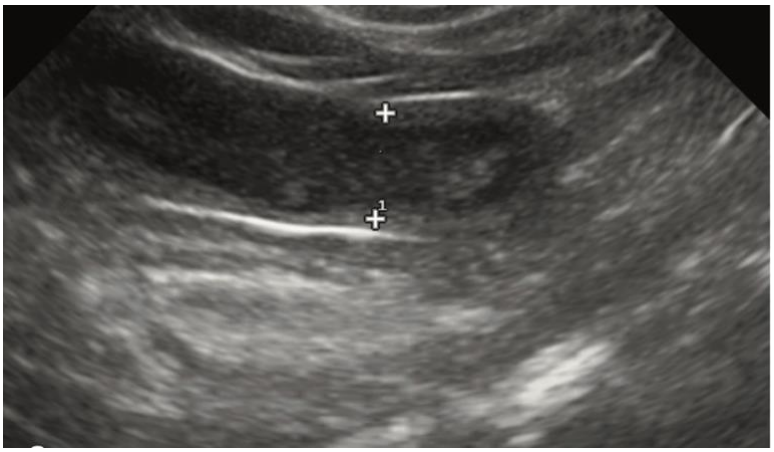

Figure 4: Ultrasound image of the uterus of a healthy postpubertal queen in anestrus. The uterus (between the measuring calipers) is imaged longitudinally and measures 0.78 cm in diameter and presents normal echotexture *(Johnson A. & Kutzler, 2022)*.

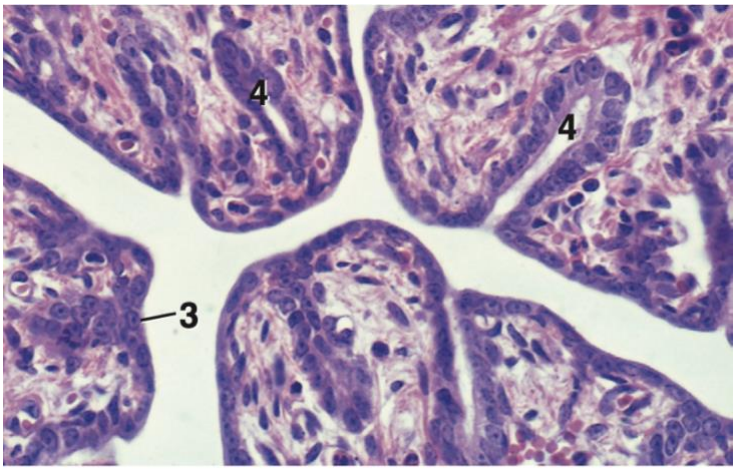

Figure 5: Histological section of the uterine horn of an anestrus queen. The lumen of the uterus in anestrus is lined by a simple cuboidal epithelium. 3- cuboidal epithelium, 4 – endometrial glands (Bacha & Linda M., 2012).

2.4 – CERVIX

The cervix is an important structure that acts as a barrier to stop the transport of sperm and the entrance of pathogenic agents. It is characterized by its relatively thick-walled and non-compliant nature. During pregnancy, the cervix serves to isolate the uterus from the external environment by creating a barrier composed of highly viscous mucus (Senger, 2012).
Compared to other species, the feline cervix has poor definition. It has thickened walls with transverse folds and contains mucus-secreting glands. The cervix protrudes as a prominent papilla into the vagina and is oriented ventrocaudally. The patency of the cervical canal varies throughout the estrous cycle and is open exclusively during estrus and parturition (Johnson A. & Kutzler, 2022).

2.5 – VAGINA

The vagina serves as the primary copulatory organ, as well as a passage for urine expulsion during micturition. Additionally, it acts as a passive birth canal during parturition. The vaginal structure comprises a poorly organized and ill-defined muscular layer with a well-developed, highly adapted mucosal epithelium. The caudal vagina is distinguished by its stratified squamous epithelium, similar

to the one found in the skin. The degree of secretory activity and the thickness of the stratified squamous epithelium in the caudal vagina undergo alterations in response to the endocrine status of the female.
In queens and bitches, it is possible to determine the stage of the reproductive cycle by microscopically observing the types of cells present in the vagina, which can be obtained through vaginal lavage or swabbing. During periods of E2 dominance, particularly during estrus, the stratified squamous epithelium undergoes a significant thickening. This thickening has two protective functions: first, it mechanically protects the vagina during copulation, and second, the thickened mucosa acts as a barrier preventing microorganisms from gaining access to the vasculature in the submucosa (Johnson A. & Kutzler, 2022).

2.6 – VESTIBULE

The vestibule represents the segment of the vagina that serves as a shared space for both the urinary and reproductive systems). It develops from the external urethral orifice to the labia of the vulva.
The queen possesses many vestibular glands, measuring approximately 5 mm in size, situated in the lateral walls of the vestibule, with small openings on the vestibular floor.
The urethral orifice emerges onto a urethral tubercle, elevated above the floor of the vestibule (Johnson A. & Kutzler, 2022).

2.7 – VULVA

The vulva constitutes the external portion of the female reproductive tract, it is composed of two labia (major and minor) that converge at the medial aspect to create two commissures, or sites of union, positioned just below the anus; they exhibit an even closure without any noticeable gap.
Under typical conditions, the labia form a closure mechanism, minimizing the entrance of foreign material into the vagina. The skin of the labia is an integral part of the integumentary system and includes numerous sebaceous and sweat glands, as well as hair follicles (Senger, 2012).
During estrus, the feline vulva may experience a slight enlargement and become edematous, but overall, it undergoes minimal physical changes. Additionally, the vulva might be slightly smaller in spayed females compared to unaltered queens.
It is noted that the vulva and the vestibule stand out as the only well-innervated reproductive organs in the queen, receiving sensory nerve fibers (Johnson A. & Kutzler, 2022).

2.8 – MAMMARY GLANDS

Queens typically possess four pairs of bilaterally symmetric mammary glands—cranial thoracic, caudal thoracic, cranial abdominal, and caudal abdominal. Each mammary gland comprises multiple lobes, drained by four to eight ducts that lead to individual openings on each teat. Occasionally, non-functional accessory teats may be found in the inguinal region, unrelated to any mammary tissue. It is important to distinguish them from functional supernumerary teats.
Feline mammary tissue expresses both E2 and P4 receptors. Stimulation of these receptors during puberty and pregnancy induces mammary epithelial proliferation and mammogenesis, respectively.
Throughout lactation, concentrations of milk components (e.g., total solids, crude protein, fat, lactose) exhibit significant variations based on mammary gland (teat) location, lactation stage, diet, and litter size (Senger, 2012).
Postnatal changes in the mammary gland occur during various phases:

- Between birth and puberty.
- Between puberty and pregnancy.
- During pregnancy.
- During lactation.

- During involution (Johnson A. & Kutzler, 2022).

CHAPTER 3 - PHYSIOLOGY OF FELINE REPRODUCTION

3.1 – ENDOCRINE CONTROL OF REPRODUCTION

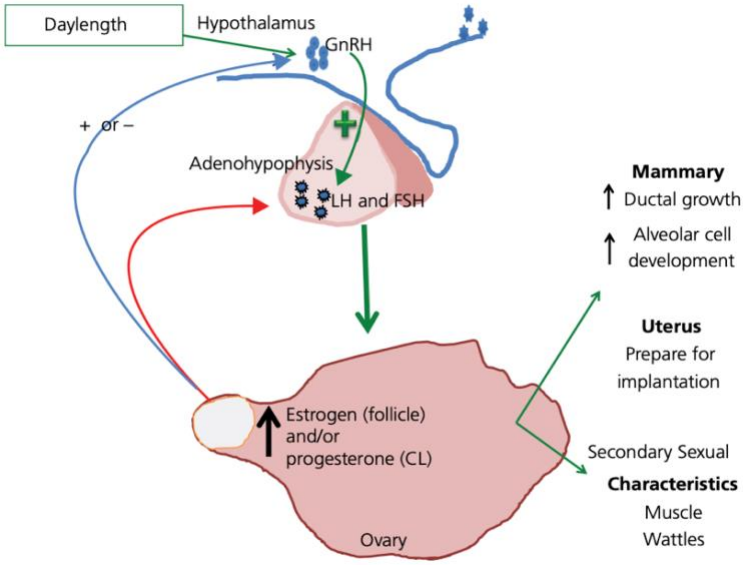

Figure 6: GnRH, produced by the hypothalamus, travels to the adenohypophysis via the hypothalamo-hypophyseal portal system, stimulating the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the adenohypophysis. FSH travels to the ovary, initiating the maturation of one or more follicles. Within the developing follicle, FSH stimulates the granulosa cells to secrete E2. E2 impacts the growth of the uterus and mammary gland and stimulates hypothalamic secretion of GnRH through positive feedback. This feedback loop reaches a point where sufficient GnRH secretion is triggered, leading to a surge in LH secretion, which culminates in the ovulation of the mature follicle. Throughout different reproductive stages, E2 can inhibit LH and FSH secretion by the adenohypophysis. Following ovulation, LH stimulates the ovulated follicle to transform into a corpus luteum (CL). The luteinized granulosa cells of the CL produce progesterone, crucial for preparing the uterus for conceptus implantation and for alveolar cell development in the mammary gland. Progesterone provides feedback to the hypothalamus and adenohypophysis, inhibiting GnRH FSH and LH secretion, respectively (Reece, 2015).

Gonadotropes located within the *pars distalis* of the adenohypophysis produce follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Reece, 2015).
In females, FSH stimulates ovarian follicular development and induces the cells of the developing follicle wall to secrete E2.; this hormone induces changes in the reproductive tract and mammary gland necessary for reproduction. LH is responsible for inducing ovulation in many species; it also causes the cells comprising the ovulated follicle to alter their phenotype, becoming P4-secreting cells and forming the CL. P4 is essential for preparing the uterus for the implantation of a fertilized egg (Reece, 2015).
The secretion of LH and FSH is stimulated by gonadotropin-releasing hormone (GnRH). This neurohormone is produced in the hypothalamus and secreted into the hypothalamo-hypophyseal portal system (figure 6). Various factors, such as day length (particularly for species that are seasonal breeders) and signals from the pregnant uterus and fetal placenta dictate when and how much GnRH is to be secreted. Age and nutrition levels also impact GnRH secretion. E2 can have a stimulatory effect on GnRH secretion when the goal is to induce ovulation of the developing follicle, while at other times, E2 can decrease GnRH secretion. P4 often provides feedback to the hypothalamus to reduce the secretion of GnRH. The control of GnRH, LH, and FSH secretion varies considerably among species (Reece, 2015).

3.2 – THE FELINE ESTRUS CYCLE AND SEASONALITY

The queen exhibits seasonal polyestrous reproductive behavior, engaging in repeated estrous cycles throughout a breeding season, unless interrupted by ovulation, pregnancy, or disease.
The photoperiod, characterized by the ratio of light to darkness, plays a role in initiating and influencing the cyclicity of queens. When the photoperiod is shortened (decreasing numbers of light during the day), there is an increase in melatonin secretion by the pineal gland (Johnson A. & Kutzler, 2022; Leyva et al., 1989).
A period of extended daylight (at least 12 hours per day) is required for follicular activity in the cat (Hurni, 1981); the initiation of cyclicity is related to an increase in daylight duration, while anestrus starts following a reduction in daylight hours.
Cats residing in a home environment are often exposed to both natural and artificial light.
In northern temperate zones, the cat breeding season typically starts around February (spanning from January to March) and ends in September (with a range from June to November).
Notably, long-haired breeds tend to manifest a shorter and more clearly defined breeding season compared to short-haired breeds (Johnson A. & Kutzler, 2022)

3.3 – PUBERTY

The onset of puberty in female domestic animals is typically attributed to either the onset of the first estrus or the first ovulation (Johnson A. & Kutzler, 2022).
There is significant variation in the rate at which a mammal matures reproductively. In domestic queens, puberty occurs at 181–560 days of age (average: 345.0 ± 0.9 days) and significantly earlier in kittens born between March and June in the Northern Hemisphere compared to all other times of the year) (Tsutsui et al., 2004). In other studies, puberty was reported to occur earlier at 13.3 ± 0.4 weeks of age (Faya et al., 2013).
The average body weight at puberty is 2.5–3.0 kg (about 80% of the adult body weight) (England, 2010). In addition to body weight, the onset of feline puberty is affected by other intrinsic factors (e.g. breed) as well as extrinsic factors (e.g. environment, season) (Jemmett & Evans, 1977; Romagnoli et al., 2019).
Photoperiod, as mentioned before, plays an important role in determining the onset of puberty in queens. A study that involved 174 queens acclimated under natural photoperiod demonstrated that the interval between birth and puberty varied significantly depending on the birth month; in particular, the interval was shorter in queens born between July and October: these had the onset of the ovarian activity the year after birth (mean 242.2 days) (Tsutsui et al., 2004).

3.4 - PHYSIOLOGY OF OVULATION

The domestic cat is typically classified as an induced ovulator, but cases of spontaneous ovulation have been documented. The frequency and rate of spontaneous ovulation vary significantly across studies, ranging from 35% to 87%, with some females rarely exhibiting spontaneous ovulation, while others consistently do so (Graham et al., 2000; Gudermuth et al., 1997; Johnson A. & Kutzler, 2022; Pelican et al., 2005).
There is a suggestion that high rates of spontaneous ovulation may contribute to the development of the cystic endometrial hyperplasia-pyometra complex in cats, possibly mediated through prolonged periods of P4 influence on the endometrium.
In the classical induced ovulation reflex, vaginal stimulation resulting from the tomcat's spined penis during copulation is transmitted through a spinal afferent nervous pathway to the queen's hypothalamus. This leads to the release of GnRH and subsequently triggers a pulsatile release of LH from the anterior pituitary gland.

The LH surge usually occurs within 10 minutes of copulation, and its amplitude correlates with the number of copulations. Peak LH levels are observed 4 hours after 8–12 copulations (Johnson A. & Kutzler, 2022). The peak LH level is notably lower when the queen engages in only four breedings within the 4-hour period, and it is further reduced with only one breeding (Concannon et al., 1980).

3.5 – PHASES OF THE ESTRUS CYCLE

The estrous cycle of the queen comprises two major phases, the follicular phase and the luteal phase, but typically is divided into five stages: proestrus, estrus, interestrus, diestrus, and anestrus.
Follicular phases comprise proestrus, estrus, and interestrus while the luteal phase comprises diestrus. Each phase is associated with distinct changes in hormones and behavior. While changes in vaginal cytology do occur, they are not as clearly demarcated as they are in the bitch (Johnson A. & Kutzler, 2022).

3.5.1 – FOLLICULAR PHASE

The follicular phase is characterized by four key events:

- Elevated gonadotropin secretion from the anterior lobe of the pituitary;
- Follicular growth and preparation for ovulation;
- Sexual receptivity;
- Ovulation.

During this phase, estradiol is the predominant hormone secreted by developing follicles, stimulating profound changes that prepare the reproductive tract for copulation. In non-primate mammals, reproductive behavior is induced by estradiol. Moreover, estradiol governs the onset of the preovulatory LH surge, a pivotal event leading to ovulation. Ovulation is a complex cascade of physiological and biochemical changes culminating in the maturation of preovulatory follicles and the release of the oocyte from the ovary(Senger, 2012).

3.5.2 – LUTEAL PHASE

The luteal phase encompasses three major processes:
luteinization (the transformation of follicular cells into luteal cells after ovulation),
synthesis and secretion (growth and development of the corpus luteum accompanied by increasing quantities of P4), and luteolysis (destruction of the corpus luteum) accompanied by rapidly declining blood P4 that results in a subsequent follicular phase.
The regression of the corpus luteum is facilitated by prostaglandin F2α, which is synthesized and secreted by the uterine endometrium in most mammals. The negative feedback exerted by P4 on the hypothalamus is removed, and the female enters a new follicular phase because the pulse frequency and amplitude of GnRH increase, allowing FSH and LH to rise (Senger, 2012).

3.5.3 – PROESTRUS

Proestrus is behaviorally defined as the period preceding estrus, during which the queen is sexually attractive to a tomcat but will not permit the coitus.
Common behaviors include head and neck rubbing against objects, frequent vocalization, and rolling over on her back (Michael, 1961). Unlike the bitch, signs such as vaginal bleeding, and vulvar swelling are not observed.
Clearing of the vaginal smear, indicating an absence of non-cellular debris, mucus, and a decrease in cell coalescence, is the most sensitive and earliest indicator of follicular activity. This occurs in approximately one-third of cats during proestrus (Shille et al., 1979).

Recognizing this phase can be challenging due to subtle behavioral signs and its short duration, typically lasting less than 24 hours (Johnson A. & Kutzler, 2022).
This phase is associated with an increase in follicle size and rising estradiol levels. At the ultrasonographic examination, follicles manifest as indistinct dark areas on the surface of the ovary (Senger, 2012).

3.5.4 – ESTRUS

Following a short proestrus, queens enter the estrous phase, during which the female permits mounting and coitus.
The rise in estradiol is rapid, increasing from a basal plasma level of approximately 15 pg/ml to ≥20 pg/ml as ovarian follicles grow into distinct, vesicular structures measuring ≥2 mm in diameter and protruding from the surface of the ovary.
In a typical cycle, the queen will have two to seven mature follicles, and this does not differ between mated and non-mated queens. Although estrus behavior is dependent on the rise in estradiol, only 8% of cats show behavioral estrus on day 1, while 80% exhibit such behavior by day 4.
Extended estrus behavior can occur due to overlapping waves of maturing follicles. As estradiol rises, superficial vaginal cells (keratinized with large, polygonal-shaped cytoplasm and signs of nuclear degeneration) remain constant at 40–60%, while anucleate cells (keratinized with large, polygonal-shaped cytoplasm without nucleus) increase from 5% to 40%, and intermediate cells (non-keratinized with large to medium-round shaped cytoplasm and intact nuclei) decrease from ~45% to <10% by day 4 of estrus (figure 7). Parabasal cells (non-keratinized with small round-shaped cytoplasm and intact nuclei) are generally low throughout the feline estrous cycle (1–6%) but are completely absent on days 4–7 of estrus. Clearing occurs in most cats (~90%) during estrus, and smears remain cleared for five days following the end of estrus (Johnson A. & Kutzler, 2022).
The duration of estrus, defined by the interval when plasma estradiol remains above 20 pg/ml, has been reported to be 7.4 ± 2.3 days in length (range 3–16 days). The amount of time that estradiol remains elevated is unaffected by coitus or ovulation (Johnson A. & Kutzler, 2022)

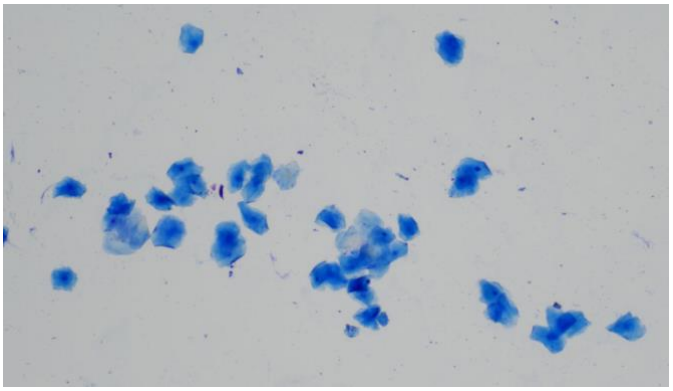

Figure 7: Vaginal cytology of subject P4 (200X). All epithelial cells present a polygonal shape (keratinized) with or without visible nuclei, respectively superficial and anucleate cells. Full keratinization is compatible with cytological estrus.

3.5.5 – INTERESTRUS

In the absence of ovulation, the queen enters the interestrus phase (also referred to as postestrus). Plasma estradiol levels drop to <20 pg/ml as mature follicles undergo atresia.
Behaviorally, the queen returns to her normal behavior during this phase.

Vaginal smears show the dominance of intermediate cells (48%), a percentage of superficial cells around 46%, and non-cellular debris (figure 8). It's noteworthy that breeding without ovulation does not impact the duration of this phase (Johnson A. & Kutzler, 2022).

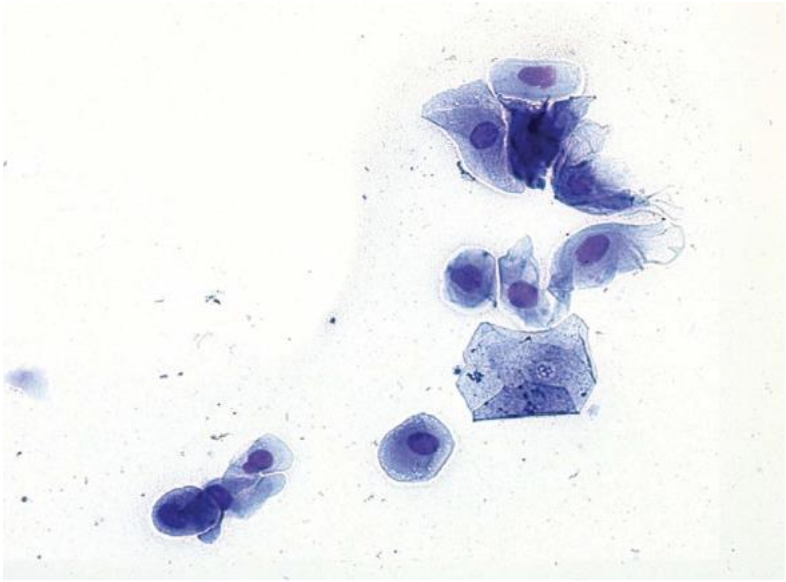

Figure 8: Vaginal cytology of a queen in interestrus. Notice the mixed-cell population (simultaneous presence of keratinized, intermediate and parabasal cells. The percentage of keratinized cells is below 50 % (Bacha & Linda M., 2012).

3.5.6 – DIESTRUS

If ovulation occurs (due to coitus, vaginal stimulation, or occurring spontaneously), the queen will enter the diestrus phase, also referred to as the luteal phase (Johnson A. & Kutzler, 2022).
Vaginal cytology typically transitions to predominantly small intermediate and parabasal cells (figure 9).
Plasma P4 begins to rise 1–2 days following ovulation from <1 ng/ml to peak levels of 25–90 ng/ml by 14–22 days post-ovulation (Paape et al., 1975).
If the female is not pregnant, P4 will remain elevated for approximately 35-45 days.
(Johnston S. D. Root Kustritz M. V. & Olson P. S., 2001); with the corpus luteum gradually regressing to form a persistent, yellow luteal scar. During a non-pregnant luteal phase, queens do not show any of the physical or behavioral changes (e.g. weight gain, mammary development, nesting) typically seen in pseudopregnant bitches (Johnson A. & Kutzler, 2022).
Queens usually begin a new estrous cycle within 7–14 days of corpus luteum regression, making the entire non-pregnant luteal phase approximately 40–50 days (Paape et al., 1975). The duration of a non-pregnant luteal phase in the cat is considerably shorter than that of pregnancy (35–45 days versus 63–67 days). Estradiol drops quickly to 8–12 pg/ml during the first five days following mating and typically remains basal. However, ovarian follicular activity can occur during diestrus (Johnson A. & Kutzler, 2022).

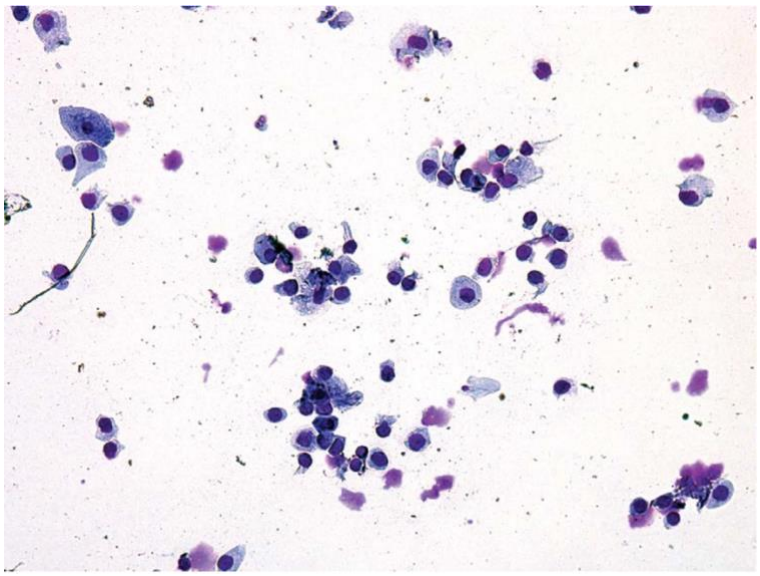

Figure 9: Vaginal cytology of a queen in diestrus. This cytology is characterized by small intermediate and parabasal cells and presence of debris in the background *(Johnson A. & Kutzler, 2022)*

3.5.7 – ANESTRUS

Anestrus is the period of reproductive quiescence and typically begins in October and ends in January or February in the Northern Hemisphere. Behaviorally and hormonally, anestrus is similar to interestrus. Plasma estradiol and P4 remain basal, and the queen does not attract males or display any estral behaviors (Johnson A. & Kutzler, 2022). Vaginal smears during anestrus are characterized by scattered small intermediate and parabasal cells (Johnson, 2022).

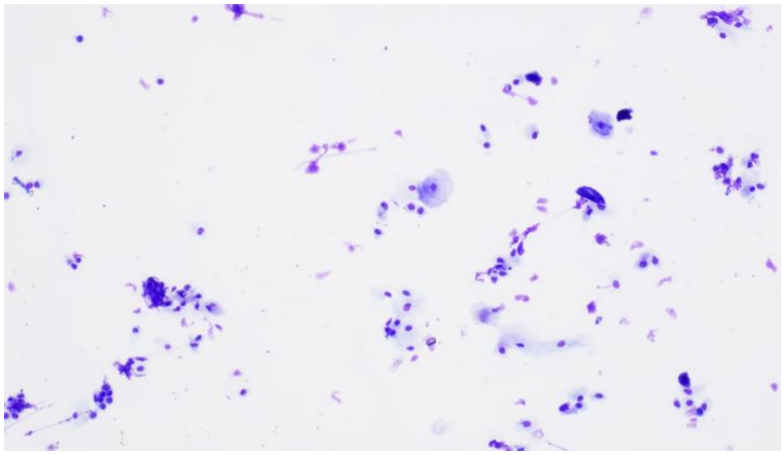

Figure 10: Vaginal cytology of subject P8 (100X - personal archives). All epithelial cells present have a round-shaped cytoplasm (non-keratinized) with visible nuclei. The amount of cytoplasm determines whether the cell is intermediate (abundant) or parabasal (scarce). The absence of keratinization along with a P4 concentration < 2ng/mL indicate anestrus.

Figure 11 shows a graphic representation of the hormonal (E2 and P4) and behavioral profiles of queens during different estrous cycle.

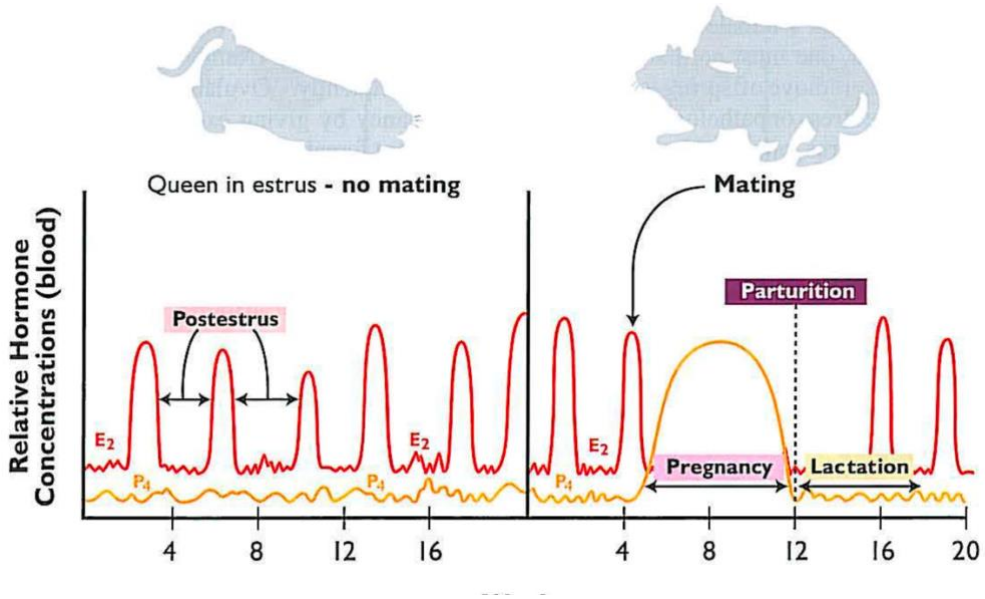

Figure 11: Graphic representation of the hormonal (E2 and P4) and behavioral profiles of the queen along different moments of the estrus cycle (estrus, postestrus=interestrus, pregnancy and lactation) (Senger, 2012).

CHAPTER 4 – MEGESTROL ACETATE AS NON-SURGICAL CONTRACEPTION IN QUEENS

4.1 – MEGESTROL ACETATE

MA (6-methyl-6-dehydro-17α-acetoxyprogesterone) is a potent progestin, exhibiting activity 25 times greater than endogenous P4 (Romagnoli & Ferre-Dolcet, 2022). It has a significant affinity for both androgen and glucocorticoid receptors, with a 75% affinity for the former and 37% for the latter. These properties make it noteworthy in hormone therapy, contraceptive development, and other potential medical applications (used in the past for dermatological issues).

Given its brief half-life, lasting only a few hours, MA distinguishes itself as the most rapidly acting progestin available in the veterinary market. It holds a singular status as the only product appropriately classified as 'short-acting' among its counterparts (Romagnoli, 2015).
When orally administered, MA is rapidly absorbed, which poses the need for daily administration to maintain drug concentrations in the plasma capable of exerting effective pharmacological activity (MSD Animal Health S.r.l. – Segrate (MI), 2004).

4.1.1 – HISTORY of MA USE

Introduced in 1975 and spanning multiple decades, MA was commercially available in the USA as a Food and Drug Administration (FDA) approved veterinary drug under the name Ovaban by Intervet Schering-Plough for use in female dogs. Its off-label use in cats was prevalent.

Additionally, an extra label formulation of MA, known as FeralStat, emerged in North America for cat use and was privately marketed by veterinarian Dr. John Caltabiano starting in 2008, outside regulatory oversight. FeralStat gained popularity among certain American cat colony managers, although its efficacy and safety in cats lack scientific data. Following Dr. Caltabiano's passing in 2011, FeralStat orders ceased to be fulfilled. Since then, compounding pharmacies have supplied MA to feral cat colony managers with a veterinary prescription.

Currently, MA is commercially available in many European countries, including the UK, the Netherlands, Belgium, France, Switzerland, and Italy, in oral formulations such as pills or syrup (Romagnoli, 2015).

Various progestins initially developed as potential human contraceptives have subsequently been repurposed and marketed for contraceptive use in dogs and/or cats in different countries. These include oral MA, oral MPA, oral delmadinone acetate, oral clormadinone acetate, and depot injectable PRG (Romagnoli S. & Concannon P.W., 2003).

MA is one of the most potent and recent synthetic progestins, active in very small oral doses in the form of drops. Its action is exerted at the ovarian level, preventing the maturation of the oocyte and, consequently, its availability for fertilization. Additionally, by inhibiting the secretion of natural E2, it prevents the onset of the behavioral manifestations that accompany the cat's heat.

4.1.2 – PHARMACODYNAMICS

The effect begins from the 10th day of administration and lasts throughout the duration of the treatment and beyond. The onset of heat occurs, after discontinuation of administration, within variable periods, which are also related to the overall duration of the treatment.

Practical experiences conducted on numerous subjects have demonstrated that, with a 365-day treatment, the subsequent heat may reappear between the 80th and 140th day after discontinuing administration (MSD Animal Health S.r.l. – Segrate (MI), 2004).

4.1.3 – DOSAGE

MA dosages are typically classified into low, medium, and high categories, determined by the dose administered per treatment, the frequency, and the duration of treatment. Research indicates that higher dosages are associated with increased risk and severity of adverse reactions. Treatment protocols utilizing MA dosages lower than the 'low' dosage of 0.625 mg/kg/week have shown effectiveness in managing estrus activity and ovulation in cats (Romagnoli, 2015).

An instance of low-dosage MA application is exemplified in the United States (FeralStat), combined with lactose powder to improve palatability. The package insert recommended a posology of approximately 0.1–0.2 mg/kg weekly (Greenberg et al., 2013) equivalent to 0.014–0.028 mg/kg administered every 24 hours (Romagnoli, 2015).

Another non-FDA-approved oral MA formulation, reported anecdotally as effective for reproductive control in feral cat colonies, suggests a dosage of 5.0 mg/week for groups of five to seven cats, which corresponds to 0.7–1.0 mg/cat/week or 0.18–0.25 mg/kg/week or 0.025–0.035 mg/kg administered every 24 hours (for approximately 4kg-cats) (Romagnoli, 2015).

The manufacturer's recommended posology for Estropill™, MSD is 0.011 mg/kg q24h (MSD Animal Health S.r.l. – Segrate (MI), 2004; Romagnoli, 2015), corresponding approximately to 5 drops/kg/day.

MA has been employed at different dosages depending on the phase of the estrus cycle the therapy is initiated:

1. Anestrus Dose:
    - Dosage: 5 mg/cat orally every 2 weeks or 2.5 mg/cat orally every week.
    - Timing: Administered during anestrus.
2. Breeding Season Dose:
    - Initiated during Interestrus:
        - Dosage: 2 mg/cat orally every day for 2 weeks.
    - Initiated during Proestrus or Estrus:
        - Dosage: 5 mg/cat orally once a day for 4 days.
3. Post-Initial Treatment:
    - After the initial treatment protocol, estrus suppression during the breeding season can be extended using the anestrus dose (Romagnoli S. & Concannon P.W., 2003).

4.1.4 – CONTRAINDICATIONS AND SIDE EFFECTS

MA is contraindicated during a normal pregnancy, as it will artificially raise the level of P4 and therefore risk delaying or inhibiting parturition. Furthermore, MA treatment is not advisable in animals with uterine disease or hemorrhage, diabetes mellitus, mammary neoplasia, and in diestrus females due to the risk of overexposure to P4 (Plumb, 2008).

All published cases of side effects due to MA in cats were associated with the use of high dosages (0.625–1.25 mg/kg q24h or 2.5–5.0 mg/kg weekly for 1 or more years). Such high dosages should be

avoided, as endocrine, uterine, and mammary side effects appear more rapidly, develop to greater severity, and may be irreversible (Romagnoli, 2015).

Undesirable reproductive side effects of progestins include cystic endometrial hyperplasia, pyometra, benign mammary gland hyperplasia, and mammary neoplasia (Aime K. Johnson & Michelle Anne Kutzler, 2022).
The occurrence of mammary carcinoma in cats receiving progestins for long periods was first reported by Hernandez and colleagues. Five cats received depot-medroxyprogesterone injections of 25 mg every 3 months, with an average duration of administration spanning 5 years. Among these, two cats developed adenocarcinoma of the mammary gland at 4 and 2 years, respectively, following the last injections (Hernandez et al., 1975).

Still, early research demonstrated the effectiveness of MA in estrus postponement in cats using 'low' doses of 2.5 mg/week (equivalent to approximately 0.625 mg/kg/week for a 4 kg cat) for up to 30 weeks with low rate of side effects – a pyometra was reported 3 years after the end of the treatment in 1/244 cats and a mammary carcinoma in 1/397; increased appetite in 33.6% of queens; and increased body weight in 13% of queens (Oen, 1977; Romagnoli, 2015).

Furthermore, the manifestation of these side effects can vary based on the specific stage of the estrous cycle during which progestin treatment is administered (Romagnoli S. & Concannon P.W., 2003).
Limited clinical studies have indicated that MA may induce less cystic endometrial hyperplasia compared to other progestational agents (Plumb, 2008).

The adverse effects linked with progestins extend beyond the reproductive system. Progestins have been reported to potentially induce adrenocortical suppression, hyperglycemia, diabetes mellitus, increased appetite (polyphagia), obesity, lethargy, apathy, immunosuppression, and potential skin issues at the injection site (e.g., hair discoloration, alopecia, skin atrophy, and calcinosis circumscripta) (Tomlinson et al.,1984)
Side effects such as adrenocortical insufficiency can arise at dosages (2.5–5 mg every other day) within 1–2 weeks of administration. Upon discontinuation of the drug, serum cortisol levels (both resting and ACTH-stimulated) typically normalize within a few weeks. While clinical signs of adrenocortical insufficiency (e.g., vomiting, lethargy) are rare, supplemental steroid support may be warranted if the animal experiences stress (e.g., surgery, trauma) (Plumb, 2008).
Although increased appetite and weight gain are not consistently observed, MA is sometimes used as an appetite stimulant. Hepatotoxicity (elevated alkaline phosphatase) in cats is a rare adverse effect of MA.
The weekly protocol of 2.5 mg of MA (Houdeshell & Hennessey, 1977) can be regarded as relatively safe for cats. Indeed, the product insert for MA-based formulations available in many European countries recommends a dosage regimen of 2.5 mg per week for up to 30 weeks (Romagnoli, 2015).
Due to the above-presented reasons, careful selection of patients is recommended before initiating treatment with a progestin. It would be particularly relevant to:

- Gather a comprehensive patient history.
- Conduct a thorough clinical assessment.
- Obtain a vaginal smear (to exclude estrus).
- Palpate the mammary glands (to check for masses).
- Palpate the abdomen (to assess uterine size).

In addition, the selection process should include uterine ultrasound (to verify normal uterine size and texture) and serum P4 assay to exclude diestrus (because supplementing exogenous P4 to endogenous high levels would be equivalent to administering a high dosage) (Romagnoli, 2015).

4.2 - OTHER NON-SURGICAL CONTRACEPTIVE METHODS IN QUEENS

Since surgical contraception may not always align with the owner's preferences and considering the increasing restrictions and animal welfare legislation in diverse countries on this traditional method, there is a demand for alternative approaches to preserve the future reproductive potential.
Numerous methods have been studied, usually aiming to interfere with endocrine regulatory mechanisms.
These methods are:

- Administration of progestins (negative feedback effect on the pituitary gland).
- GnRH agonist implants (downregulation of the hypothalamus-pituitary–gonadal axis).
- Melatonin implants.
- Immunization against endogenous GnRH or LH.

4.2.1 – HORMONAL CONTRACEPTION

PROGESTINS

Progestins, synthetic derivatives of P4, exert their effects similarly to endogenous P4 by reducing GnRH pulsatility, therefore lowering gonadotrophin secretion. Common progestins in domestic felids include MPA, MA, and PRG. They are both available in long-acting parenteral and oral formulations (tablet or liquid) (Romagnoli S. & Concannon P.W., 2003).
Administering progestins requires particular attention to patient selection, dosing, and precisely scheduling the administration in order to minimize potential side effects associated with high dosages, prolonged use, or females with previous subclinical issues. Hence, recommending the lowest effective dose and frequency to suppress estrus is crucial. However, interrupting an ongoing estrous cycle might require a higher dosage (Jöchle & Jöchle, 1975)
Avoiding contact with male cats is advisable until all signs of estrus have ceased. The ideal progestin combines high anti-gonadotrophic activity with low progestogenic effects. Medroxyprogesterone acetate (MPA), while quite efficient, is considered less safe due to its dual activity (both high anti-gonadotrophic and progestogenic). Proligestone (PRG), a third-generation progestin, has a milder impact on the endometrium and mammary epithelium than other progestins. (Evans & Sutton, 1989; Findik et al., 1999).
Although long-acting injectable progestins are convenient, low doses of oral megestrol acetate allow for immediate discontinuation if side effects arise.
Administering progestins during anestrus or seasonal interestrous is safer than during estrus when the progestogenic activity may be heightened by elevated concentrations of endogenous estradiol.
For queens with an unknown reproductive history, confirming the cycle stage through vaginal cytology and P4 serum concentrations before treatment is recommended. Despite the traditional exclusion of prepubertal queens from progestin treatments, there is no evidence of adverse effects on the immature gonadal axis (Lopez Merlo et al., 2016). Generally, healthy young, post-pubertal, anestrous queens subjected to low-dose treatments of short durations scarcely experience any side effects (Johnson A. & Kutzler, 2022).

MEDROXYPROGESTERONE ACETATE

MPA - 17α-hydroxy-6α-methylprogesterone acetate - is a synthetic derivative of P4, possessing greater potency than natural P4. It also acts as an agonist for androgen and glucocorticoid receptors, although its affinity for these receptors is notably lower than that of MA (Romagnoli, 2015).
MPA is accessible in both parenteral and oral formulations in many countries. Its half-life is approximately 12–17 hours after oral administration and 40–50 days following intramuscular injection (Johnson A. & Kutzler, 2022; Romagnoli, 2015).

In one of the initial scientific inquiries into MPA use in cats, estrus suppression was achieved through oral administration of doses ranging from 0.05 to 0.01 mg/kg every 24 hours for 12 months in two groups of six queens each (Harris & Wolchuk, 1963).
Differently from MA, reports of diabetogenic side effects in cats treated with high doses of MPA are lacking. This discrepancy may be attributed to MPA's diminished affinity for the glucocorticoid receptor compared to MA. The available data on MPA side effects in queens highlight the limitations of extrapolating effects between drugs and across species (Romagnoli, 2015; Schindler et al., 2008).
Current research indicates that oral doses of 0.02 mg/kg every 24 hours for MA and 0.05 mg/kg every 24 hours for MPA are suitable for administration, both in feral cat colonies and in pet cats (Romagnoli, 2015). It is recommended to avoid doses exceeding 2.5 mg/kg of MPA in breeding cats, as injectable doses ranging from 3.0 to 10 mg/kg or higher may pose significant health risks to all cats (Hernandez et al., 1975; Loretti et al., 2005) and should be strictly avoided.
There is a dearth of scientific information regarding the safe duration of progestin treatment using low doses of MPA. Decisions regarding treatment duration should consider individual health conditions: while a healthy young adult queen may tolerate low-dose MPA treatment safely for longer than one year, an adult female with age-related uterine and/or mammary gland changes should likely not receive treatment for more than one year (Romagnoli, 2015).

PROLIGESTONE

PRG, also known as 14α7α propylidene dioxyprogesterone, is structurally equivalent to P4. Besides its progestagenic effect, it exhibits anti-gonadotropic and anti-estrogenic properties. PRG exhibits greater anti-gonadotrophic activity and fewer gestational and anti-estrogenic effects compared to MPA and MA. PRG is sometimes preferred to the former and latter in domestic carnivores due to its repeated and widespread use, and minimal reported side effects (Goericke-Pesch et al., 2014; Kutzler & Wood, 2006; Max et al., 2014; Romagnoli & Sontas, 2010) in comparison to other compounds, and minimal impact on the uterus, mammary gland, and endocrine system, administered at a low dose ranging from 10 mg/kg to 30 mg/kg (Findik et al., 1999). The subcutaneous dose of PRG ranges from 100 to 250 mg per cat (approximately 25–30 mg/kg), administered at the onset of proestrus, during interestrus, or anestrus at intervals of 3, then 4 months and subsequently at a 5-month interval schedule (Findik et al., 1999; Romagnoli S. & Concannon P.W., 2003).
According to the manufacturer, when administered during proestrus, estrus signs disappear within 1–7 days after treatment. Suppression duration typically ranges from 4.5 to 10 months (Findik et al., 1999). The manufacturer also recommends allowing breeding females to undergo a normal cycle between injections (Johnson A. & Kutzler, 2022)

GNRH AGONISTS

GnRH is a decapeptide derived from the hypothalamus. Its physiological function involves stimulating pituitary activity for gonadotropin secretion, primarily through pulsatile release.
In the bitch, inducing a fertile estrus requires injecting GnRH every 70–90 minutes for over 8 days) (Johnson A. & Kutzler, 2022). However, this frequent injection schedule presents a significant limitation, rendering protocols utilizing GnRH and its agonists unsuitable for clinical application in cats (and dogs).
For adult queens, long-acting GnRH agonist implants are used, like Deslorelin (Suprelorin^{TM}, Virbac) - which offer effective long-term suppression of the feline estrous cycle. These implants are positioned subcutaneously between the scapulae or near the umbilicus, typically with the cat restrained in standing or dorsal recumbency ((Johnson A. & Kutzler, 2022).
Long-term GnRH agonist administration desensitizes and down-regulates pituitary GnRH receptors, inhibiting gonadotropin synthesis and secretion. However, initial treatment with GnRH agonists may have a short stimulatory effect, known as a 'flare-up', particularly in female animals (Gobello, 2012).

Studies have demonstrated the reversibility of the 4.7 mg Deslorelin acetate implant in suppressing estrus in queens, restoring normal fertility post-treatment (Gobello, 2012). The duration of estrus suppression varies widely, ranging from 16 to 37 months (Goericke-Pesch et al., 2013). Although a 9.4 mg Deslorelin implant extended suppression for 18.5 months (Banchi et al., 2022), the safety and efficacy of repeated treatments require further investigation.
Deslorelin implants' safety and efficacy have been assessed in prepubertal and postnatal kittens, postponing puberty and estrus until later ages without altering growth rates.
Initial undesirable increases in estradiol concentrations post-deslorelin implantation have been noted in queens. Combining MA (5 mg) before and after deslorelin implantation prevented estrus behavior associated with the flare-up in most cases, though it did not prevent estradiol increases(Banchi et al., 2022). The need for progestin treatment before and after implantation with deslorelin to suppress estrus during the flare-up remains questionable.

MELATONIN

Melatonin (or N-acetyl-5-methoxytryptamine), a hormone produced by the pineal gland during periods of darkness, plays a crucial role in regulating the reproductive cycle in cats. During anestrus and interestrus melatonin reaches its peak concentrations (Leyva et al., 1989).
Studies have investigated the effects of exogenous melatonin administration on estrus suppression in cats (Johnson A. & Kutzler, 2022).
Administering long-term release melatonin implants or oral tablets to queens exposed to 14 hours of light resulted in estrus suppression lasting approximately 63.8 ± 5 days (Faya et al., 2013). In separate studies, melatonin implants were effective in suppressing estrus during interestrus for durations ranging from 61.1 ± 6.8 days to 113.3 ± 6.1 days (Gimenez et al., 2009). However, in one study, estrus suppression was achieved in only 68% of queens, with one queen becoming pregnant despite treatment. Similar results were observed in purebred queens, with 81% experiencing estrus suppression for an average of 103.9 days (Schäfer-Somi, 2017).
Interestingly, administering melatonin to prepubertal queens did not significantly affect the time of puberty onset, compared to controls (Faya et al., 2013).
Initiating melatonin treatment from the end of estrus until early interestrus appears crucial, as the duration of suppression appears to correlate with the stage of the estrous cycle at the onset of treatment (Faya et al., 2011).
Notably, no clinically detectable side effects, including impacts on growth rate, were observed in prepubertal cats following melatonin administration (Johnson A. & Kutzler, 2022).

4.2.2 – IMMUNOCONTRACEPTION

Current research is exploring alternative contraceptive methods, such as gene silencing, targeted cytotoxin delivery, and immunocontraception. Immunocontraception involves vaccination against specific reproductive proteins like GnRH, the LH receptor, or zona pellucida proteins.
Initially, zona pellucida vaccines were developed, but they proved ineffective in preventing gestation, and granulomatous reactions to the adjuvant were observed (Johnson A. & Kutzler, 2022; Levy, 2011). Subsequent adjustments in formulation were made, incorporating a different antigen-carrier protein and increased antigen concentration. The updated formula elicited a robust humoral immune response, along with self-limiting masses at the injection site in some cats (Johnson A. & Kutzler, 2022; Vansandt et al., 2017).
GonaCon™, an immunocontraceptive vaccine registered for wild mammalian species, employs a GnRH-hemocyanin conjugate. Recent findings suggest that a single dose of GonaCon provides contraception lasting at least 1 year in 30% of 20 treated female cats (Fischer et al., 2018; Johnson A. & Kutzler, 2022).

In early trials with female domestic cats, this vaccine demonstrated contraceptive effects lasting over 3 years. However, this level of contraceptive efficacy is not currently recommended for use in queens (Johnson A. & Kutzler, 2022).

CHAPTER 5 - MATERIALS AND METHODS

5.1 - AIM OF THE STUDY

This study aimed to investigate the efficacy and safety of a low-dose MA protocol in suppressing cyclicity in queens and mating behavior and fertility of tomcats and the interval between the end of treatment and the resumption of reproductive function in treated cats.

5.2 - ANIMALS

Enrollment criteria for the study were:

a) healthy.

b) post-pubertal.

c) absence of a history of reproductive problems.

d) not in estrus.

e) not in diestrus (P4 < 2ng/ml).

The owners were explained the aim of the study and presented with the opportunity to have their cats enrolled. At the end of the trial, owners could opt for the surgical sterilization of the animal. Owners were further informed of MA's mode of action and administration. All owners read and signed an informed consent for the enrollment of their cat in the study.

5.3 - STUDY DESIGN

The following protocol was conducted at the Veterinary Teaching Hospitals of the University of Padova (Legnaro, PD, Italy) and the University of Ljubljana (Ljubljana, Slovenia).

1) Pre-treatment appointment.
2) Monthly check-up appointment.
3) Post-treatment appointment.
4) Confirmation of resumption of reproductive activity post-treatment.
5) Surgical sterilization at the end of the study, if requested by the owner.

Pre and post-treatment appointments comprised:

a) History.
b) General physical examination.
c) Reproductive tract examination.
d) Vaginal cytology.
e) Blood collection for hematology and biochemistry (following sedation, if necessary).
f) Abdominal and reproductive ultrasound.
g) Urinalysis.

If all enrollment criteria were met, owners received instructions for the administration of a MA commercially available veterinary product (Estropill™, MSD Animal Health S.r.l).

At monthly checkups, all subjects underwent a comprehensive clinical examination, which included vaginal cytology in queens and inspection of the penile mucosa (checking for the presence of penile spikes) in toms, and reproductive ultrasound. At each appointment, a checklist was filled out with the main data collected (Annex 4).

HISTORY

The following information was collected at the first appointment: signalment, behavior, eating and sleeping habits, lifestyle (indoor/outdoor), and reproductive history (age at puberty, date of last heat, estrus behavior and length, number of previous pregnancies, parturition, litter size, and past reproductive problems).

GENERAL PHYSICAL EXAMINATION

The general clinical examination focused on the following aspects:

- Skeletal state and body conformation.
- Nutrition and muscle tone.
- Weight and body condition score (BCS); The subject's weight was assessed at the monthly checkups with the same scale at the Padua's and Ljubljana's VTH.
- Level of consciousness and attitude.
- Skin and subcutaneous tissue.
- Mucous membrane color and capillary refill time.
- Lymph nodes.
- Body temperature.
- Respiratory rate and respiratory assessment.
- Heart rate and cardiac auscultation.
- Abdominal palpation.

a. REPRODUCTIVE TRACT EXAMINATION

Visual examination and manual palpation were performed on external genitalia and mammary glands with non-sterile disposable latex gloves.

The macroscopic aspects of the vulva and the vulvar lips were assessed to determine abnormalities such as lesions (external or vestibular), abnormal discharge, or inflammation.

The animals were then placed in dorsal recumbency and the mammary glands were first visually examined (shape and color). Subsequently, a manual examination of the mammary gland was performed, moving from the nipple to the base, using the thumb and index finger to determine consistency and the eventual presence of nodules, hyperplasia, inflammation or swollen areas, abnormal discharges from the nipples and fibrosis.

In tomcats, the penis was protruded from the prepuce and the penile mucosa was examined to highlight the presence of spikes. Testicles were visually and manually examined.

b. VAGINAL CYTOLOGY

A vaginal smear was collected from queens using non-sterile disposable latex gloves. A cotton-stripped swab was damped with running water and introduced horizontally approximately 1-2 cm into the vagina of the queen and then pulled out.

The cotton swab was then rolled on a glass microscope slide (both rolling the side of the cotton swab in a horizontal direction and pressing just the tip to impress some “dots” on the slide, to collect the cells located deeply in the vaginal canal).

Afterwards, the slide was stained using the Diff-Quik stain (MGG quick stain, BioOptica), a commercial Romanowsky-type stain with a methanol-based fixative, an eosin-based acid solution, and a thiazine-based basic solution (Figure 12). The slide was immersed 5 times in all 3 solutions in the above-mentioned order and then washed with running water, being careful not to direct the stream of water directly onto the smear, naturally dried and assembled.

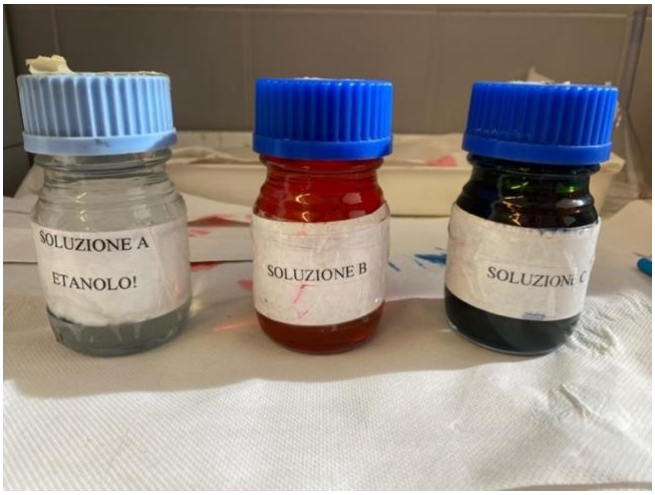

Figure 12: Diff-Quik stain (methanol-based fixative, an eosin-based acid solution and a thiazine-based basic solution from left to right) used to stain vaginal smears in VTH of Padova.

The slide was then analyzed under a light microscope (Eclipse Ci^{TM}, Nikon, Tokyo), at low magnifications (40x and 100x) to assess cellularity and background “clearing”(Figure 13). Cellular types and the degree of keratinization were examined at higher magnifications (200x and 400x) (Figure 14).

Figure 13: Light microscope (Eclipse CiTM, Nikon, Tokyo) used for vaginal smear's observation in the VTH of Padova.

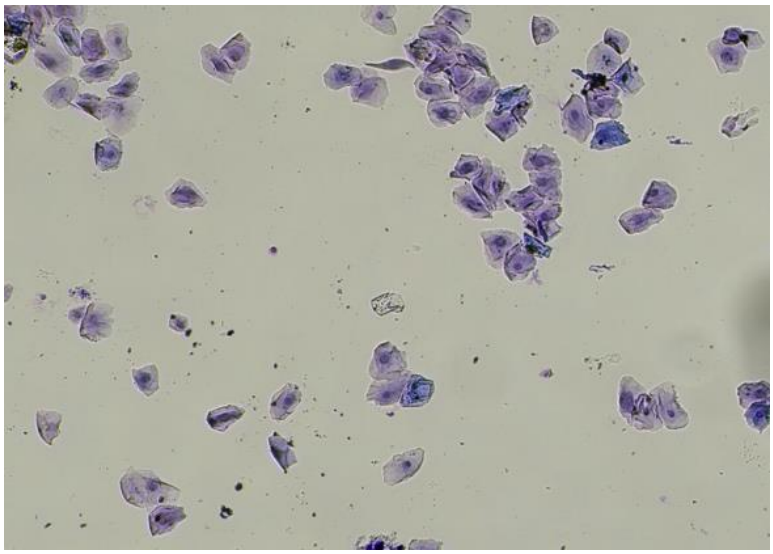

Figure 14: Example of vaginal cytology of estrus from subject P7 (personal archives, 2023)

The estrus cycle of each queen was staged based on the ratio between cornified/keratinized (superficial and anucleate) and non-cornified/non-keratinized (intermediate and parabasal) cells, along with the presence of polymorphonuclear cells.

- Superficial epithelial cells are characterized by their large size, oval and flattened shapes, with small to pyknotic nuclei and a low nucleus-to-cytoplasm ratio (N/C ratio); superficial epithelial cells are referred to as anucleate when the nucleus is absent.
- Intermediate epithelial cells range in size from small to medium to large, although not as large as superficial cells. They are round to oval in shape with smooth cytoplasmic and nuclear membranes, along with round and slightly flattened vesicular nuclei.
- Parabasal epithelial cells represent the smallest and thickest cells, featuring a high N/C ratio. They exhibit smooth, rounded cytoplasmic and nuclear membranes, and spherical nuclei. These cells stain deeply cyanophilic and are consistently arranged in layers, typically ranging from one to five cells deep (Ills et al., 1979).

Diagnosis of estrus was based on the detection of >70% cornified cells in a slide with moderate to high cellularity and a clear background. Anestrus was characterized by a vast predominance of intermediate and parabasal cells, along with scarce cellularity. Interestrus (postestrus) was defined cytologically by a mixed population of cornified and non-cornified cells, with the latter in higher proportion. Diestrus was characterized mainly by intermediate epithelial cells and a low number of superficial cells (Ills et al., 1979).

c. BLOOD ANALYSIS

Blood was sampled for hematology, biochemistry, and P4 assay. Blood was obtained from the jugular vein; the animal was positioned in sternal recumbency with the forelegs and neck extended; the hair was clipped and the area disinfected. A 22G needle attached to a 5 ml syringe was used and the collected blood was immediately transferred into 2 vacutainer tubes:

- One plain tube for biochemistry and P4 assay.
- One EDTA K2 tube for hematology.

The sample inserted in the tube with anticoagulant was gently turned up down after filling, to allow the anticoagulants to mix with the blood. It was subsequently analyzed in the VTHs' laboratories, along with a blood smear to confirm the platelet count given by the machine according to the presence or absence of platelet aggregation in the smear. Whereas the plain tube was left upright to allow coagulation and then centrifugated at 1000G to separate the serum from the coagulated fraction. Serum was used for biochemistry (BT 1500TM; Biotecnica, Rome – VTH of Padova, RX Daytona Plus (Randox Laboratories Ltd, UK)- VTH of Ljubljana) and P4 assay (Automated Immunoassay Analyser-360TM, Tosoh, Tokyo – VTH of Padova, MiniVidas (MiniVidas analyzer, BioMerieux S.A., Lyon, France) – VTH of Ljubljana) (Figure 15). P4 levels were determined through a fluorescence enzyme immunoassay (FEIA) and complemented with the results of vaginal cytology to determine the estrus cycle phase. This determination allowed us to ensure that MA therapy was not initiated in diestrus, this way avoiding an overload of P4. If P4 turned out to be above 2 ng/ml in the first appointment, treatment beginning was postponed for the amount of time necessary to rule out pregnancy and avoid P4 overexposure due to endogenous P4 production resulting from a non-fertile mating or spontaneous ovulation.

The samples obtained for biochemistry and hematology demonstrated the health state of the animal. Pre and post-treatment results were compared to confirm the safety of the treatment.

Figure 15: Automated Immunoassay Analyser-360TM, Tosoh, Tokyo used for P4 assay at the VTH of Padova

ABDOMINAL AND REPRODUCTIVE ULTRASOUND

Queens were placed in dorsal recumbency U-shaped pillows, the fur was clipped in the abdominal area and ultrasound gel was used to favor visualization. The ultrasound was conducted using an ultrasound unit and a micro convex probe of 5-8 MHz (Affinity 50^{TM}, Philips, Amsterdam – VTH of Padova (figure 16,17), GE LogiqPro7 – VTH of Ljubljana) executing both longitudinal and transverse scanning planes of the reproductive organs examined for queens, while a linear probe of 3-11MHz was used for male reproductive organs.

The uterus was appraised for the presence of fluid within the lumen and any other pathological alteration, and the ovaries were appraised for the presence of fluid-filled structures compatible with follicles, corpora lutea, or cysts. The thickness of the uterine wall, as well as the length and height of both ovaries, were measured.

The remaining organs of the abdominal cavity were also assessed with particular attention to the kidneys and the bladder for which images were acquired.

Figure 16: Ultrasound unit (Affinity 50TM, Philips, Amsterdam) used for abdominal and reproductive ultrasound in the VTH of Padova.

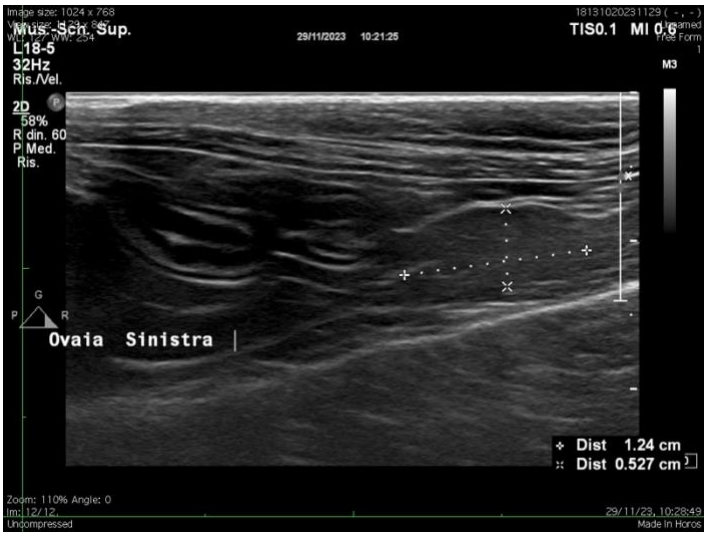

Figure 17: Ultrasound of left ovary of a queen, performed in VTH of Padua with the ultrasound unit (Affinity 50TM, Philips, Amsterdam). The ovary is identified between the calipers and measurements can be appraised in the right lower corner. No fluid-filled structures (follicles or CL) are observed in the ovary (personal archives).

d. URINALYSIS

During abdominal ultrasound, a cystocentesis was conducted to obtain a urine sample.

A 22 G needle attached to a 5ml syringe was employed, and the collected sample was placed into a urine tube (vacUaptaca^{TM}). Subsequently, the sample underwent macroscopic, physicochemical

(urinary stripes and density), protein creatinine ratio, and sediment analysis. Pre- and post-treatment results were compared to confirm the safety of the treatment.

e. SEDATION

If retained necessary, in order to bypass avoidable stress to the animals and to increase the safety of the operators, a sedation was performed after ascertaining the good general state of the subjects through the general clinic examination. The protocol included Ketamine (1.0 mg/kg IM), butorphanol (0.2-0.4 mg/kg IM), and Dexmedetomidine (8 μg/kg IM).

5.4 - INDICATION FOR ADMINISTERING ESTROPILL™

Following the evaluation of the general health of the animal and good conditions of the reproductive tract, owners were provided with detailed instructions on the proper administration of the megestrol acetate medication (Estropill™, MSD Animal Health S.r.l) including posology: 5 drops of the product per kg of body weight of the animal, either directly into the oral cavity or mixed with food (figure 18). Emphasis was put on the importance of consistent daily administration, ideally around the same time each day. When administered with food, careful attention was advised to ensure the ingestion of the entire portion, thereby assuring the complete assimilation of the prescribed dose.

Figure 18: formulation of MA,
Estropill™ (MSD, Animal Health s.r.l)

5.5 - FOLLOW-UPS

The duration of the treatment was decided according to the owner's requests and expectations, spanning either 4, 5, or 6 months. Subsequently, owners were instructed to present their animals for monthly examinations at the VTH, during which general physical exam, ultrasound evaluations, vaginal cytology, and manual palpation of the mammary glands for females and inspection of the penis and testis for males were conducted. These assessments aimed to verify the effectiveness and safety of the administered dosage. In cases where adverse reactions were suspected, the treatment would be promptly ceased.

5.6 - END OF THE TRIAL

At the first manifestation of heat behavior post-treatment by the queens, owners were instructed to refer to the VTH for cytological confirmation of estrus. Beginning and end dates of treatment with Estropill™ (MSD Animal Health S.r.l) and characteristics of heat were registered. Subsequently, the owners were offered the choice to surgically sterilize (ovariohysterectomy or orchiectomy) their animal.

In the cases where the surgery was performed, the reproductive organs were analyzed histologically, to further establish the safety of the therapy. These histology results, however, do not constitute part of this thesis.

5.7 - STATISTICAL ANALYSIS

Subjects were divided into 3 groups depending on treatment duration (G4 = 4 months-treatment, G5= 5 months-treatment and G6=6 months-treatment). Data collected regarding the timing of the resumption of ovarian activity and body weight underwent statistical analysis. Mean and standard deviation were calculated for both variables on the entire population and each treatment group. The interval from the end of treatment to the first estrus post-treatment (days) was compared between groups performing independent sample comparison of means.
Weight (kg) was analyzed through a Student *t*-test for paired data comparing pre and post-treatment values (by group). The same statistical test was employed to compare queens‘ weight at the end of treatment and at the moment of return in heat. The significance level (α) was set at 0.05.
Results from ultrasound examinations and hematobiochemical parameters before, during, and after treatment were used for monitoring treatment safety and to ensure general and reproductive health status of all subjects before, during, and after treatment.

CHAPTER 7 - RESULTS

ANIMALS

Twenty-one intact (18 female and 3 male) privately owned cats were recruited for the study. Cat recruitment was based on owners requesting short-term control of reproduction either prior to resorting to surgical sterilization (pet cats) or to achieve a pause in the reproductive career (breeding cats).

Even if the primary aim of the study focused on the Megestrol Acetate effect in queens, the possibility of enrolling 3 tomcats arose following the owner's request during the study period. As little to no information is available in the literature on low-dosage MA therapy in males, the 3 tomcats were included in the study, receiving the same protocol as queens, with the intent of understanding if Estropill™ at this dosage would constitute a valid option for undesired reproductive behavior control.

Eight subjects were presented to the Veterinary Teaching Hospital (VTH) of the University of Padova and the remainder 13 to the VTH of the University of Ljubljana.

Subjects were identified by a letter and a number, corresponding respectively to the VTH where they were followed during treatment (P = Padua, L= Ljubljana) and the chronological moment when they started the treatment (1- 6 for Padua, 1-13 for Ljubljana).

The enrollment and study period overlapped and went from the spring of 2022 (Subject P1: 14/04/22) to the winter of 2023 (The last subject to finish the treatment was P8: 12/02/24).

The study population was composed of healthy post-pubertal cats of different ages (mean 2.4 ± 1.67 years), ranging from 0.75 (9 months, Subject P3) to 6 years of age (subject L6).

The subjects belonged to different breeds, specifically:

- European shorthair cat (n = 4);
- British shorthair cat (n = 5);
- Norwegian Forest cat (n =1);
- Persian (n =1);
- Siamese (n =1);
- Sphynx (n = 3);
- Ragdoll (n = 1);
- Maine Coon (n = 4);
- Bengal (n =1);

Table n° 1 shows the demographics of the enrolled subjects.

Table 1: Breed, sex, age, and weight before the treatment (WBT) of the subjects enrolled.

NAME	BREED	SEX (F= female, M= male)	AGE (years)	WBT (kg)
P1	European shorthair cat	F	1	4.2
P2	Persian	F	6	3.2
P3	European shorthair cat	F	0.75	3.2
P4	Bengal	F	0.9	3.7
P5	Norwegian Forest cat	F	5.6	4.2
P6	British shorthair cat	M	1.4	4.2
P7	British shorthair cat	F	3.2	3.4
P8	British shorthair cat	F	2.4	2.9
L1	British shorthair cat	F	2.25	3.5
L2	Maine Coon	F	1.25	3.7
L3	Maine Coon	F	1.75	4.8
L4	Siamese	F	1.91	2.3
L5	Sphynx	F	1.5	2.8
L6	Sphynx	F	6	4.3
L7	British shorthair cat	F	3	4.3
L8	European shorthair cat	F	1	2.9
L9	European shorthair cat	F	1	2.2
L10	Ragdoll	F	4	5.6
L11	Maine Coon	F	2.1	5.1
L12	Sphynx	M	1.5	3.1
L13	Maine Coon	M	1.75	6.2

The 21 cats were treated for the following periods of time previously decided with the owner:

- 4 months' period (cat P1, P2, P3, P5, L12).
- 5 months' period (cat P4, P6, L10, L11, L13).
- 6 months' period (cat P4, P7, P8, PL1, L2, L3, L4, L5, L6, L7, L8, L9, L10).

Table 2 summarizes the group of treatment, and the beginning and end of the treatment for each subject enrolled.

Table 2: Duration, start, and end dates of the treatment with Estropill™ for each subject enrolled.

NAME	TREATMENT DURATION (months)	TREATMENT BEGINNING	TREATMENT END
P1	4	14/04/22	14/08/22
P2	4	22/07/22	21/11/22
P3	4	24/02/23	24/06/23
P4	5	04/04/23	30/08/23
P5	4	20/04/23	16/08/23
P6	5	02/10/23	02/03/24
P7	6	04/08/23	01/02/24
P8	6	12/08/23	12/02/24
L1	6	04/04/23	04/10/23
L2	6	07/04/23	07/10/23
L3	6	07/04/23	07/10/23
L4	6	04/05/23	04/11/23
L5	6	05/05/23	05/11/23
L6	6	10/05/23	10/11/23
L7	6	17/05/23	17/11/23
L8	6	19/05/23	19/11/23
L9	6	19/05/23	19/11/23
L10	5	07/06/23	07/11/23
L11	5	23/06/23	23/11/23
L12	4	5/04/23	27/07/23
L13	5	7/04/23	01/09/23

EFFICACY OF THE TREATMENT AND RESUMPTION OF OVARIAN ACTIVITY

Seventeen/18 treated queens consistently exhibited behavioral and cytological anestrus during the treatment while one queen displayed vocalization, lordosis, rubbing, and increased affection after 10 days of treatment (Subject P4); her case is discussed afterwards.

When the owners noticed initial signs of heat following the end of the trial, the queens were scheduled to be reexamined for cytological confirmation of estrus.

The time until the resumption of ovarian activity post-treatment (PT) varied greatly, ranging from a minimum of 23 days observed in subject P2 to a maximum of 184 days in subject P5. Due to the consistent delay in heat return exhibited by subject P5, exceeding the mean plus three times the standard deviation (50.12±17.08), this queen was identified as an outlier and excluded from the statistical analysis regarding the resumption of ovarian activity. At the same time, she was included in the weight analysis.

Results regarding the number of days between the end of the trial and the onset of ovarian activity are shown in Table 3.

Table 3: End of treatment and onset of first estrus dates and interval in days between them for each queen treated.

SUBJECT	TREATMENT END	FIRST ESTRUS AFTER MA	INTERVAL BETWEEN END OF TREATMENT AND ONSET OF ESTRUS (DAYS)
P1	14/08/22	1/11/22	77
P2	21/11/22	14/12/22	23
P3	24/06/23	21/07/23	27
P5	16/08/23	20/02/24	184
P4	30/08/23	12/10/23	42
P7	01/02/24	9/03/24	38
P8	12/02/24	9/03/24	27
L1	04/10/23	8/12/23	64
L2	07/10/23	11/12/23	64
L3	07/10/23	28/11/23	51
L4	04/11/23	03/01/24	59
L5	05/11/23	15/01/25	70
L6	10/11/23	16/01/24	66
L7	17/11/23	16/01/24	59
L8	19/11/23	12/01/24	53
L9	19/11/23	15/12/23	26
L10	7/11/23	8/01/24	61
L11	23/11/23	8/01/24	45

The mean duration from the end of treatment to the first signs of heat was 50.12 ± 17.08 days[1]. Cat P1 was the one who took the longest to exhibit estrus signs: 77 days.
Categorizing the queens into the three treatment groups (G4 - n=4 cats, G5 - n=3 cats, and G6 - n=11 cats) the meantime until first signs of estrus were exhibited by the queens PT were 42.33 ± 30.08, 49.3 ± 10.21, and 52.45 ± 15.5 days, respectively.

No significant difference was found between treatment groups, as the p-value was above the significance level adopted (α=0.05) - Table 4.

[1] This calculation did not include cat P5, which was considered an outlier - based on a cut-off of Mean ± 3*SD = 164.4 days - since it took 184 days to resume ovarian activity. If cat P5 had been included in the data analysis, the mean would have resulted in 57.5±36.6 days.

Table 4: P value and statistical significance for the analysis of time until ovarian resumption between treatment groups (G4, G5, and G6).

Group Comparison	*P* value	Significant?
G4 vs G5	0.638	No
G4 vs G6	0.423	No
G5 vs G6	0.687	No

Regarding the males, in 2/3 tomcats (L12 and L13), urinary marking and display of interest towards females disappeared after 1 month of treatment. In the same subjects, penile spikes disappeared within 3 months of MA treatment and were again detected 100 days from the end of the treatment. The third tomcat (P6) never fully ceased mounting behavior, and his penile spikes, although slightly reduced in size from the 2nd month of treatment (Figure 19, 20), never disappeared. Moreover, cat P6 mated with a queen (who had already finished treatment and had returned in heat) between the 4th and 5th month of his treatment, resulting in a successful pregnancy of 6 healthy kittens. The subject's P6 case is presented in detail subsequently.

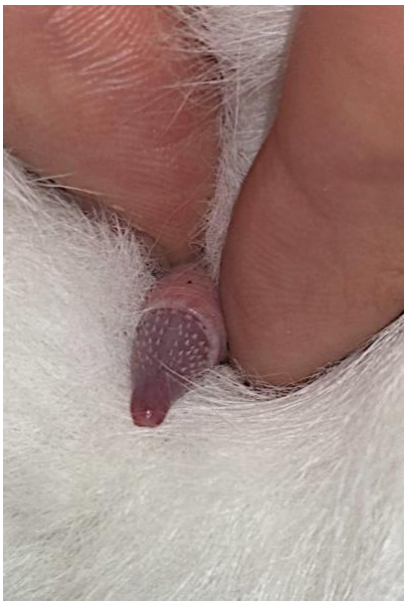

Figure 19: Penile spikes of subject P6 before treatment (28/09/23)- VTH of Padova.

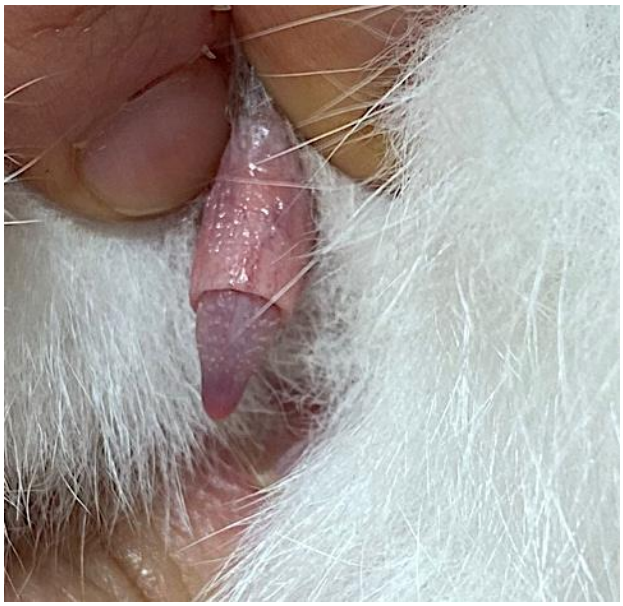

Figure 20: Penile spikes of subject P6 2 months after treatment (5/12/23)- VTH of Padova.

SIDE EFFECTS

No reproductive side effects were observed during the treatment period. Also, hematobiochemical, urinalysis and ultrasonographic parameters did not reveal any clinically significant changes and no relevant difference was noticed between pre- and post-treatment results (Annex 1, 2, 3).
On ultrasound, all subjects showed a normal echogenicity of the uterus and ovaries at every monthly checkup. Subject P2 presented bilateral ovarian fluid-filled structures at the pre-treatment appointment (approximately 0.7cm), presumably paraovarian cysts of no clinical interest, which kept unaltered throughout treatment and didn't affect the subsequent resumption of ovarian activity (Figures 21, 22); following her 1st heat PT subject P2 underwent surgical sterilization and histology analysis and diagnosis of the ovaries was cyst from rete ovarii (Annex 5), while the uterus was normal. No uterine abnormalities were found in the uterus of any queen, including subject P2.

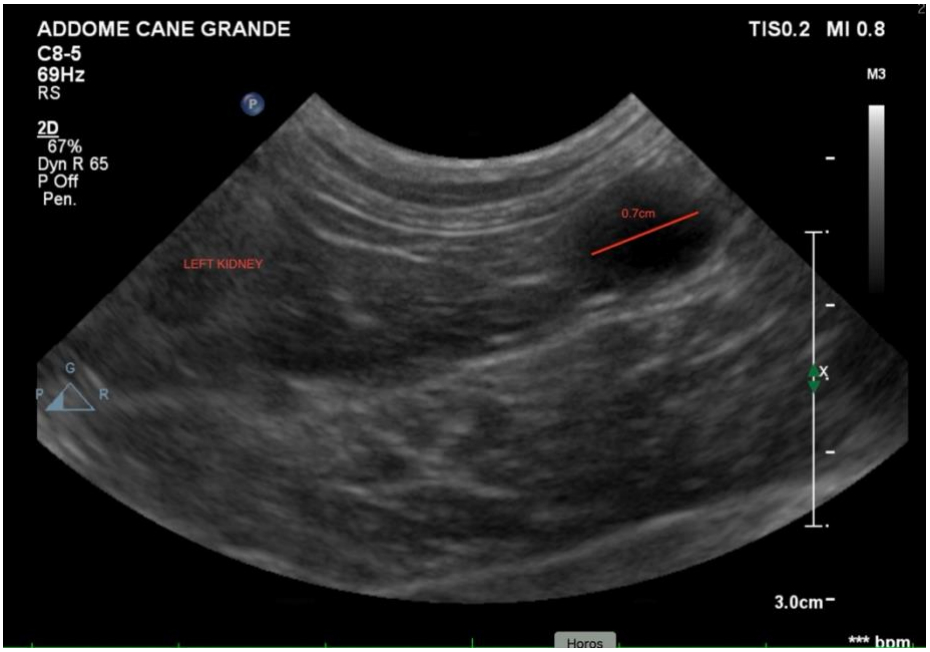

Figure 21: Left ovary, Subject P2 (intact queen, persian, 6y), before treatment (21.07.22). Presence of a fluid-filled (anechogenic) structure measuring 0.7cm; VTH of Padova, private archives (2022).

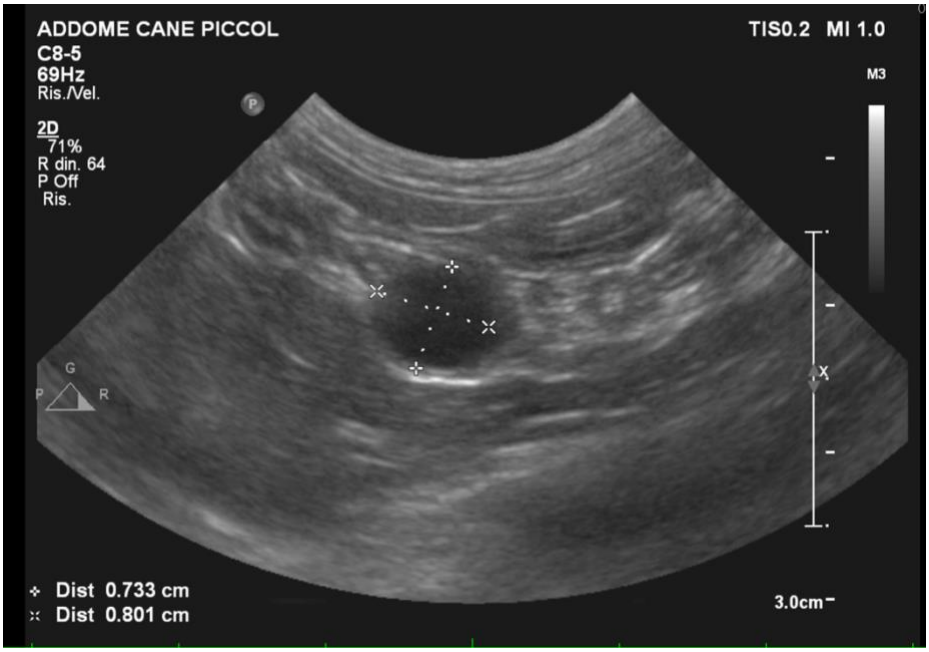

Figure 22: Right ovary, Subject P2, after the end of treatment (15.12.22). Presence of a fluid-filled (anechogenic) structure measuring 0.7-0.8 cm in diameter, VTH of Padova private archives (2022).

Hematobiochemical exams and urinalysis showed no relevant variation from physiological values during treatment. Three subjects from the Ljubljana VTH manifested a slight and temporary enlargement of mammary glands (L6, L6, L10) that resolved around one month PT, and had no clinical complications related. Changes regarding texture, size, or discharge from the mammary glands were not verified in any other of the subjects involved.

While no meaningful reproductive side effects were noticed during the treatment period, an important tendency was observed regarding general health: every subject exhibited an increase in appetite during treatment resulting in weight gain (Table 5).

Table 5: Body weight of each subject before (WBT)[2], during (check-ups) and after treatment (WAT) and at first estrus PT in kg.

NAME	WBT (kg)	Weight at 1st check-up (kg)	Weight at 2nd check-up (kg)	Weight at 3rd check-up (kg)	Weight at 4th check-up (kg)	Weight at 5th check-up (kg)	Weight at 6th check-up (kg)	WAT (kg)	Weight at 1st heat PT (kg)
P1	4.2	-	4.5	4.2	-	-	-	4.2	4
P2	3.2	3.7	3.7	4	-	-	-	4	4
P3	3.2	3.7	3.7	3.8	-	-	-	3.8	3.7
P4	3.7	4.9	4.2	5.5	5.5	5.6	-	5.6	5.5
P5	4.2	-	4.7	4.7	-	-	-	4.7	-
P7	3.4	3.6	4	4	4.1	-	-	4.2	4.6
P8	2.9	3	3.2	3.1	-	3.1	-	3.1	3.4
L1	3.5	3.8	4	4,2	4,4	4.3	4	3.7	3.7
L2	3.6	4.3	4.3	4.5	5	4.8	4.7	4	4
L3	4.8	5.3	5	5.2	5.3	5	4.9	4.9	4.9
L4	2.3	2.3	2.5	2.5	2.8	2.8	3	2.6	-
L5	2.8	3.0	3	3.2	3.5	3.6	3.7	3.2	-
L6	3.4	3.6	3.5	3.6	3,6	3.6	3.3	3.1	-
L7	4.3	4.5	4.6	5	4.7	4.8	4.7	4.6	-
L8	2.9	3.2	3.6	3.8	3.7	3.8	3.8	3.5	-
L9	2.2	2.4	2.5	2.5	2.7	2.6	2.5	2.3	2.3
L10	5.6	5.6	5.8	5.7	5.9	5.5	4	5.2	-
L11	5.1	5.3	5.4	6	5.7	5.4	4.7	5.1	-
P6	4.2	4.3	4.2	-	4.4	-	-	3.6	-
L12	3.1	3.3	3.2	3.3	-	-	-	3	-
L13	6.2	6.6	6.5	6.7	6.3	6.6	-	6.3	-

The mean weight before treatment (WBT) was 3.76 ± 1.04 kg, whereas it increased to 4.06 ± 1.09 kg PT, referred to as weight after treatment (WAT), indicating a total mean weight gain (WG) of approximately 0.31 kg.
The mean of weight at the last appointment (WLA, still during MA treatment) was 4.26 ± 0.99 kg, with a total mean WG of 0.5 kg.

[2] WAT : weight after treatment.
WBF: weight before treatment.
WLA : weight at last appointment.
PT: Post treatment.
WG : weight gain.

The WLA differs from WAT[3]: subjects were still under MA therapy in the latter, while WAT was registered at the appointment some days after the end of the treatment, depending on owner compliance.
Month-to-month analysis revealed a consistent increase in weight compared to the initial WBT, which increased even further towards the end of treatment, albeit with fewer subjects involved. The weight gain percentages (compared to WBT) are as follows:

- after one month, 6.57%.
- after two months, 8.3%.
- after three months, 12.18%.
- after four months, 16.7%.
- after five months, 14.55%.
- and after six months, 2.3%.

These percentages were obtained by calculating the mean of the values resulting with the formula: (weight at month X – WBT)/WBT * 100 (for each subject).

Weight gain (mean and SD) was calculated separately for the three treatment groups (G4, G5, G6) to compare WBT and WLA to determine weight gain during the treatment (Table 5, figure 23). Some animals lost weight from the last appointment (still under MA therapy) to the first appointment PT (not anymore under MA therapy).

G4: 4 months

- The mean WBT was 3.58 ± 0.57kg.
- The mean WLA was 4±0.5 kg.
- The mean WAT was 3.94 ± 0.62 kg.
- A significant statistical difference was observed between WBT and WLA, in the direction of WLA > WBT (*p*-value = 0.021).

G5: 5 months

- The mean WBT was 4.95±1.02 kg.
- The mean WLA was 5.5 ± 0.8 kg.
- The mean WAT was 5.32±0.7 kg.
- No significant statistical difference was observed between WBT and WLA (*p*-value = 0.097).
- Between the 2nd and the 3rd months of treatment, an important relative increase in weight was noticed: + 15,47% compared to WBT.

G6: 6 months

- The mean WBT was 3.29±0.79 kg.
- The mean WLA was 3.84 ± 0.8 kg
- The mean WAT was 3.56±0.81 kg.
- A significant statistical difference was observed between WBT and WLA, in the direction of WLA > WBT (*p*-value = 0.0004).
- Between the 3rd and the 4th months of treatment, an important relative increase in weight was observed: + 20.53% compared to the initial weight.

[3] WAT: weight after treatment, WBT: weight before treatment, WLA: weight at last appointment, PT: Post-treatment, WG: weight gain.

Table 4: Percentage of weight gain (WG) compared to weight before treatment (WBT) for each subject; the last column shows the percentage of WG on the appointment to confirm heat post-treatment (PT), second last represents the percentage of WG at the first appointment after terminating the treatment.

NAME	GROUP	WBT	% WG 1st month	% WG 2nd month	% WG 3rd month	% WG 4th month	% WG 5th month	% WG 6th month	% WG at 1st visit PT	% WG at 1st heat PT
P1	G4	4.2	-	7.14%	-	-	-	-	0.00%	-4.76%
P2	G4	3.2	14.06%	15.63%	25.00%	-	-	-	25.00%	25.00%
P3	G4	3.2	15.63%	15.63%	18.75%	-	-	-	18.75%	15.63%
P4	G5	3.7	32.43%	40.54%	48.65%	48.65%	51.35%	-	51.35%	48.65%
P5	G4	4.2	0.00%	11.90%	11.90%	-	-	-	11.90%	-
P7	G6	3.4	5.88%	17.65%	17.65%	21.76%	-	-	23.53%	35.29%
P8	G6	2.9	3.45%	8.97%	8.28%	-	8.28%	-	7.59%	17,24%
L1	G6	3.5	8.57%	14.29%	20.00%	25.71%	22.86%	1.29%	5.71%	5,71%
L2	G6	3.7	16.22%	16.22%	21.62%	35.14%	29.73%	27.03%	8.11%	8,11%
L3	G6	4.8	10.42%	4.17%	8.33%	10.42%	4.17%	2.08%	2.08%	-8,33%
L4	G6	2.3	0.00%	8.70%	8.70%	21.74%	21.74%	30.43%	13.04%	-
L5	G6	2.8	7.14%	7.14%	14.29%	25.00%	28.57%	32.14%	14.29%	-
L6	G6	3.4	5.88%	2.94%	5.88%	5.88%	5.88%	-2.94%	-8.82%	-
L7	G6	4.3	4.65%	6.98%	16.28%	9.30%	11.63%	9.30%	6.98%	-
L8	G6	2.9	10.34%	24.14%	31.03%	27.59%	31.03%	31.03%	20.69%	-
L9	G6	2.2	9.09%	13.64%	13.64%	22.73%	18.18%	13.64%	4.55%	4,55%
L10	G5	5.6	0.00%	3.57%	1.79%	5.36%	-1.79%	-	-7.14%	-
L11	G6	5.1	3.92%	5.88%	17.65%	11.76%	5.88%	-	0.00%	-
P6	G5	4.2	3.83%	-0.48%	-	5.26%	-	-	-12.92%	-
L12	G4	5.1	6.45%	3.23%	6.45%	-	-	-	-3.23%	-
L13	G5	6.2	6.45%	4.84%	8.06%	1.61%	6.45%	-	1.61%	-

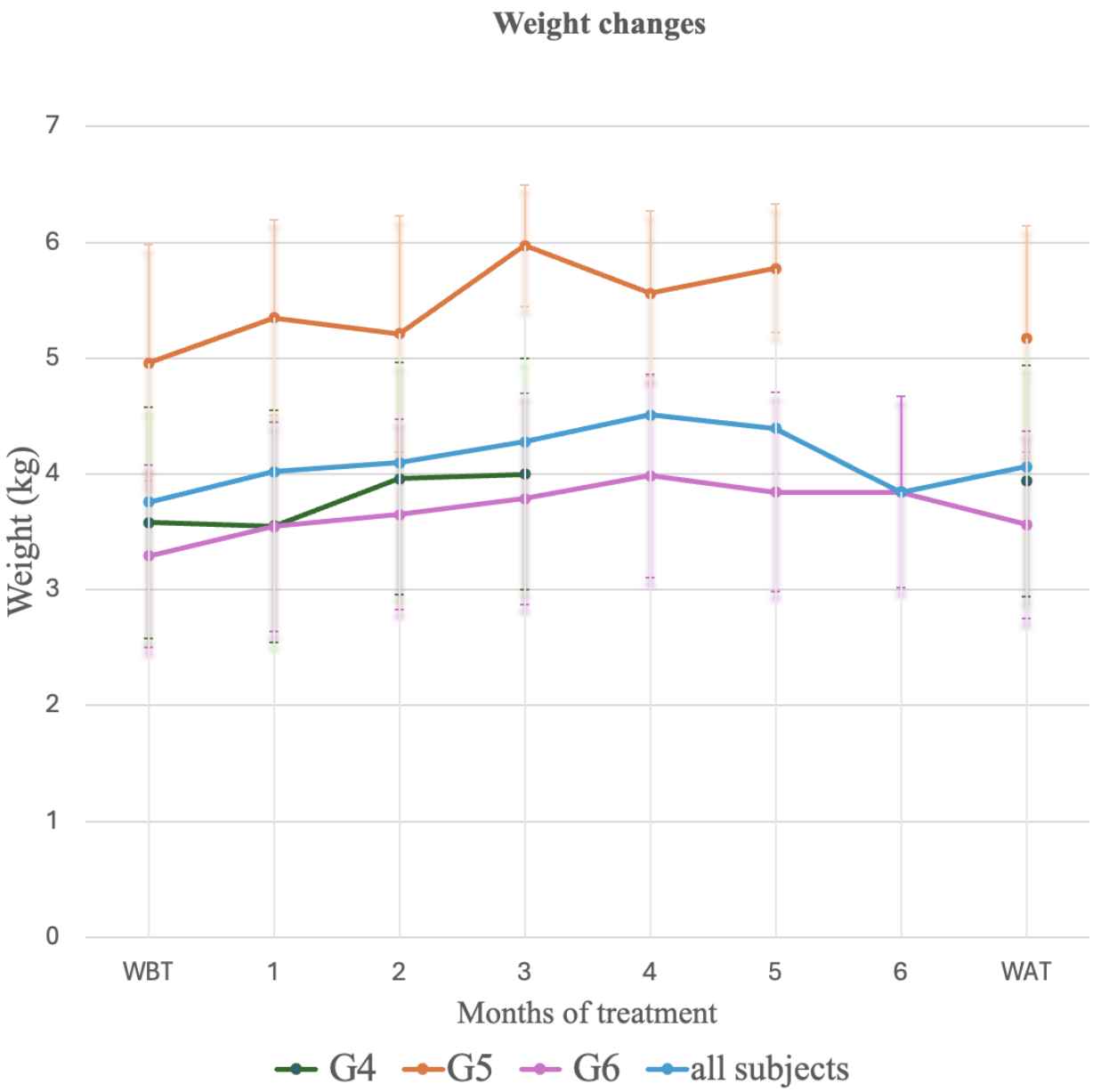

Figure 23: Graphic representation of the mean body weight changes of all subjects and divided per group (G4, G5, and G6) along the treatment period; on the horizontal axis, the months of treatment (WBT= Weight before treatment, WAT= weight after treatment, 1 = 1st month, etc) and on the vertical axis the weight (kg).

Of the 10 queens whose weight was available at the onset of the first estrus PT, 7 experienced weight loss from the end of treatment. Two exhibited an increase in body weight and one did not show any change. WLA and weight at 1st heat PT were not significantly different (p-value = 0.097).

All three tomcats treated with MA had also gained weight during the treatment:
P6 had a peak of +5.26% in the 4th month of treatment (which is the last weight available for this subject when still under treatment with MA), L12 +6.45% in the 3rd month of treatment and L13 +8.06% in the 3rd month of treatment while at the last appointment before ending the treatment L12 was at +6.45% and L13 at +6.45%. These percentages were obtained through the formula (WLA-WBT)/WBT * 100.

After terminating with MA, all 3 tomcats lost weight: WAT < WLA. Subject P6 registered a decrease of 18.18% (WLA = 4.4 kg vs WAT= 3.6 kg) compared to WAT, subject L12 a decrease of 9.09%

(WLA = 3.3 kg vs WAT=3 kg), and subject L13 4.5% (WLA=6.6 kg vs WAT=6.3 kg). These percentages were obtained through the formula (WAT-WLA)/WLA * 100.

SERUM PROGESTERONE

Serum P4 was assayed before treatment to ensure that queens were not diestrus (P4 > 2ng/ml) at administration to avoid overdosage of P4.
Four queens from VTH of Padova at the pre-treatment appointment had serum P4 > 2ng/ml, without having mated with a male during the previous heat (subject P1, P3, P5, P8). They were classified as spontaneous ovulators and owners were told to wait 20-30 days, depending on the queen's P4 value, so that the queen could be in interestrus or anestrus when the treatment with megestrol acetate would be started. Table 7 shows serum P4 concentration before and after treatment.

Table 7: Serum P4 concentration of the 18 studied queens before and after treatment (ng/ml).

NAME	P4 BEFORE TREATMENT (ng/ml)	P4 AFTER TREATMENT (ng/ml)
P1	2.71	1.5
P2	1.21	0.78
P3	20.03	0.47
P4	<0.1	0.45
P5	>40	0.45
P7	0.9	<0.1
P8	2.46	0.86
L1	0.6	0.6
L2	0.51	0.51
L3	< 0.5	< 0.5
L4	0.83	0.83
L5	0.54	0.54
L6	< 0.5	< 0.5
L7	< 0.5	< 0.5
L8	0.55	0.55
L9	0.57	0.57
L10	0.71	0.71
L11	< 0.5	< 0.5

The mean and standard deviation of P4 values before and after treatment excluding the 4 spontaneous ovulating queens was 0.61± 0.25 ng/ml and 0.62 ±0.29 ng/ml, respectively. The mean and SD were calculated considering 0.5 ng/ml for values indicated as "< 0.5" and 0.1 ng/ml for values "<0.1". These two different minimum detectable values for the P4 assay are due to the two different P4 analyzers employed in the two VTHs.

CHAPTER 8 - DISCUSSION

This study investigated the efficiency of a low-dose protocol of MA (Estropill^{TM}, MSD) in inhibiting ovarian activity in female cats and mitigating marking and mounting behaviors in male cats. The enrollment of male subjects was not anticipated at the moment of protocol design; however, this route was later pursued due to the owner's request which gave us the opportunity to study the effect of the drug also in males, despite the small sample size (n=3).
Historically, the use of MA has been surrounded by controversy, largely due to its frequent administration at dosages higher than necessary. However, this study demonstrates that even at minimal doses, MA effectively produces the desired effects.
The results of this study indicated that the administration of MA at the dose of 11 µg /kg per os once daily was both effective and safe in suppressing cyclicity in queens. All treated female cats resumed estrus within an average of 50±17 (range 23-77) days post-treatment, with no significant variation based on either the season of treatment end or treatment duration of (42.3±30.1 – *p-value* 0.638, 49.3±10.21 – *p-value* = 0.423, and 52.5±15.5 - *p-value* = 0.687 days for 4, 5, and 6-month treatments, respectively).
In Chapter 4.2.5, pertaining to contraindications and side effects, it was noted that adverse effects were observed with standard dosages ranging from 2.5–5 mg administered every other day—a dosage approximately 30 times greater than that employed in this study. Considering the mean weight of our pool of cats before treatment initiation (3.75 kg) and multiplying it by the dosage of 11.5 µg/day/kg, we arrived at a daily dosage of 0.043 mg/cat/day. In comparison, a dosage of 2.5 mg every other day equates to 1.25 mg/day/cat, which, when divided by 0.043 mg, results in a factor of approximately 29— meaning that the dosage associated with reported side effects is nearly 29 times greater than the dosage employed in our study.

Subject P4 has been cited as a particular case of manifestation of heat behavior such as rubbing against people and objects, vocalization, and increased affection, during MA treatment, this was probably due to the important weight gain (+ 32%) that presumably led to an underdosage of MA, further confirming that the dose employed is close to the minimum effective dose of the drug; subsequently, the dosage was adapted to the newly gain weight and the queen was cytologically confirmed in anestrous. This case will be discussed in detail below.

Serum P4 levels were assayed before treatment to ensure subjects were not in diestrus – which would have led to an overdosage of P4. Effective control of ovarian activity was observed throughout the treatment period, cytologically and when P4 was reassessed PT[4], all values were physiological for cats in anestrous.

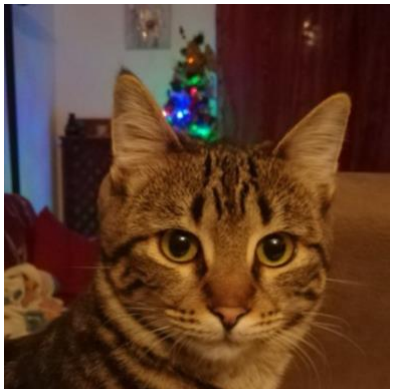
Figure 24: cat P1, intact queen, 12 months, domestic shorthair, seen at Padova.

The most significant cases are explained herewith:

Cat P1 (figure 24):
Intact 12-month-old domestic shorthair queen of 4.2 kg who was treated with MA for 4 months (14/09/22 - 01/11/22) at the VTH of Padova. On the first appointment (21/03/22), before starting the treatment with MA, the owner reported that her last heat had been approximately 2 weeks before. The general clinical evaluation was normal as well as the ultrasound of the ovaries and the uterus (Figure 25). Serum P4 was 2.71 ng/ml accompanied by a vaginal cytological pattern characteristic of diestrus. She was considered a spontaneous ovulator since she lived exclusively indoors and had no contact with other cats, meaning she could not have mated. Owners were advised to start the treatment 20 days from the day of the first appointment to ensure it

[4] WAT: weight after treatment, WBT: weight before treatment, WLA: weight at last appointment, PT: Post-treatment WG: weight gain

would not occur overdosage of P4. As the last heat had been 2 weeks before, this low P4 value is presumed to indicate the end of the luteal phase rather than its beginning.

During the treatment, no side effects were reported besides higher appetite. PT ultrasound images were normal (Figure 26) and the queen came back in heat 77 days PT- the heat was normal. Subsequently, she was spayed.

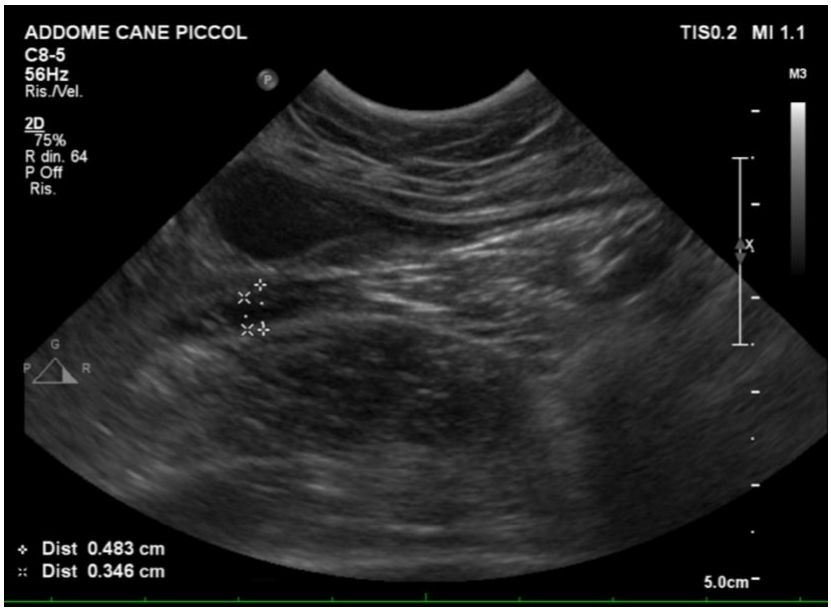

Figure 25: Uterine ultrasound of subject P1 at the first appointment before treatment (31/03/22); dist 0.483cm corresponds to the outer diameter of the uterus while dist 0.346cm is the inner diameter. VTH of Padova

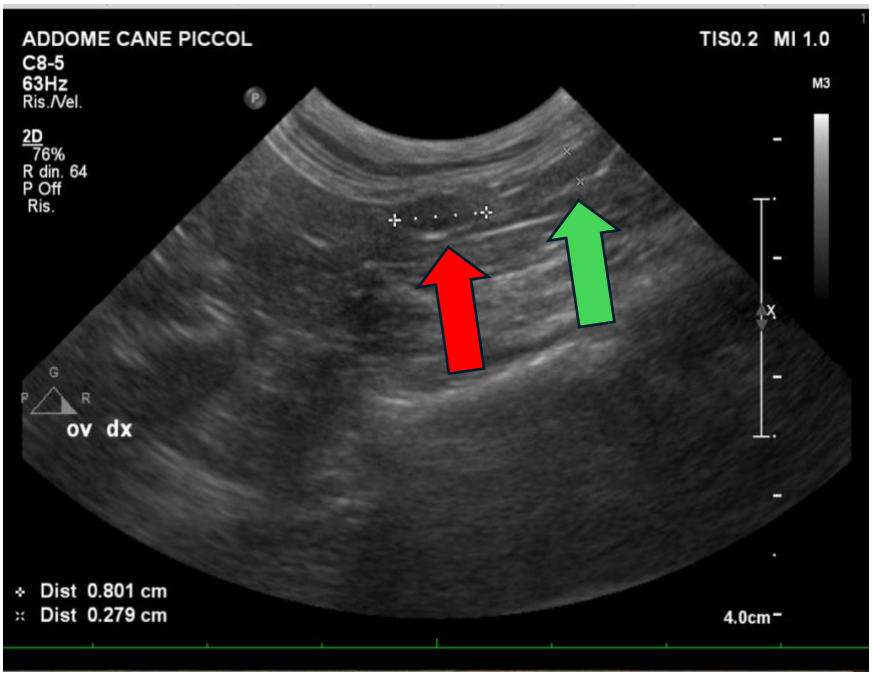

Figure 26 : Reproductive ultrasound of subject P1 at the first appointment PT (27/09/22); dist 0.801 cm corresponds to the length of the ovary (red arrow) while dist 0.279 cm is the diameter of the uterus (green arrow). VTH of Padova

Cat P4 (Figure 27):
Intact female Bengal of 11 months, 3.7 kg before the treatment, treated with MA for 5 months (04/04/23 – 30/08/23) at the VTH of Padova.
Treatment began on April 4th, 2023, at a dose of 0.05 ml/day (weight 3.7 kg). Ten days into treatment, owners observed signs of heat behavior, such as vocalization, lordosis, and continuous rubbing against objects and people. Additionally, the queen's appetite consistently increased.

Figure 27: Cat P4, intact female Bengal, 11month, group of treatment G5 - VTH of Padova.

Without any veterinary consultation, the dose was increased to 0.06 ml/day by the owners, leading to a calming effect within 2 days. She was rechecked on the 4th of May and vaginal cytology was performed (figure 28) and diagnosed as estrous. The owners were instructed not to intervene in such a way without prior consultation with the veterinarian. Subsequently, during an additional check-up on May 11th, 2023, a weight gain of 1.2 kg was noticed (weight 4.9kg), and it was agreed to maintain the dose at 0.06 ml/day after anestrous was confirmed cytologically (Figure 29).
By the second monthly check-up on June 19th, 2023, she had gained another 300g (weight 5.2 kg) but showed no signs of estrus, so the dose remained unchanged.
At the third monthly check-up on July 20th, 2023, she had gained another 300g (weight 5.5 kg) and began displaying estrus behavior five days previously (July 15th, 2023). The dose was increased to 0.07 ml/day, but she did not respond. A vaginal smear confirmed interestrus. Due to the history, vaginal cytology, and lack of response, the dose was further increased to 0.08 ml/day, and a follow-up visit was scheduled for two weeks later.
On August 2nd, 2023 appointment, subject P4 was in anestrus (confirmed cytologically). Treatment was concluded on this day, with a weight of 5.6 kg (+ 1.9 kg from the start of the trial). The dosage the owner administered in the last month varied around 0.09 ± 0.005 ml between days even if the indication was for 0.08 ml/day.
Following the end of the treatment, the queen came back in heat on 12/10/23, had normal estrus manifestations, and was subsequently spayed when in anestrous.

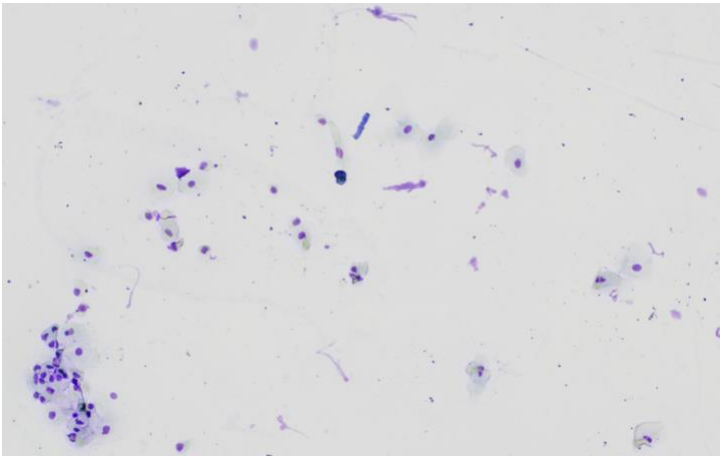

Figure 28: Vaginal cytology of subject P4, estrus (04/05/23) – VTH of Padova.

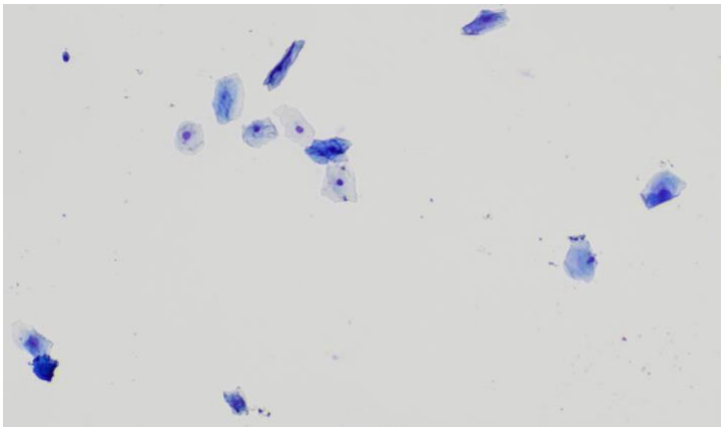

Figure 29: Vaginal cytology of subject P4, anestrous (11/05/23)- VTH of Padova.

Cat P5 (Figure 30):
Intact Norwegian Forest queen from Padova VTH, 5.6 years, treated with MA for 4 months (20/04/23 - 18/08/23). Due to her anxious and aggressive temperament, it was decided to perform only the first and last appointments, and a single checkup at mid-trial.

This queen was considered an outlier for the extended time interval from treatment end to resumption of ovarian activity, as she took 184 days to return to heat PT. A remark must be made on the matter - this queen was housed in an outside enclosure and possibly not checked as frequently and thoroughly as necessary to detect heat.
The owner declared a return in heat only on 20/02/24, more than 6 months PT.
The estrus was not confirmed cytologically and no record of weight is available for that moment due to a very low owner compliance.

Figure 30: Cat P5, intact Norvegian Forest female, 5.6y, group of treatment G4 - VTH of Padova

Cat P8 (Figure 31):
Intact female British shorthair, 2.4 years, 2.9 kg, who was treated with MA for 6 months (12/08/23 - 12/02/24) in VTH of Padova.

This queen was examined for the first time on 03/08/23, exhibiting a serum P4 of 2.41 ng/ml. The owner admitted the possibility of a mating with an intact male cat living together, who later on was enrolled in the study as subject P6. However, the owner could not provide a date for this alleged mating.

Treatment began on 12/08/23, after the owner saw her back in heat and ruled out pregnancy, and continued for 6 months. Following the end of the treatment, the queen came back in heat 27 days PT (confirmed cytologically), exhibiting similar estrous behavior as pre-treatment. During this first heat PT, she was mated again by subject P6 (10/03/24) and is at the moment awaiting pregnancy diagnosis at 30 days post-mating.

Figure 31: Cat P8, intact queen, 2.4y, British shorthair, Group of treatment G6 - VTH of Padova.

Male cats:
Three tomcats were enrolled in this study, with the aim of understanding if MA at the dosage of 11.5 µg/kg/ day (approximately 5 drops/kg/day PO) would represent an effective alternative to surgical castration for temporary suppression of fertility.
The results obtained are heterogeneous:

Cat P6 (Figure 32):
Intact male British shorthair 1-year-old treated for 5 months (02/10/23 - 02/03/24) at the VTH of Padova. The subject is owned by a British shorthair cat breeder and during treatment was housed with 3 intact females.
At each monthly check-up, penile spikes were present, slightly decreased in size, mostly from the 2nd month of treatment, but never fully disappeared whereas urine marking attenuated over time.

Figure 32: Cat P6, intact male, 1y, British shorthair. Group of treatment G5 - VTH of Padova.

Between the 3rd and 4th checkups (approximately during the last week of January 2024), subject P6 mated 2 intact British shorthair females (not treated with MA). One conceived and has queened 6 healthy kittens (Figures 33A and B).

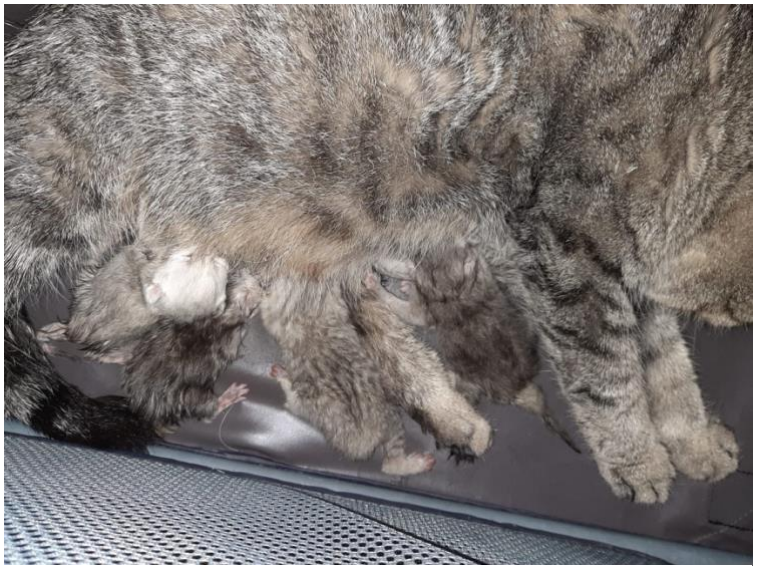

Figure 33A: litter queened subsequently successful mating happened during treatment of P6. VTH of Padova.

Figure 33 B: six healthy kittens obtained from subject P6 during treatment – VTH of Padova.

Cat L12
Intact male Sphynx,1.4 years old from Ljubljana's VTH, treated with MA for 4 months (05/04/23 - 27/07/23).

Cat L13
Intact male Maine Coon, 1.75 years old from Ljubljana's VTH treated with MA for 5 months (07/04/23 - 01/09/23).

For both subjects L12 and L13 the effects of MA were similar:

- No reduction in testicles' size was observed;
- Urine marking disappeared around 1 week PT;
- Interest towards females disappeared around 1 month PT;
- Penile spikes disappeared around the third month of treatment;
- Around 100 days after the end of the treatment, penile spikes were again visible for both cats (respectively 103- and 102-days PT);
- L12 showed interest towards females at the beginning of December (3 months PT) while no information is available for L13.

The main outcome of this study, apart from the efficacy and safety of the protocol, is the WG experienced by all the subjects during the treatment, accompanied by an increased appetite in all of them except for subjects P2 and P5.
The higher appetite was noticed by the owners, who observed an increased request for food and restlessness around mealtimes. Appetite was assessed subjectively and reported during monthly checkups. For this reason, we cannot be sure whether or not all subjects experienced increased appetite since it is possible that animals fed *ad libitum* were not monitored closely for this aspect.

Further investigation with a focus on weight fluctuations could be interesting, standardizing the diet aspects to better understand patterns of WG[5] in cats treated with MA.
The data regarding weight is not available for all subjects at all appointments planned on the study protocol due to insufficient owner compliance. Weight Gain profile was not one of the established aims of the study, therefore it was not regarded as an important outcome during data collection, but rather as additional information for general health assessment and a required value for calculation of the sedatives' dose, if deemed necessary.

A pattern of weight loss between the last checkup during the treatment and the first appointment PT was noticed for all males. This result was not statistically analyzed as only 3 tomcats were enrolled but results show that cat P6 showed weight loss after 26 days PT, while L12 and L13 were visited more than 3 months after the end of the treatment, depicting also a weight reduction, compared to the last checkup during MA.
It needs also to be highlighted that even when the treatment was not fully effective, WG was nevertheless present, suggesting that MA's efficacy is not directly correlated with its metabolical effects – evident in the case of subject P6, who did not respond to therapy, though showing the greatest weight loss PT (- 0.8kg).

The statistical analysis conducted in this study focused on two key findings: the average number of days for queens to return to estrus among the three different treatment groups, which did not yield statistically significant differences (G4 p-value = 0.638, G5 p-value = 0.423, G6 p-value = 0.687), and the observed weight gain across all groups. Notably, groups G4 (p-value = 0.021) and G6 (p-value = 0.0004) demonstrated significant weight gain, while G5 (p-value = 0.097) did not exhibit a statistically significant difference. However, a p-value below 0.1 suggests a tendency for weight gain in G5 subjects. An increased number of experimental cats might have made G5 weight results significant. The same goes for the comparison between WLA and WAT (at first heat) for queens, in which a trend for weight decrease was found (p-value=0.097).
Since the number of subjects enrolled in the study was small, we could only observe a general trend of weight gain and subsequent weight loss toward the original WBT. This is not sufficient to fully understand if, with time, subjects would return to the initial weight or maintain the weight reached during the treatment and surely needs further investigations and analysis, with more subjects enrolled and a longer follow-up after the end of treatment.

Upon closer examination of the situations involving cats P6 and P8, an additional consideration may be made. As previously noticed, the queen P8 at her first heat cycle following treatment end engaged in mating behavior with the tom P6. This demonstrates the reversibility of the treatment, as the tom P6 successfully mated both during the treatment period and post-treatment (leading to the queening of 6 healthy kittens), while subject P8 exhibited receptivity after just 27 days PT.

[5] WAT: weight after treatment, WBT: weight before treatment, WLA: weight at last appointment. PT: Post-treatment, WG: weight gain.

CHAPTER 9 - CONCLUSIONS

This research shows that the low-dose MA treatment can be a good option for moderating sexual-related behaviors in males and an effective alternative for suppressing cyclicity in adult queens for up to 6 months.
The time of resumption of ovarian activity is estimated to be 7 weeks after treatment is withdrawn.
The efficacy of a low-dose MA treatment in tomcats needs further research since in one out of three tomcats the treatment did not prevent urine marking, mating behavior, and fertility. On the contrary, PT fertility was observed in one of the male subjects who achieved conception and subsequent queening of a liter of healthy kittens.
The dosage employed is close to the minimum effective dose of the drug, demonstrated by the fact that weight gain led to underdosing and subsequently heat manifestation, which allows a high degree of treatment safety.
No reproductive or systemic side effects were observed apart from an increase in appetite, noticed in all subjects but two, and an increase in BW evident in all subjects during the treatment which was statistically significant in 2 groups (G4 and G6) out of three. A slight reduction in weight between the last appointment during treatment and the first appointment PT was noticed in some subjects, suggesting the need to further investigate weight fluctuation during and after treatment, with a more extended follow-up.

REFERENCES

4. Bacha, W. J. , B., & Linda M. (2012). *Color Atlas of Veterinary Histology* (third).
5. Banchi, P., Rota, A., Bertero, A., Domain, G., Ali Hassan, H., Lannoo, J., & Van Soom, A. (2022). Trends in Small Animal Reproduction: A Bibliometric Analysis of the Literature. *Animals : An Open Access Journal from MDPI*, *12*(3). https://doi.org/10.3390/ani12030336
6. Concannon, P., Hodgson, B., & Lein, D. (1980). Reflex LH Release in Estrous Cats Following Single and Multiple Copulations. *Biology of Reproduction*, *23*(1), 111–117. https://doi.org/10.1095/biolreprod23.1.111
7. Evans, J. M., & Sutton, D. J. (1989). The use of hormones, especially progestagens, to control oestrus in bitches. *Journal of Reproduction and Fertility. Supplement*, *39*, 163–173.
8. Faya, M., Carranza, A., Miotti, R., Ponchón, T., Furlan, P., & Gobello, C. (2013). Fecal estradiol-17β and testosterone in prepubertal domestic cats. *Theriogenology*, *80*(6), 584–586. https://doi.org/10.1016/J.THERIOGENOLOGY.2013.05.026
9. Findik, M., Erünal, N., & Aslan, S. (1999). *The use of proligestone, megestrol acetate, and GnRH on queens with the aim of hormonal contraception*. https://journals.tubitak.gov.tr/veterinary
10. Fischer, A., Benka, V. A., Briggs, J. R., Driancourt, M.-A., Maki, J., Mora, D. S., Morris, K. N., Myers, K. A., Rhodes, L., Vansandt, L. M., Weedon, G. R., Wolf, J., & Levy, J. K. (2018). Effectiveness of GonaCon as an immunocontraceptive in colony-housed cats. *Journal of Feline Medicine and Surgery*, *20*(8), 786–792. https://doi.org/10.1177/1098612X18758549
11. Gimenez, F., Stornelli, M. C., Tittarelli, C. M., Savignone, C. A., Dorna, I. V., de la Sota, R. L., & Stornelli, M. A. (2009). Suppression of estrus in cats with melatonin implants. *Theriogenology*, *72*(4), 493–499. https://doi.org/10.1016/j.theriogenology.2009.04.004
12. Gobello, C. (2012). Effects of GnRH antagonists vs agonists in Domestic Carnivores, a Review. *Reproduction in Domestic Animals*, *47*(s6), 373–376. https://doi.org/10.1111/rda.12025
13. Goericke-Pesch, S., Georgiev, P., Atanasov, A., Albouy, M., Navarro, C., & Wehrend, A. (2013). Treatment of queens in estrus and after estrus with a GnRH-agonist implant containing 4.7 mg deslorelin; hormonal response, duration of efficacy, and reversibility. *Theriogenology*, *79*(4), 640–646. https://doi.org/10.1016/j.theriogenology.2012.11.018
14. Goericke-Pesch, S., Wehrend, A., & Georgiev, P. (2014). Suppression of Fertility in Adult Cats. *Reproduction in Domestic Animals*, *49*(s2), 33–40. https://doi.org/10.1111/rda.12301
15. Graham, L. H., Swanson, W. F., & Brown, J. L. (2000). Chorionic gonadotropin administration in domestic cats causes an abnormal endocrine environment that disrupts oviductal embryo transport. *Theriogenology*, *54*(7), 1117–1131. https://doi.org/10.1016/S0093-691X(00)00420-9
16. Greenberg, M., Lawler, D., Zawistowski, S., & Jöchle, W. (2013). Low-dose megestrol acetate revisited: A viable adjunct to surgical sterilization in free-roaming cats? *The Veterinary Journal*, *196*(3), 304–308. https://doi.org/10.1016/J.TVJL.2013.01.038
17. Gudermuth, D. F., Newton, L., Daels, P., & Concannon, P. (1997). Incidence of spontaneous ovulation in young, group-housed cats based on serum and faecal concentrations of progesterone. *Journal of Reproduction and Fertility. Supplement*, *51*, 177–184.

18. Hernandez, F. J., Fernandez, B. B., Chertack, M., & Gage, P. A. (1975). Feline mammary carcinoma and progestogens. *Feline Practice*, *5*(5), 45–48.
19. Houdeshell, J. W., & Hennessey, P. W. (1977). Megestrol acetate for control of estrus in the cat. *Veterinary Medicine, Small Animal Clinician : VM, SAC*, *72*(6), 1013–1017.
20. Hurni, H. (1981). Daylength and breeding in the domestic cat. *Laboratory Animals*, *15*(3), 229–233. https://doi.org/10.1258/002367781780893803
21. Ills, J. N. M., Valli, V. E., & Lumsden, J. H. (1979). *Cyclical Changes of Vaginal Cytology in the Cat*.
22. Jemmett, J. E., & Evans, J. M. (1977). A survey of sexual behavior and reproduction of female cats. *Journal of Small Animal Practice*, *18*(1), 31–37. https://doi.org/10.1111/j.1748-5827.1977.tb05821.x
23. Jöchle, W., & Jöchle, M. (1975). Reproductive and behavioral control in the male and female cat with progestins: Long-term field observations in individual animals. *Theriogenology*, *3*(5), 179–185. https://doi.org/10.1016/0093-691X(75)90002-3
24. Johnson, A. K. (2022). Normal feline reproduction: The queen. In *Journal of Feline Medicine and Surgery* (Vol. 24, Issue 3, pp. 204–211). SAGE Publications Ltd. https://doi.org/10.1177/1098612X221079706
25. Johnson A., & Kutzler, M. A. (2022). *Feline Reproduction*. https://doi.org/10.1079/9781789247107.0000
26. Johnston S. D. Root Kustritz M. V. & Olson P. S. (2001). *Canine and feline theriogenology* (Saunders, Ed.).
27. Kutzler, M., & Wood, A. (2006). Non-surgical methods of contraception and sterilization. *Theriogenology*, *66*(3), 514–525. https://doi.org/10.1016/j.theriogenology.2006.04.014
28. Levy, J. K. (2011). Contraceptive Vaccines for the Humane Control of Community Cat Populations. In *American Journal of Reproductive Immunology* (Vol. 66, Issue 1, pp. 63–70). https://doi.org/10.1111/j.1600-0897.2011.01005.x
29. Leyva, H., Madley, T., & Stabenfeldt, G. H. (1989). Effect of light manipulation on ovarian activity and melatonin and prolactin secretion in the domestic cat. *Journal of Reproduction and Fertility. Supplement*, *39*, 125–133.
30. Lopez Merlo, M., Faya, M., Blanco, P. G., Carransa, A., Barbeito, C., & Gobello, C. (2016). Failure of a single dose of medroxyprogesterone acetate to induce uterine infertility in postnatally treated domestic cats. *Theriogenology*, *85*(4), 718–723. https://doi.org/10.1016/j.theriogenology.2015.10.013
31. Loretti, A. P., da Silva Ilha, M. R., Ordás, J., & de las Mulas, J. M. (2005). Clinical, pathological, and immunohistochemical study of feline mammary fibroepithelial hyperplasia following a single injection of depot medroxyprogesterone acetate. *Journal of Feline Medicine and Surgery*, *7*(1), 43–52. https://doi.org/10.1016/j.jfms.2004.05.002
32. Max, A., Jurka, P., Dobrzy´nski, A. D., & Rijsselaere, T. (2014). NON-SURGICAL CONTRACEPTION IN FEMALE DOGS AND CATS. In *Acta Sci. Pol., Zootechnica* (Vol. 13, Issue 1).
33. Michael, R. P. (1961). Observations Upon the Sexual Behaviour of the Domestic Cat (Felis Catus L.) Under Laboratory Conditions. *Behavior*, *18*(1–2), 1–24. https://doi.org/10.1163/156853961X00259
34. MSD Animal Health S.r.l. – Segrate (MI). (2004). *RIASSUNTO DELLE CARATTERISTICHE DEL PRODOTTO*.
35. Oen, E. O. (1977). The oral administration of megestrol acetate to postpone oestrus in cats. *Nordisk Veterinaermedicin*, *29*(6), 287–291.

36. Paape, S. R., Suille, V. M., Seto, H., & Stabenfeldt, G. U. (1975). Luteal Activity in the Pseudopregnant Cat. In *BIOLOGY OF REPRODUCTION* (Vol. 13). https://academic.oup.com/biolreprod/article/13/4/470/2841522
37. Pelican, K. M., Brown, J. L., Wildt, D. E., Ottinger, M. A., & Howard, J. G. (2005). Short-term suppression of follicular recruitment and spontaneous ovulation in the cat using levonorgestrel versus a GnRH antagonist. *General and Comparative Endocrinology*, *144*(2), 110–121. https://doi.org/10.1016/j.ygcen.2005.04.014
38. Plumb, D. C. (2008). *Veterinary Drug Handbook Sixth Edition*. www.vetfield.sitew.org
39. Reece, W. O. (2015). *Dukes' Physiology of Domestic Animals, 13th Edition*.
40. Romagnoli, S. (2015). Progestins to control feline reproduction: Historical abuse of high doses and potentially safe use of low doses. In *Journal of Feline Medicine and Surgery* (Vol. 17, Issue 9, pp. 743–752). SAGE Publications Ltd. https://doi.org/10.1177/1098612X15594987
41. Romagnoli, S., Bensaia, C., Ferré-Dolcet, L., Sontas, H. B., & Stelletta, C. (2019). Fertility parameters and reproductive management of Norwegian Forest Cats, Maine Coon, Persian and Bengal cats raised in Italy: a questionnaire-based study. *Journal of Feline Medicine and Surgery*, *21*(12), 1188–1197. https://doi.org/10.1177/1098612X18824181
42. Romagnoli S., & Concannon P.W. (2003). *Clinical Use of Progestins in Bitches and Queens: A Review Introduction and Terminology*. www.ivis.org
43. Romagnoli, S., & Ferre-Dolcet, L. (2022). Reversible Control of Reproduction In Queens: Mastering the use of reproductive drugs to manipulate cyclicity. In *Journal of Feline Medicine and Surgery* (Vol. 24, Issue 9, pp. 853–870). SAGE Publications Ltd. https://doi.org/10.1177/1098612X221118754
44. Romagnoli, S., & Sontas, H. (2010). Prevention of breeding in the female. In *BSAVA Manual of Canine and Feline Reproduction and Neonatology*.
45. Schäfer-Somi, S. (2017). Effect of melatonin on the reproductive cycle in female cats: a review of clinical experiences and previous studies. *Journal of Feline Medicine and Surgery*, *19*(1), 5–12. https://doi.org/10.1177/1098612X15610369
46. Schindler, A. E., Campagnoli, C., Druckmann, R., Huber, J., Pasqualini, J. R., Schweppe, K. W., & Thijssen, J. H. H. (2008). Reprint of Classification and pharmacology of progestins. *Maturitas*, *61*(1–2), 171–180. https://doi.org/10.1016/j.maturitas.2008.11.013
47. Senger, P. L. (Phillip L.). (2012). *Pathways to pregnancy & parturition* (3rd ed.).
48. Shille, V. M., Lundström, K. E., & Stabenfeldt, G. H. (1979). Follicular Function in the Domestic Cat as Determined by Estradiol-17β Concentrations in Plasma: Relation to Estrous Behavior and Cornification of Exfoliated Vaginal Epithelium. *Biology of Reproduction*, *21*(4), 953–963. https://doi.org/10.1095/biolreprod21.4.953
49. Tomlinson, M. J., Barteaux, L., Ferns, L. E., & Angelopoulos, E. (1984). *THE CANADIAN VETERINARY JOURNAL LA REVUE VETERINAIRE CANADIENNE Feline Mammary Carcinoma: A Retrospective Evaluation of 17 Cases*.
50. Tsutsui, T., Nakagawa, K., Hirano, T., Nagakubo, K., Shinomiya, M., Yamamoto, K., & Hori, T. (2004). Breeding Season in Female Cats Acclimated Under a Natural Photoperiod and Interval Until Puberty. In *J. Vet. Med. Sci* (Vol. 66, Issue 9).
51. Vansandt, L., Kutzler, M., Fischer, A., Morris, K., & Swanson, W. (2017). Safety and effectiveness of a single and repeat intramuscular injection of a Gn RHvaccine (GonaCon™) in adult female domestic cats. *Reproduction in Domestic Animals*, *52*(S2), 348–353. https://doi.org/10.1111/rda.12853

ANNEX 1 – URINALYSIS (VTH of Padova)

Subject P1 – Before treatment

Test	Risultato	U. M.	Valori di normalità
ESAME URINE			
ESAME CHIMICO-FISICO			
Metodo di prelievo	Minzione spontanea		
Volume Urine Raccolte	5	ml	
Colore	Giallo		
Aspetto	Torbido		
Peso Specifico	1,050		1,015 - 1,080
pH	7		5 - 7,5
Leucociti	500 (+++)	CELLS/µL	Assenti
Nitriti	Assenti		Assenti
Proteine	0,3 (+)	g/L	> Assenti
Glucosio	Assente	mmol/L	> Assente
Chetoni	Assenti	mmol/L	> Assenti
Urobilinogeno	< 16	µmol/L	> < 16
Bilirubina	Assente	µmol/L	Assente
Eritrociti	Assenti	CELLS/µL	> Assenti
SEDIMENTO			
Annotazioni: Presenza di numerosi cristalli di struvite.			

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA URINARIA			
RAPP. PROT. URIN./CREAT.URIN.	0,09		< 0,5

Subject P1 – After treatment

Test	Risultato	U. M.	Valori di normalità
ESAME URINE			
ESAME CHIMICO-FISICO			
Metodo di prelievo	Cistocentesi		
Volume Urine Raccolte	3	ml	
Colore	Giallo		
Aspetto	Limpido		
Peso Specifico	1,045		1,015 - 1,080
pH	5		5 - 7,5
Leucociti	500 (+++)	CELLS/µL	Assenti
Nitriti	Assenti		Assenti
Proteine	0,3 (+)	g/L	> Assenti
Glucosio	Assente	mmol/L	> Assente
Chetoni	Assenti	mmol/L	> Assenti
Urobilinogeno	< 16	µmol/L	> < 16
Bilirubina	Assente	µmol/L	Assente
Eritrociti	Assenti	CELLS/µL	> Assenti
SEDIMENTO			
Annotazioni:			
Presenza di numerose gocciole lipidiche.			

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA URINARIA			
Rapporto PU/CU Ricerca	0,1		<0,5

Subject P2 – before treatment

Test	Risultato	U. M.	Valori di normalità
ESAME URINE			
ESAME CHIMICO-FISICO			
Metodo di prelievo	Cistocentesi		
Volume Urine Raccolte	2,5	ml	
Colore	Giallo		
Aspetto	Torbido		
Peso Specifico	1,076		1,015 - 1,080
pH	6		5 - 7,5
Leucociti	500 (+++)	CELLS/µL	Assenti
Nitriti	Assenti		Assenti
Proteine	0,3 (+)	g/L	> Assenti
Glucosio	Assente	mmol/L	> Assente
Chetoni	Assenti	mmol/L	> Assenti
Urobilinogeno	< 16	µmol/L	> < 16
Bilirubina	Assente	µmol/L	Assente
Eritrociti	25 (++)	CELLS/µL	> Assenti
SEDIMENTO			
Annotazioni: alcuni leucociti e numerose gocce lipidiche			

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA URINARIA			
Rapporto PU/CU Ricerca	0,1		< 0,5

Subject P2 – After treatment

Test	Risultato	U. M.	Valori di normalità
ESAME URINE			
ESAME CHIMICO-FISICO			
Metodo di prelievo			
Volume Urine Raccolte	5	ml	
Colore	Giallo		
Aspetto	Torbido		
Peso Specifico	1,062		1,015 - 1,080
pH	6		5 - 7,5
Leucociti	10-25 (+)	CELLS/µL	Assenti
Nitriti	Assenti		Assenti
Proteine	0,3 (+)	g/L	> Assenti
Glucosio	Assente	mmol/L	> Assente
Chetoni	Assenti	mmol/L	> Assenti
Urobilinogeno	< 16	µmol/L	> < 16
Bilirubina	Assente	µmol/L	Assente
Eritrociti	Assenti	CELLS/µL	> Assenti
SEDIMENTO			
Annotazioni: Presenza di rari leucociti e di numerose gocce lipidiche.			

Subject P3 – Before treatment

Test	Risultato	U. M.	Valori di normalità
ESAME URINE			
ESAME CHIMICO-FISICO			
Metodo di prelievo			
Volume Urine Raccolte	2	ml	
Colore	Giallo		
Aspetto	Limpido		
Peso Specifico	**1058**		1,015 - 1,080
pH	6		5 - 7,5
Leucociti	75 (++)	CELLS/µL	Assenti
Nitriti	Assenti		Assenti
Proteine	0,3 (+)	g/L	> Assenti
Glucosio	Assente	mmol/L	> Assente
Chetoni	Assenti	mmol/L	> Assenti
Urobilinogeno	< 16	µmol/L	> < 16
Bilirubina	Assente	µmol/L	Assente
Eritrociti	50 (+++)	CELLS/µL	> Assenti
SEDIMENTO			

Annotazioni:
Moderata ematuria e leucocituria, moderata presenza di cellule epiteliali (transizione e vacuolizzate)

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA URINARIA			
Rapporto PU/CU Ricerca	0,1		

Subject P3 – After treatment

Test	Risultato	U. M.	Valori di normalità
ESAME URINE			
ESAME CHIMICO-FISICO			
Metodo di prelievo	Cistocentesi		
Volume Urine Raccolte	4	ml	
Colore	Giallo		
Aspetto	Limpido		
Peso Specifico	1,054		1,015 - 1,080
pH	6		5 - 7,5
Leucociti	75 (++)	CELLS/µL	Assenti
Nitriti	Assenti		Assenti
Proteine	1,0 (++)	g/L	> Assenti
Glucosio	Assente	mmol/L	> Assente
Chetoni	Assenti	mmol/L	> Assenti
Urobilinogeno	< 16	µmol/L	> < 16
Bilirubina	Assente	µmol/L	Assente
Eritrociti	50 (+++)	CELLS/µL	> Assenti
SEDIMENTO			

Annotazioni:
Diffuse gocce lipidiche, moderata leucocituria e lieve presenza di cellule epiteliali di sfaldamento

Subject P4 – Before treatment

Test	Risultato	U. M.	Valori di normalità
ESAME URINE			
ESAME CHIMICO-FISICO			
Metodo di prelievo			
Volume Urine Raccolte	4,5	ml	
Colore	Giallo		
Aspetto	Limpido		
Peso Specifico	1,080		1,015 - 1,080
pH	6		5 - 7,5
Leucociti	10-25 (+)	CELLS/µL	Assenti
Nitriti	Assenti		Assenti
Proteine	0,3 (+)	g/L	> Assenti
Glucosio	Assente	mmol/L	> Assente
Chetoni	Assenti	mmol/L	> Assenti
Urobilinogeno	< 16	µmol/L	> < 16
Bilirubina	Assente	µmol/L	Assente
Eritrociti	Assenti	CELLS/µL	> Assenti
SEDIMENTO			

Annotazioni:
Numerose gocce lipidiche.

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA URINARIA			
Rapporto PU/CU Ricerca	0,1		

Subject P4 – After treatment

Test	Risultato	U. M.	Valori di normalità
ESAME URINE			
ESAME CHIMICO-FISICO			
Metodo di prelievo	Cistocentesi		
Volume Urine Raccolte	4	ml	
Colore	Giallo		
Aspetto	Limpido		
Peso Specifico	**1,084**		1,015 - 1,080
pH	7		5 - 7,5
Leucociti	Assenti	CELLS/µL	Assenti
Nitriti	Assenti		Assenti
Proteine	0,3 (+)	g/L	> Assenti
Glucosio	Assente	mmol/L	> Assente
Chetoni	Assenti	mmol/L	> Assenti
Urobilinogeno	< 16	µmol/L	> < 16
Bilirubina	Assente	µmol/L	Assente
Eritrociti	Assenti	CELLS/µL	> Assenti
SEDIMENTO			
Annotazioni: Diffuse gocce lipidiche.			

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA URINARIA			
Rapporto PU/CU Ricerca	0,1		

Subject P5 – Before treatment

Test	Risultato	U. M.	Valori di normalità
ESAME URINE			
ESAME CHIMICO-FISICO			
Metodo di prelievo	Cistocentesi		
Volume Urine Raccolte	5	ml	
Colore	Giallo		
Aspetto	Torbido		
Peso Specifico	1,040		1,015 - 1,080
pH	7		5 - 7,5
Leucociti	Assenti	CELLS/µL	Assenti
Nitriti	Assenti		Assenti
Proteine	0,3 (+)	g/L	> Assenti
Glucosio	56 (++++)	mmol/L	> Assente
Chetoni	Assenti	mmol/L	> Assenti
Urobilinogeno	< 16	µmol/L	> < 16
Bilirubina	Assente	µmol/L	Assente
Eritrociti	50 (+++)	CELLS/µL	> Assenti
SEDIMENTO			

Annotazioni:
Marcata ematuria e abbondanti gocce lipidiche

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA URINARIA			
Rapporto PU/CU Ricerca	0,3		

Subject P5 – After treatment

Test	Risultato	U. M.	Valori di normalità
ESAME URINE			
ESAME CHIMICO-FISICO			
Metodo di prelievo	Cistocentesi		
Volume Urine Raccolte	3	ml	
Colore	Giallo		
Aspetto	Torbido		
Peso Specifico	1,036		1,015 - 1,080
pH	7		5 - 7,5
Leucociti	10-25 (+)	CELLS/µL	Assenti
Nitriti	Assenti		Assenti
Proteine	0,3 (+)	g/L	> Assenti
Glucosio	56 (++++)	mmol/L	> Assente
Chetoni	Assenti	mmol/L	> Assenti
Urobilinogeno	< 16	µmol/L	> < 16
Bilirubina	Assente	µmol/L	Assente
Eritrociti	Assenti	CELLS/µL	> Assenti
SEDIMENTO			
Annotazioni: Diffuse gocce lipidiche.			

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA URINARIA			
Rapporto PU/CU Ricerca	0,2		

Subject P6 – Before treatment

Test	Risultato	U. M.	Valori di normalità
ESAME URINE			
ESAME CHIMICO-FISICO			
Metodo di prelievo	Cistocentesi		
Volume Urine Raccolte	2	ml	
Colore	Giallo		
Aspetto	Limpido		
Peso Specifico	**1,096**		1,015 - 1,080
pH	5		5 - 7,5
Leucociti	Assenti	CELLS/µL	Assenti
Nitriti	Assenti		Assenti
Proteine	0,3 (+)	g/L	> Assenti
Glucosio	Assente	mmol/L	> Assente
Chetoni	Assenti	mmol/L	> Assenti
Urobilinogeno	17 (+)	µmol/L	> < 16
Bilirubina	Assente	µmol/L	Assente
Eritrociti	Tracce No Emolisi	CELLS/µL	> Assenti
SEDIMENTO			

Annotazioni:
Lieve presenza di cilindri granulari e di cilindri cellulari, diffuse gocce lipidiche.

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA URINARIA			
Rapporto PU/CU Ricerca	0,1		

Subject P6 – After treatment (not available)

Subject P7 – Before treatment

Test	Risultato	U. M.	Valori di normalità
ESAME URINE			
ESAME CHIMICO-FISICO			
Metodo di prelievo	Cistocentesi		
Volume Urine Raccolte	2,5	ml	
Colore	Giallo		
Aspetto	Limpido		
Peso Specifico	1,070		1,015 - 1,080
pH	6		5 - 7,5
Leucociti	Assenti	CELLS/µL	Assenti
Nitriti	Assenti		Assenti
Proteine	Assenti	g/L	> Assenti
Glucosio	Assente	mmol/L	> Assente
Chetoni	Assenti	mmol/L	> Assenti
Urobilinogeno	< 16	µmol/L	> < 16
Bilirubina	Assente	µmol/L	Assente
Eritrociti	Assenti	CELLS/µL	> Assenti
SEDIMENTO			
Annotazioni: numerose gocce lipidiche			

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA URINARIA			
Rapporto PU/CU Ricerca	0,1		

Subject P7 – After treatment

Test	Risultato	U. M.	Valori di normalità
ESAME URINE			
ESAME CHIMICO-FISICO			
Metodo di prelievo	Cistocentesi		
Volume Urine Raccolte	2	ml	
Colore	Giallo		
Aspetto	Limpido		
Peso Specifico	**1,096**		1,015 - 1,080
pH	5		5 - 7,5
Leucociti	Assenti	CELLS/µL	Assenti
Nitriti	Assenti		Assenti
Proteine	0,3 (+)	g/L	> Assenti
Glucosio	Assente	mmol/L	> Assente
Chetoni	Assenti	mmol/L	> Assenti
Urobilinogeno	< 16	µmol/L	> < 16
Bilirubina	Assente	µmol/L	Assente
Eritrociti	Tracce No Emolisi	CELLS/µL	> Assenti
SEDIMENTO			
Annotazioni:			
Lieve ematuria e diffuse gocce lipidiche.			

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA URINARIA			
Rapporto PU/CU Ricerca	0,1		

Subject P8 – Before treatment

Test	Risultato	U. M.	Valori di normalità
ESAME URINE			
ESAME CHIMICO-FISICO			
Metodo di prelievo	Cistocentesi		
Volume Urine Raccolte	2	ml	
Colore	Giallo		
Aspetto	Limpido		
Peso Specifico	1,060		1,015 - 1,080
pH	6		5 - 7,5
Leucociti	10-25 (+)	CELLS/µL	Assenti
Nitriti	Assenti		Assenti
Proteine	0,3 (+)	g/L	> Assenti
Glucosio	Assente	mmol/L	> Assente
Chetoni	Assenti	mmol/L	> Assenti
Urobilinogeno	< 16	µmol/L	> < 16
Bilirubina	Assente	µmol/L	Assente
Eritrociti	Assenti	CELLS/µL	> Assenti
SEDIMENTO			
Annotazioni: Lieve leucocituria e diffuse gocce lipidiche			

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA URINARIA			
Rapporto PU/CU Ricerca	0,1		

Subject P8 – After Treatment

Test	Risultato	U. M.	Valori di normalità
ESAME URINE			
ESAME CHIMICO-FISICO			
Metodo di prelievo	Cistocentesi		
Volume Urine Raccolte	3	ml	
Colore	Giallo		
Aspetto	Limpido		
Peso Specifico	1,062		1,015 - 1,080
pH	6		5 - 7,5
Leucociti	Assenti	CELLS/µL	Assenti
Nitriti	Assenti		Assenti
Proteine	0,3 (+)	g/L	> Assenti
Glucosio	Assente	mmol/L	> Assente
Chetoni	Assenti	mmol/L	> Assenti
Urobilinogeno	17 (+)	µmol/L	> < 16
Bilirubina	Assente	µmol/L	Assente
Eritrociti	Assenti	CELLS/µL	> Assenti
SEDIMENTO			
Annotazioni: Diffuse gocce lipidiche.			

ANNEX 2 – BLOOD ANALYSIS – Complete blood count (VTH of Padova)

Subject P1 – Before treatment

EMOGRAMMA

WBC	9,91	7,39-16,21	10^3/µl
RBC	9,06	5,56-8,44	10^6/µl
Hgb	14,1	8,1-12,2	g/dl
Hct	38,3	21,9-34	%
MCV	42,2	37,1-43	fl
MCH	15,6	13,6-15,4	pg
MCHC	37,0	34,4-38,3	g/dl
CHCM	39,1	34,3-38,4	g/dL
RDW	15,5	15,5-17,8	%
CH	16,5	13,6-15,5	pg
CHDW	2,44	2,25-2,68	pg
HDW	2,11	2,1-3,09	g/dL
PLT	250	147-361	10^3/µl
MPV	14,1	13,5-19,6	fl
PCT	0,35	0,26-0,52	%
PDW	65,4	61,9-69,6	%

FORMULA LEUCOCITARIA

% neutrofili segmentati	69,3	53,4-80,7	%
% linfociti	22,1	13-36,1	%
% monociti	1,6	1,6-3,5	%
% eosinofili	6,8	1,1-5,8	%
% basofili	0,2	0-0,2	%
% Luc	0	0,1-0,5	%

LEUCOCITI

Neutrofili segmentati	6860	4060-11640	/µl
linfociti	2190	1362-3520	/µl
monociti	160	140-440	/µl
eosinofili	670	120-620	/µl
basofili	10	0-20	/µl
LUC	0	10-57	/µl

Subject P1 – after treatment

EMOGRAMMA

WBC	6,52	7,39-16,21	10^3/µl
RBC	8,49	5,56-8,44	10^6/µl
Hgb	12,2	8,1-12,2	g/dl
Hct	35,8	21,9-34	%
MPV	15,6	13,5-19,6	fl
MCV	42,2	37,1-43	fl
MCH	14,3	13,6-15,4	pg
MCHC	34,0	34,4-38,3	g/dl
CHCM	35,1	34,3-38,4	g/dL
RDW	16,0	15,5-17,8	%
CH	14,8	13,6-15,5	pg
CHDW	2,25	2,25-2,68	pg
HDW	1,92	2,1-3,09	g/dL
PLT	263	147-361	10^3/µl
PCT	0,41	0,26-0,52	%
PDW	66,1	61,9-69,6	%

FORMULA LEUCOCITARIA

% neutrofili segmentati	57,2	53,4-80,7	%
% linfociti	30,3	13-36,1	%
% monociti	1,9	1,6-3,5	%
% eosinofili	10,3	1,1-5,8	%
% basofili	0,1	0-0,2	%
% Luc	0,10	0,1-0,5	%

LEUCOCITI

Neutrofili segmentati	3730	4060-11640	/µl
linfociti	1980	1362-3520	/µl
monociti	130	140-440	/µl
eosinofili	670	120-620	/µl
basofili	10	0-20	/µl
LUC	10	10-57	/µl

Stima PLT: ☐ inadeguata ☒ adeguata ☐ aumentata

Presenti aggregati piastrinici +

Subject P2 – before treatment

EMOGRAMMA

WBC	8,81	7,39-16,21	10^3/µl
RBC	10,19	5,56-8,44	10^6/µl
Hgb	13,7	8,1-12,2	g/dl
Hct	38,3	21,9-34	%
MPV	12,5	13,5-19,6	fl
MCV	37,6	37,1-43	fl
MCH	13,5	13,6-15,4	pg
MCHC	35,8	34,4-38,3	g/dl
CHCM	37,7	34,3-38,4	g/dL
RDW	15,3	15,5-17,8	%
CH	14,2	13,6-15,5	pg
CHDW	2,09	2,25-2,68	pg
HDW	2,27	2,1-3,09	g/dL
PLT	209	147-361	10^3/µl
PCT	0,26	0,26-0,52	%
PDW	71,8	61,9-69,6	%

FORMULA LEUCOCITARIA

% neutrofili segmentati	84,4	53,4-80,7	%
% linfociti	11,4	13-36,1	%
% monociti	1,7	1,6-3,5	%
% eosinofili	2,2	1,1-5,8	%
% basofili	0,1	0-0,2	%
% Luc	0,10	0,1-0,5	%

LEUCOCITI

Neutrofili segmentati	7440	4060-11640	/µl
linfociti	1010	1362-3520	/µl
monociti	150	140-440	/µl
eosinofili	190	120-620	/µl
basofili	10	0-20	/µl
LUC	10	10-57	/µl

Stima PLT: ☐ inadeguata ☒ adeguata ☐ aumentata

Presenti aggregati piastrinici ++

Note:

Subject P2 – After treatment

EMOGRAMMA

WBC	7,18	7,39-16,21	10^3/µl
RBC	10,95	5,56-8,44	10^6/µl
Hgb	15,4	8,1-12,2	g/dl
Hct	43,7	21,9-34	%
MPV	16,2	13,5-19,6	fl
MCV	39,9	37,1-43	fl
MCH	14,0	13,6-15,4	pg
MCHC	35,2	34,4-38,3	g/dl
CHCM	36,0	34,3-38,4	g/dL
RDW	15,3	15,5-17,8	%
CH	14,4	13,6-15,5	pg
CHDW	2,16	2,25-2,68	pg
HDW	2,14	2,1-3,09	g/dL
PLT	22	147-361	10^3/µl
PCT	0,04	0,26-0,52	%
PDW	76,4	61,9-69,6	%

FORMULA LEUCOCITARIA

% neutrofili segmentati	71,8	53,4-80,7	%
% linfociti	20,5	13-36,1	%
% monociti	1,7	1,6-3,5	%
% eosinofili	5,3	1,1-5,8	%
% basofili	0,4	0-0,2	%
% Luc	0,30	0,1-0,5	%

LEUCOCITI

Neutrofili segmentati	5150	4060-11640	/µl
linfociti	1470	1362-3520	/µl
monociti	120	140-440	/µl
eosinofili	380	120-620	/µl
basofili	30	0-20	/µl
LUC	20	10-57	/µl

Subject P3 – Before treatment

EMOGRAMMA

WBC	7,37	7,39-16,21	10^3/µl
RBC	9,61	5,56-8,44	10^6/µl
Hgb	11,8	8,1-12,2	g/dl
Hct	34,2	21,9-34	%
MPV	15,3	13,5-19,6	fl
MCV	35,7	37,1-43	fl
MCH	12,2	13,6-15,4	pg
MCHC	34,3	34,4-38,3	g/dl
CHCM	34,1	34,3-38,4	g/dL
RDW	15,7	15,5-17,8	%
CH	12,1	13,6-15,5	pg
CHDW	1,80	2,25-2,68	pg
HDW	2,16	2,1-3,09	g/dL
PLT	130	147-361	10^3/µl
PCT	0,20	0,26-0,52	%
PDW	73,8	61,9-69,6	%

FORMULA LEUCOCITARIA

% neutrofili segmentati	62,8	53,4-80,7	%
% linfociti	21,6	13-36,1	%
% monociti	3,5	1,6-3,5	%
% eosinofili	11,4	1,1-5,8	%
% basofili	0,4	0-0,2	%
% Luc	0,30	0,1-0,5	%

LEUCOCITI

Neutrofili segmentati	4630	4060-11640	/µl
linfociti	1590	1362-3520	/µl
monociti	260	140-440	/µl
eosinofili	840	120-620	/µl
basofili	30	0-20	/µl
LUC	20	10-57	/µl

Stima PLT: ☐ inadeguata ☒ adeguata ☐ aumentata

Presenti aggregati piastrinici ++

Subject P3 – After treatment

EMOGRAMMA

WBC	5,79	7,39-16,21	10^3/µl
RBC	11,16	5,56-8,44	10^6/µl
Hgb	14,0	8,1-12,2	g/dl
Hct	41,9	21,9-34	%
MPV	11,7	13,5-19,6	fl
MCV	37,5	37,1-43	fl
MCH	12,6	13,6-15,4	pg
MCHC	33,5	34,4-38,3	g/dl
CHCM	32,8	34,3-38,4	g/dL
RDW	14,0	15,5-17,8	%
CH	12,3	13,6-15,5	pg
CHDW	1,70	2,25-2,68	pg
HDW	2,14	2,1-3,09	g/dL
PLT	327	147-361	10^3/µl
PCT	0,38	0,26-0,52	%
PDW	59,6	61,9-69,6	%

FORMULA LEUCOCITARIA

% neutrofili segmentati	66,1	53,4-80,7	%
% linfociti	21,4	13-36,1	%
% monociti	3,6	1,6-3,5	%
% eosinofili	8,6	1,1-5,8	%
% basofili	0,1	0-0,2	%
% Luc	0,20	0,1-0,5	%

LEUCOCITI

Neutrofili segmentati	3830	4060-11640	/µl
linfociti	1240	1362-3520	/µl
monociti	210	140-440	/µl
eosinofili	500	120-620	/µl
basofili	0	0-20	/µl
LUC	10	10-57	/µl

Subject P4 – Before treatment

EMOGRAMMA

WBC	12,11	7,39-16,21	10³/µl
RBC	7,78	5,56-8,44	10^6/µl
Hgb	11,2	8,1-12,2	g/dl
Hct	31,0	21,9-34	%
MPV	16,4	13,5-19,6	fl
MCV	39,9	37,1-43	fl
MCH	14,4	13,6-15,4	pg
MCHC	36,0	34,4-38,3	g/dl
CHCM	34,8	34,3-38,4	g/dL
RDW	16,3	15,5-17,8	%
CH	13,9	13,6-15,5	pg
CHDW	2,32	2,25-2,68	pg
HDW	2,04	2,1-3,09	g/dL
PLT	8	147-361	10³/µl
PCT	0,01	0,26-0,52	%
PDW	91,4	61,9-69,6	%

FORMULA LEUCOCITARIA

% neutrofili segmentati	45,4	53,4-80,7	%
% linfociti	42,8	13-36,1	%
% monociti	2,7	1,6-3,5	%
% eosinofili	8,7	1,1-5,8	%
% basofili	0,1	0-0,2	%
% Luc	0,20	0,1-0,5	%

LEUCOCITI

Neutrofili segmentati	5500	4060-11640	/µl
linfociti	5190	1362-3520	/µl
monociti	330	140-440	/µl
eosinofili	1060	120-620	/µl
basofili	20	0-20	/µl
LUC	30	10-57	/µl

Stima PLT: [x] inadeguata [ ] adeguata [ ] aumentata

Presenti aggregati piastrinici [+]

Subject P4 – After treatment

EMOGRAMMA

WBC	12,81	7,39-16,21	10³/µl
RBC	7,87	5,56-8,44	10^6/µl
Hgb	11,8	8,1-12,2	g/dl
Hct	32,9	21,9-34	%
MPV	22,7	13,5-19,6	fl
MCV	41,9	37,1-43	fl
MCH	15,0	13,6-15,4	pg
MCHC	35,9	34,4-38,3	g/dl
CHCM	33,9	34,3-38,4	g/dL
RDW	16,0	15,5-17,8	%
CH	14,2	13,6-15,5	pg
CHDW	2,23	2,25-2,68	pg
HDW	1,97	2,1-3,09	g/dL
PLT	102	147-361	10³/µl
PCT	0,23	0,26-0,52	%
PDW	58,8	61,9-69,6	%

FORMULA LEUCOCITARIA

% neutrofili segmentati	59,5	53,4-80,7	%
% linfociti	26,5	13-36,1	%
% monociti	2,4	1,6-3,5	%
% eosinofili	11,4	1,1-5,8	%
% basofili	0	0-0,2	%
% Luc	0,20	0,1-0,5	%

LEUCOCITI

Neutrofili segmentati	7620	4060-11640	/µl
linfociti	3390	1362-3520	/µl
monociti	310	140-440	/µl
eosinofili	1460	120-620	/µl
basofili	0	0-20	/µl
LUC	20	10-57	/µl

Stima PLT: [ ] inadeguata [x] adeguata [ ] aumentata

Presenti aggregati piastrinici [+++]

Subject P5 – Before treatment

EMOGRAMMA

WBC	16,60	7,39-16,21	10^3/µl
RBC	9,62	5,56-8,44	10^6/µl
Hgb	13,9	8,1-12,2	g/dl
Hct	40,4	21,9-34	%
MPV	16,7	13,5-19,6	fl
MCV	42,0	37,1-43	fl
MCH	14,5	13,6-15,4	pg
MCHC	34,5	34,4-38,3	g/dl
CHCM	34,4	34,3-38,4	g/dL
RDW	16,0	15,5-17,8	%
CH	14,5	13,6-15,5	pg
CHDW	2,39	2,25-2,68	pg
HDW	1,97	2,1-3,09	g/dL
PLT	137	147-361	10^3/µl
PCT	0,23	0,26-0,52	%
PDW	70,4	61,9-69,6	%

Stima PLT: ☐ inadeguata ☒ adeguata ☐ aumentata

FORMULA LEUCOCITARIA

% neutrofili segmentati	81,0	53,4-80,7	%
% linfociti	14,0	13-36,1	%
% monociti	0,5	1,6-3,5	%
% eosinofili	4,3	1,1-5,8	%
% basofili	0,1	0-0,2	%
% Luc	0,10	0,1-0,5	%

LEUCOCITI

Neutrofili segmentati	13450	4060-11640	/µl
linfociti	2320	1362-3520	/µl
monociti	90	140-440	/µl
eosinofili	720	120-620	/µl
basofili	10	0-20	/µl
LUC	10	10-57	/µl

Presenti aggregati piastrinici ++

Subject P5 – After treatment (not available)

Subject P6 – Before treatment

EMOGRAMMA

WBC	14,52	7,39-16,21	10^3/µl
RBC	7,53	5,56-8,44	10^6/µl
Hgb	11,9	8,1-12,2	g/dl
Hct	33,9	21,9-34	%
MPV	15,2	13,5-19,6	fl
MCV	45,0	37,1-43	fl
MCH	15,8	13,6-15,4	pg
MCHC	35,2	34,4-38,3	g/dl
CHCM	35,3	34,3-38,4	g/dL
RDW	14,4	15,5-17,8	%
CH	15,9	13,6-15,5	pg
CHDW	2,35	2,25-2,68	pg
HDW	2,20	2,1-3,09	g/dL
PLT	229	147-361	10^3/µl
PCT	0,35	0,26-0,52	%
PDW	67,8	61,9-69,6	%

Stima PLT: ☐ inadeguata ☒ adeguata ☐ aumentata

FORMULA LEUCOCITARIA

% neutrofili segmentati	56,0	53,4-80,7	%
% linfociti	37,3	13-36,1	%
% monociti	2,4	1,6-3,5	%
% eosinofili	4,1	1,1-5,8	%
% basofili	0,1	0-0,2	%
% Luc	0,10	0,1-0,5	%

LEUCOCITI

Neutrofili segmentati	8140	4060-11640	/µl
linfociti	5410	1362-3520	/µl
monociti	350	140-440	/µl
eosinofili	590	120-620	/µl
basofili	10	0-20	/µl
LUC	20	10-57	/µl

Subject P6 – After treatment

EMOGRAMMA

WBC	9,80	7,39-16,21	10^3/µl
RBC	8,55	5,56-8,44	10^6/µl
Hgb	15,0	8,1-12,2	g/dl
Hct	41,9	21,9-34	%
MPV	16,3	13,5-19,6	fl
MCV	48,9	37,1-43	fl
MCH	17,5	13,6-15,4	pg
MCHC	35,8	34,4-38,3	g/dl
CHCM	34,0	34,3-38,4	g/dL
RDW	12,9	15,5-17,8	%
CH	16,7	13,6-15,5	pg
CHDW	2,36	2,25-2,68	pg
HDW	2,08	2,1-3,09	g/dL
PLT	110	147-361	10^3/µl
PCT	0,18	0,26-0,52	%
PDW	72,9	61,9-69,6	%

MORFOLOGIA RBC

Macro PLT	+	

Stima PLT: ☐ inadeguata ☒ adeguata ☐ aumentata

FORMULA LEUCOCITARIA

% neutrofili segmentati	72,4	53,4-80,7	%
% linfociti	24,0	13-36,1	%
% monociti	1,5	1,6-3,5	%
% eosinofili	1,9	1,1-5,8	%
% basofili	0	0-0,2	%
% Luc	0,10	0,1-0,5	%

LEUCOCITI

Neutrofili segmentati	7100	4060-11640	/µl
linfociti	2360	1362-3520	/µl
monociti	150	140-440	/µl
eosinofili	190	120-620	/µl
basofili	0	0-20	/µl
LUC	10	10-57	/µl

Subject P7 – Before treatment

EMOGRAMMA

WBC	14,61	7,39-16,21	10^3/µl
RBC	9,31	5,56-8,44	10^6/µl
Hgb	12,6	8,1-12,2	g/dl
Hct	38,8	21,9-34	%
MPV	14,6	13,5-19,6	fl
MCV	41,6	37,1-43	fl
MCH	13,6	13,6-15,4	pg
MCHC	32,6	34,4-38,3	g/dl
CHCM	32,2	34,3-38,4	g/dL
RDW	13,8	15,5-17,8	%
CH	13,4	13,6-15,5	pg
CHDW	2,00	2,25-2,68	pg
HDW	2,07	2,1-3,09	g/dL
PLT	288	147-361	10^3/µl
PCT	0,42	0,26-0,52	%
PDW	65,2	61,9-69,6	%

FORMULA LEUCOCITARIA

% neutrofili segmentati	72,1	53,4-80,7	%
% linfociti	22,8	13-36,1	%
% monociti	1,3	1,6-3,5	%
% eosinofili	3,6	1,1-5,8	%
% basofili	0,1	0-0,2	%
% Luc	0,10	0,1-0,5	%

LEUCOCITI

Neutrofili segmentati	10530	4060-11640	/µl
linfociti	3340	1362-3520	/µl
monociti	190	140-440	/µl
eosinofili	530	120-620	/µl
basofili	10	0-20	/µl
LUC	10	10-57	/µl

Stima PLT: ☐ inadeguata ☒ adeguata ☐ aumentata

Presenti aggregati piastrinici +

Note:

Subject P7 – After treatment

EMOGRAMMA

WBC	9,42	7,39-16,21	10^3/µl
RBC	6,83	5,56-8,44	10^6/µl
Hgb	9,4	8,1-12,2	g/dl
Hct	27,5	21,9-34	%
MPV	17,8	13,5-19,6	fl
MCV	40,3	37,1-43	fl
MCH	13,7	13,6-15,4	pg
MCHC	34,1	34,4-38,3	g/dl
CHCM	33,0	34,3-38,4	g/dL
RDW	14,2	15,5-17,8	%
CH	13,3	13,6-15,5	pg
CHDW	1,94	2,25-2,68	pg
HDW	1,93	2,1-3,09	g/dL
PLT	57	147-361	10^3/µl
PCT	0,10	0,26-0,52	%
PDW	66,8	61,9-69,6	%

FORMULA LEUCOCITARIA

% neutrofili segmentati	62	53,4-80,7	%
% linfociti	30	13-36,1	%
% monociti	2	1,6-3,5	%
% eosinofili	0	1,1-5,8	%
% basofili	0	0-0,2	%
% Luc	0	0,1-0,5	%

LEUCOCITI

Neutrofili banda	0		/µl
Neutrofili segmentati	5840	4060-11640	/µl
linfociti	2826	1362-3520	/µl
monociti	188	140-440	/µl
eosinofili	0	120-620	/µl
basofili	0	0-20	/µl
LUC	0	10-57	/µl

Stima PLT: ☐ inadeguata ☒ adeguata ☐ aumentata

Presenti aggregati piastrinici +++

Note:

Subject P8 – Before treatment

EMOGRAMMA

WBC	12,45	7,39-16,21	10^3/µl
RBC	9,25	5,56-8,44	10^6/µl
Hgb	12,8	8,1-12,2	g/dl
Hct	38,2	21,9-34	%
MPV	14,4	13,5-19,6	fl
MCV	41,3	37,1-43	fl
MCH	13,8	13,6-15,4	pg
MCHC	33,4	34,4-38,3	g/dl
CHCM	32,7	34,3-38,4	g/dL
RDW	14,9	15,5-17,8	%
CH	13,5	13,6-15,5	pg
CHDW	1,95	2,25-2,68	pg
HDW	2,14	2,1-3,09	g/dL
PLT	374	147-361	10^3/µl
PCT	0,54	0,26-0,52	%
PDW	63,1	61,9-69,6	%

Stima PLT: ☐ inadeguata ☒ adeguata ☐ aumentata

Note:

FORMULA LEUCOCITARIA

% neutrofili segmentati	58,0	53,4-80,7	%
% linfociti	24,3	13-36,1	%
% monociti	3,3	1,6-3,5	%
% eosinofili	14,0	1,1-5,8	%
% basofili	0,2	0-0,2	%
% Luc	0,20	0,1-0,5	%

LEUCOCITI

Neutrofili segmentati	7220	4060-11640	/µl
linfociti	3030	1362-3520	/µl
monociti	410	140-440	/µl
eosinofili	1740	120-620	/µl
basofili	30	0-20	/µl
LUC	20	10-57	/µl

Subject P8 – After treatment

EMOGRAMMA

WBC	8,03	7,39-16,21	10^3/µl
RBC	9,13	5,56-8,44	10^6/µl
Hgb	13,8	8,1-12,2	g/dl
Hct	38,2	21,9-34	%
MPV	16,2	13,5-19,6	fl
MCV	41,9	37,1-43	fl
MCH	15,1	13,6-15,4	pg
MCHC	36,2	34,4-38,3	g/dl
CHCM	32,7	34,3-38,4	g/dL
RDW	14,2	15,5-17,8	%
CH	13,7	13,6-15,5	pg
CHDW	1,93	2,25-2,68	pg
HDW	1,94	2,1-3,09	g/dL
PLT	262	147-361	10^3/µl
PCT	0,42	0,26-0,52	%
PDW	61,0	61,9-69,6	%

Stima PLT: ☐ inadeguata ☐ adeguata ☒ aumentata

Note:

FORMULA LEUCOCITARIA

% neutrofili segmentati	56	53,4-80,7	%
% linfociti	26	13-36,1	%
% monociti	2	1,6-3,5	%
% eosinofili	16	1,1-5,8	%
% basofili	0	0-0,2	%
% Luc	0	0,1-0,5	%

LEUCOCITI

Neutrofili banda	0		/µl
Neutrofili segmentati	4496	4060-11640	/µl
linfociti	2087	1362-3520	/µl
monociti	160	140-440	/µl
eosinofili	1284	120-620	/µl
basofili	0	0-20	/µl
LUC	0	10-57	/µl

Presenti aggregati piastrinici +++

ANNEX 3 – BLOOD ANALYSIS – Biochemistry (VTH of Padova)

Subject P1 – Before treatment

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA			
Fosforo	4,52	mg/dl	4,1 - 6,0
Magnesio	2,51	mg/dl	2,11 - 2,74
Calcio	**9,83**	mg/dl	8,40 - 9,52
Fosfatasi Alcalina	**116**	UI/L	38,0 - 102,5
Bilirubina totale	**0,08**	mg/dl	0,15 - 0,38
AST (GOT)	33	U/l	20 - 58
ALT (GPT)	62	U/l	31 - 84
GGT	**0,31**	U/l	0,50 - 2,52
Creatinina	1,13	mg/dl	1,10 - 1,80
Azotemia	46	mg/dl	46 - 87
Albumina	**38,46**	g/L	29,8 - 36,3
Proteine Totali	75,03	g/L	67 - 82
Rapporto A/G	**1,05**		0,47 - 0,71
Globuline	37	g/L	35,2 - 48,1
Colesterolo	**99**	mg/dl	110 - 157
Trigliceridi	62	mg/dl	44 - 128
Glicemia	145	mg/dl	106 - 173
CK	**670**	U/l	175 - 493
Cloro	**115**	mmol/l	116 - 124
Sodio (Na+) ISE	147	mEq/L	142 - 149
Potassio (K+) ISE	**3,63**	mEq/L	3,92 - 4,55
Indice di Emolisi	4,93	mg/dl	
Indice di Lipemia	0,00000	mg/dl	
Indice di Ittero	0,00000	mg/dl	

Subject P1 – After treatment

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA			
Fosforo	4,77	mg/dl	4,1 - 6,0
Magnesio	2,22	mg/dl	2,11 - 2,74
Calcio	9,15	mg/dl	8,40 - 9,52
Fosfatasi Alcalina	**4**	UI/L	38,0 - 102,5
Bilirubina totale	**0,76**	mg/dl	0,15 - 0,38
AST (GOT)	31	U/l	20 - 58
ALT (GPT)	47	U/l	31 - 84
GGT	**0,00000**	U/l	0,50 - 2,52
Creatinina	1,44	mg/dl	1,10 - 1,80
Azotemia	46	mg/dl	46 - 87
Albumina	**37,65**	g/L	29,8 - 36,3
Proteine Totali	75,30	g/L	67 - 82
Globuline	38	g/L	35,2 - 48,1
Rapporto A/G	**1,00**		0,47 - 0,71
Colesterolo	113	mg/dl	110 - 157
Trigliceridi	54	mg/dl	44 - 128
Glicemia	164	mg/dl	106 - 173
CK	**742**	U/l	175 - 493
Cloro	**115**	mmol/l	116 - 124
Sodio (Na+) ISE	**134**	mEq/L	142 - 149
Potassio (K+) ISE	**3,33**	mEq/L	3,92 - 4,55
Indice di Emolisi	219,16	mg/dl	
Indice di Lipemia	0,00000	mg/dl	
Indice di Ittero	0,24	mg/dl	

Subject P2 – Before treatment

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA			
Fosforo	**3,94**	mg/dl	4,1 - 6,0
Magnesio	2,53	mg/dl	2,11 - 2,74
Calcio	**10,44**	mg/dl	8,40 - 9,52
Fosfatasi Alcalina	**104**	UI/L	38,0 - 102,5
Bilirubina totale	0,16	mg/dl	0,15 - 0,38
AST (GOT)	32	U/l	20 - 58
ALT (GPT)	55	U/l	31 - 84
GGT	2,15	U/l	0,50 - 2,52
Creatinina	1,52	mg/dl	1,10 - 1,80
Azotemia	53	mg/dl	46 - 87
Albumina	**39,25**	g/L	29,8 - 36,3
Proteine Totali	76,29	g/L	67 - 82
Globuline	37	g/L	35,2 - 48,1
Rapporto A/G	**1,06**		0,47 - 0,71
Colesterolo	**105**	mg/dl	110 - 157
Trigliceridi	**43**	mg/dl	44 - 128
Glicemia	**86**	mg/dl	106 - 173
CK	**162**	U/l	175 - 493
Cloro	117	mmol/l	116 - 124
Sodio (Na+) ISE	149	mEq/L	142 - 149
Potassio (K+) ISE	3,98	mEq/L	3,92 - 4,55
Indice di Emolisi	0,00000	mg/dl	
Indice di Lipemia	0,00000	mg/dl	
Indice di Ittero	0,00000	mg/dl	

Subject P2 – After treatment

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA			
Fosforo	4,84	mg/dl	4,1 - 6,0
Magnesio	2,34	mg/dl	2,11 - 2,74
Calcio	**9,84**	mg/dl	8,40 - 9,52
Fosfatasi Alcalina	76	UI/L	38,0 - 102,5
Bilirubina totale	0,21	mg/dl	0,15 - 0,38
AST (GOT)	43	U/l	20 - 58
ALT (GPT)	45	U/l	31 - 84
GGT	**0,00000**	U/l	0,50 - 2,52
Creatinina	1,31	mg/dl	1,10 - 1,80
Azotemia	49	mg/dl	46 - 87
Albumina	**38,14**	g/L	29,8 - 36,3
Proteine Totali	75,53	g/L	67 - 82
Globuline	37	g/L	35,2 - 48,1
Rapporto A/G	**1,02**		0,47 - 0,71
Colesterolo	120	mg/dl	110 - 157
Trigliceridi	46	mg/dl	44 - 128
Glicemia	106	mg/dl	106 - 173
CK	**1108**	U/l	175 - 493
Cloro	**108**	mmol/l	116 - 124
Sodio (Na+) ISE	147	mEq/L	142 - 149
Potassio (K+) ISE	4,19	mEq/L	3,92 - 4,55
Indice di Emolisi	10,51	mg/dl	
Indice di Lipemia	0,00000	mg/dl	
Indice di Ittero	0,01	mg/dl	

Subject P3 – Before treatment

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA			
Fosforo	**6,62**	mg/dl	4,1 - 6,0
Magnesio	2,21	mg/dl	2,11 - 2,74
Calcio	**9,62**	mg/dl	8,40 - 9,52
Fosfatasi Alcalina	**112**	UI/L	38,0 - 102,5
Bilirubina totale	0,33	mg/dl	0,15 - 0,38
AST (GOT)	**17**	U/l	20 - 58
ALT (GPT)	42	U/l	31 - 84
GGT	<0,5	U/l	0,50 - 2,52
Creatinina	1,10	mg/dl	1,10 - 1,80
Azotemia	66	mg/dl	46 - 87
Albumina	**37,74**	g/L	29,8 - 36,3
Proteine Totali	77,44	g/L	67 - 82
Globuline	40	g/L	35,2 - 48,1
Colesterolo	**97**	mg/dl	110 - 157
Trigliceridi	118	mg/dl	44 - 128
Glicemia	**98**	mg/dl	106 - 173
CK	**145**	U/l	175 - 493
Rapporto A/G	**0,95**		0,47 - 0,71
Cloro	**114**	mmol/l	116 - 124
Sodio (Na+) ISE	**139**	mEq/L	142 - 149
Potassio (K+) ISE	**3,69**	mEq/L	3,92 - 4,55
Indice di Lipemia	150,13	mg/dl	
Indice di Emolisi	56,85	mg/dl	
Indice di Ittero	0,20	mg/dl	

Subject P3 – After treatment

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA			
Fosforo	4,74	mg/dl	4,1 - 6,0
Magnesio	2,70	mg/dl	2,11 - 2,74
Calcio	**10,52**	mg/dl	8,40 - 9,52
Fosfatasi Alcalina	58	UI/L	38,0 - 102,5
Bilirubina totale	**0,69**	mg/dl	0,15 - 0,38
AST (GOT)	35	U/l	20 - 58
ALT (GPT)	62	U/l	31 - 84
GGT	**ND**	U/l	0,50 - 2,52
Creatinina	1,42	mg/dl	1,10 - 1,80
Azotemia	52	mg/dl	46 - 87
Amiloide Sierica A	**6**	µg/ml	< 5,0
Albumina	**39,92**	g/L	29,8 - 36,3
Proteine Totali	71,99	g/L	67 - 82
Globuline	**32**	g/L	35,2 - 48,1
Rapporto A/G	**1,24**		0,47 - 0,71
Colesterolo	**109**	mg/dl	110 - 157
Trigliceridi	57	mg/dl	44 - 128
Glicemia	**99**	mg/dl	106 - 173
CK	409	U/l	175 - 493
Cloro	**113**	mmol/l	116 - 124
Sodio (Na+) ISE	145	mEq/L	142 - 149
Potassio (K+) ISE	4,28	mEq/L	3,92 - 4,55
Indice di Emolisi	215,32	mg/dl	
Indice di Lipemia	0,00000	mg/dl	

Subject P4 – Before treatment

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA			
Fosforo	**6,42**	mg/dl	4,1 - 6,0
Magnesio	2,64	mg/dl	2,11 - 2,74
Calcio	9,00	mg/dl	8,40 - 9,52
Fosfatasi Alcalina	**146**	UI/L	38,0 - 102,5
Bilirubina totale	**0,50**	mg/dl	0,15 - 0,38
AST (GOT)	**65**	U/l	20 - 58
ALT (GPT)	**102**	U/l	31 - 84
GGT	**0,00000**	U/l	0,50 - 2,52
Creatinina	1,31	mg/dl	1,10 - 1,80
Azotemia	53	mg/dl	46 - 87
Albumina	35,88	g/L	29,8 - 36,3
Proteine Totali	71,83	g/L	67 - 82
Globuline	36	g/L	35,2 - 48,1
Rapporto A/G	**1,00**		0,47 - 0,71
Colesterolo	**102**	mg/dl	110 - 157
Trigliceridi	**28**	mg/dl	44 - 128
Glicemia	**184**	mg/dl	106 - 173
CK	**3270**	U/l	175 - 493
Cloro	**115**	mmol/l	116 - 124
Sodio (Na+) ISE	**151**	mEq/L	142 - 149
Potassio (K+) ISE	**5,37**	mEq/L	3,92 - 4,55
Indice di Emolisi	140,01	mg/dl	
Indice di Lipemia	42,23	mg/dl	
Indice di Ittero	0,17	mg/dl	

Subject P4 – After treatment

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA			
Fosforo	4,27	mg/dl	4,1 - 6,0
Magnesio	2,62	mg/dl	2,11 - 2,74
Calcio	9,14	mg/dl	8,40 - 9,52
Fosfatasi Alcalina	75	UI/L	38,0 - 102,5
Bilirubina totale	0,25	mg/dl	0,15 - 0,38
AST (GOT)	**13**	U/l	20 - 58
ALT (GPT)	**30**	U/l	31 - 84
GGT	1,88	U/l	0,50 - 2,52
Creatinina	1,28	mg/dl	1,10 - 1,80
Azotemia	56	mg/dl	46 - 87
Albumina	**39,31**	g/L	29,8 - 36,3
Proteine Totali	**65,55**	g/L	67 - 82
Globuline	**26**	g/L	35,2 - 48,1
Rapporto A/G	**1,50**		0,47 - 0,71
Colesterolo	**105**	mg/dl	110 - 157
Trigliceridi	**154**	mg/dl	44 - 128
Glicemia	**226**	mg/dl	106 - 173
CK	**128**	U/l	175 - 493
Cloro	116	mmol/l	116 - 124
Sodio (Na+) ISE	144	mEq/L	142 - 149
Potassio (K+) ISE	4,18	mEq/L	3,92 - 4,55
Indice di Emolisi	19,84	mg/dl	
Indice di Lipemia	73,00	mg/dl	
Indice di Ittero	0,44	mg/dl	

Subject P5 – Before treatment

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA			
Fosforo	4,22	mg/dl	4,1 - 6,0
Magnesio	2,36	mg/dl	2,11 - 2,74
Calcio	**9,85**	mg/dl	8,40 - 9,52
Fosfatasi Alcalina	80	UI/L	38,0 - 102,5
Bilirubina totale	**0,07**	mg/dl	0,15 - 0,38
AST (GOT)	31	U/l	20 - 58
ALT (GPT)	62	U/l	31 - 84
GGT	2,37	U/l	0,50 - 2,52
Creatinina	1,10	mg/dl	1,10 - 1,80
Azotemia	56	mg/dl	46 - 87
Albumina	**41,26**	g/L	29,8 - 36,3
Proteine Totali	79,01	g/L	67 - 82
Globuline	38	g/L	35,2 - 48,1
Rapporto A/G	**1,09**		0,47 - 0,71
Colesterolo	**88**	mg/dl	110 - 157
Trigliceridi	**34**	mg/dl	44 - 128
Glicemia	**355**	mg/dl	106 - 173
CK	430	U/l	175 - 493
Cloro	**107**	mmol/l	116 - 124
Sodio (Na+) ISE	146	mEq/L	142 - 149
Potassio (K+) ISE	4,19	mEq/L	3,92 - 4,55
Indice di Emolisi	16,07	mg/dl	
Indice di Lipemia	0,00000	mg/dl	
Indice di Ittero	0,00000	mg/dl	

Subject P5 – After treatment (not available)

Subject P6 – Before treatment

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA			
Fosforo	4,52	mg/dl	4,1 - 6,0
Magnesio	2,18	mg/dl	2,11 - 2,74
Calcio	9,20	mg/dl	8,40 - 9,52
Fosfatasi Alcalina	**271**	UI/L	38,0 - 102,5
Bilirubina totale	0,16	mg/dl	0,15 - 0,38
AST (GOT)	**13**	U/l	20 - 58
ALT (GPT)	38	U/l	31 - 84
GGT	1,70	U/l	0,50 - 2,52
Creatinina	1,40	mg/dl	1,10 - 1,80
Azotemia	53	mg/dl	46 - 87
Amiloide Sierica A	**7**	µg/ml	< 5,0
Paraoxonase	87	U/L	> 75 -
Albumina	31,11	g/L	29,8 - 36,3
Proteine Totali	**65,41**	g/L	67 - 82
Globuline	**34**	g/L	35,2 - 48,1
Rapporto A/G	**0,91**		0,47 - 0,71
Colesterolo	**103**	mg/dl	110 - 157
Trigliceridi	**40**	mg/dl	44 - 128
Glicemia	130	mg/dl	106 - 173
CK	**152**	U/l	175 - 493
Cloro	**111**	mmol/l	116 - 124
Sodio (Na+) ISE	**153**	mEq/L	142 - 149
Potassio (K+) ISE	4,02	mEq/L	3,92 - 4,55
Indice di Emolisi	6,51	mg/dl	

Subject P6 – After treatment

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA			
Fosforo	**3,38**	mg/dl	4,1 - 6,0
Magnesio	2,22	mg/dl	2,11 - 2,74
Calcio	9,36	mg/dl	8,40 - 9,52
Fosfatasi Alcalina	67	UI/L	38,0 - 102,5
Bilirubina totale	0,19	mg/dl	0,15 - 0,38
AST (GOT)	24	U/l	20 - 58
ALT (GPT)	40	U/l	31 - 84
GGT	**2,60**	U/l	0,50 - 2,52
Creatinina	**1,86**	mg/dl	1,10 - 1,80
Azotemia	55	mg/dl	46 - 87
Blood Urea Nitrogen	25,70	mg/dl	
Albumina	35,31	g/L	29,8 - 36,3
Proteine Totali	75,58	g/L	67 - 82
Globuline	40	g/L	35,2 - 48,1
Rapporto A/G	**0,88**		0,47 - 0,71
Colesterolo	**96**	mg/dl	110 - 157
Trigliceridi	44	mg/dl	44 - 128
Glicemia	**101**	mg/dl	106 - 173
CK	**146**	U/l	175 - 493
Cloro	**115**	mmol/l	116 - 124
Sodio (Na+) ISE	147	mEq/L	142 - 149
Potassio (K+) ISE	4,04	mEq/L	3,92 - 4,55
Indice di Emolisi	28,64	mg/dl	
Indice di Lipemia	1,57	mg/dl	

Subject P7 – Before treatment

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA			
Fosforo	**4,07**	mg/dl	4,1 - 6,0
Magnesio	2,26	mg/dl	2,11 - 2,74
Calcio	9,00	mg/dl	8,40 - 9,52
Fosfatasi Alcalina	**137**	UI/L	38,0 - 102,5
Bilirubina totale	**0,12**	mg/dl	0,15 - 0,38
AST (GOT)	41	U/l	20 - 58
ALT (GPT)	**118**	U/l	31 - 84
GGT	0,82	U/l	0,50 - 2,52
Creatinina	1,39	mg/dl	1,10 - 1,80
Azotemia	52	mg/dl	46 - 87
Albumina	**29,34**	g/L	29,8 - 36,3
Proteine Totali	70,00	g/L	67 - 82
Globuline	41	g/L	35,2 - 48,1
Rapporto A/G	**0,72**		0,47 - 0,71
Colesterolo	**72**	mg/dl	110 - 157
Trigliceridi	**22**	mg/dl	44 - 128
Glicemia	**76**	mg/dl	106 - 173
CK	**1567**	U/l	175 - 493
Cloro	117	mmol/l	116 - 124
Sodio (Na+) ISE	149	mEq/L	142 - 149
Potassio (K+) ISE	4,17	mEq/L	3,92 - 4,55
Indice di Emolisi	14,83	mg/dl	
Indice di Lipemia	0,00000	mg/dl	
Indice di Ittero	0,00000	mg/dl	

Subject P7 – After treatment

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA			
Fosforo	5,15	mg/dl	4,1 - 6,0
Magnesio	2,15	mg/dl	2,11 - 2,74
Calcio	**8,34**	mg/dl	8,40 - 9,52
Fosfatasi Alcalina	**108**	UI/L	38,0 - 102,5
Bilirubina totale	**0,12**	mg/dl	0,15 - 0,38
AST (GOT)	21	U/l	20 - 58
ALT (GPT)	40	U/l	31 - 84
GGT	1,09	U/l	0,50 - 2,52
Creatinina	1,50	mg/dl	1,10 - 1,80
Azotemia	47	mg/dl	46 - 87
Albumina	31,22	g/L	29,8 - 36,3
Proteine Totali	72,22	g/L	67 - 82
Globuline	41	g/L	35,2 - 48,1
Rapporto A/G	**0,76**		0,47 - 0,71
Colesterolo	**101**	mg/dl	110 - 157
Trigliceridi	**22**	mg/dl	44 - 128
Glicemia	157	mg/dl	106 - 173
CK	**173**	U/l	175 - 493
Cloro	118	mmol/l	116 - 124
Sodio (Na+) ISE	145	mEq/L	142 - 149
Potassio (K+) ISE	4,55	mEq/L	3,92 - 4,55
Indice di Emolisi	1,46	mg/dl	
Indice di Lipemia	39,48	mg/dl	
Indice di Ittero	0,00000	mg/dl	

Subject P8 – Before treatmen

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA			
Fosforo	**3,63**	mg/dl	4,1 - 6,0
Magnesio	2,32	mg/dl	2,11 - 2,74
Calcio	8,96	mg/dl	8,40 - 9,52
Fosfatasi Alcalina	**126**	UI/L	38,0 - 102,5
Bilirubina totale	**0,07**	mg/dl	0,15 - 0,38
AST (GOT)	25	U/l	20 - 58
ALT (GPT)	54	U/l	31 - 84
GGT	1,31	U/l	0,50 - 2,52
Creatinina	1,26	mg/dl	1,10 - 1,80
Azotemia	71	mg/dl	46 - 87
Albumina	32,51	g/L	29,8 - 36,3
Proteine Totali	71,64	g/L	67 - 82
Globuline	39	g/L	35,2 - 48,1
Rapporto A/G	**0,83**		0,47 - 0,71
Colesterolo	**87**	mg/dl	110 - 157
Trigliceridi	**34**	mg/dl	44 - 128
Glicemia	**81**	mg/dl	106 - 173
CK	**167**	U/l	175 - 493
Cloro	117	mmol/l	116 - 124
Sodio (Na+) ISE	**152**	mEq/L	142 - 149
Potassio (K+) ISE	4,03	mEq/L	3,92 - 4,55
Indice di Emolisi	0,90	mg/dl	
Indice di Lipemia	0,90	mg/dl	
Indice di Ittero	0,00000	mg/dl	

Subject P8 – After treatment

Test	Risultato	U. M.	Valori di normalità
BIOCHIMICA			
Fosforo	4,42	mg/dl	4,1 - 6,0
Calcio	8,82	mg/dl	8,40 - 9,52
Fosfatasi Alcalina	53	UI/L	38,0 - 102,5
Bilirubina totale	**0,11**	mg/dl	0,15 - 0,38
AST (GOT)	**14**	U/l	20 - 58
ALT (GPT)	**24**	U/l	31 - 84
GGT	1,60	U/l	0,50 - 2,52
Creatinina	1,36	mg/dl	1,10 - 1,80
Azotemia	64	mg/dl	46 - 87
Albumina	33,10	g/L	29,8 - 36,3
Proteine Totali	**63,55**	g/L	67 - 82
Globuline	**30**	g/L	35,2 - 48,1
Rapporto A/G	**1,09**		0,47 - 0,71
Colesterolo	132	mg/dl	110 - 157
Trigliceridi	**32**	mg/dl	44 - 128
Glicemia	125	mg/dl	106 - 173
CK	**124**	U/l	175 - 493
Cloro	119	mmol/l	116 - 124
Sodio (Na+) ISE	147	mEq/L	142 - 149
Potassio (K+) ISE	4,38	mEq/L	3,92 - 4,55
Indice di Emolisi	35,84	mg/dl	
Indice di Lipemia	19,21	mg/dl	
Indice di Ittero	0,03	mg/dl	

Annotazioni:
H.I.L. INDEX
Indice di Emolisi 0.0 - 125 mg/dl nessuna interferenza

ANNEX 4 – CLINICAL CHECKLIST

SUBJECT N.
Weight:

OWNER
Date :

Objective clinical examination and ultrasound	❌ / ✓	**Note**
Skeletal state and constitution		
Body state of nutrition and muscle tone		
Level of consciousness and particular signs and attitude		
Skin and subcutaneous tissue		
Mucous membrane color and capillary refill time		
Lymph nodes		
Body temperature		
Respiratory rate		
Heart rate		
Great organic functions		
Reproductive tract examination		
Ultrasound evaluation		
Sedazione		

Sampling collection	❌ / ✓	**Note**
Blood sample		
Urine sample		
Blood serum (for Leptin evaluation)		
Vaginal smear		
Ormonal dosage		

ANNEX 5 - Histological diagnosis of the ovary and uterus of subject P2

Servizio Diagnostico di Anatomia Patologica Veterinaria

BCA Dipartimento di Biomedicina Comparata ed Alimentazione

Referto: AR667	**Data:** 22/02/2023	
Veterinario: Dipartimento MAPS	Viale dell'Universita' 16, 35020 Legnaro PD	Ospedale Veterinario Universitario Didattico
[redacted]		
Specie: Gatto (Felis silvestris catus)	**Razza:** Persiano	**Sesso:** Femmina sterilizzata, **Età:** 6 anni 7 mesi
Esame AR667/1: Istologico Standard		
Campione AR667/1/1: Sistema riproduttore Ovaio		
Campione inviato: Ovaio sinistro (con filo da sutura)		
Tessuto presente nel campione: ovaio in toto		
Diagnosi: cisti della rete ovarii		
Aspetto microscopico: presenza di formazioni cistiche di diametro variabile, alcune localizzate all'interno del parenchima ovarico, altre a livello dell'ilo. Le cisti sono delimitate da un monostrato di epitelio cubico o appiattito, multifocalmente ciliato, privo di caratteri di atipia. Il restante parenchima è caratterizzato da follicoli in diverso stadio di sviluppo, in assenza di corpi lutei.		
Campione AR667/1/2: Sistema riproduttore Ovaio		
Campione inviato: Ovaio destro		
Tessuto presente nel campione: ovaio in toto		
Diagnosi: cisti della rete ovarii		
Aspetto microscopico: quadro simile a quello osservato nel campione 1.		
Campione AR667/1/3: Sistema riproduttore Utero		
Campione inviato: utero in toto		
Tessuto presente nel campione: utero in toto		
Diagnosi: campione nella norma		
Aspetto microscopico: sezioni caratterizzate unicamente da lievi emorragie mucosali superficiali multifocali e dalla presenza di alcuni emosiderofagi sparsi in profondità nella propria-sottomucosa .		

ACKNOWLEDGMENTS

Un breve ma sentito ringraziamento a color oche mi hanno accompagnato in questo lungo e meraviglioso percorso, alla mia famiglia, i miei amici di sempre, gli animatori AC, i miei “bimbi”, i miei compagni di uni, le mie coinquiline, la mia dolce metà.. GRAZIE, senza di voi non sarei la persona che sono ora.

Un ringraziamento speciale al Professor Romagnoli, per l’infinita pazienza e per essere sempre stato disponibile a darmi una mano ed insegnarmi. Grazie a Maria, sei una santa donna, te l’ho detto molte volte. Senza di te non ce l’avrei fatta. Un grazie ed un abbraccio a Maja, che mi ha ospitato a Ljubljiana e mi ha fatto sentire a casa, coinvolgendomi nella realtà della clinica slovena.

Un grazie all’Agripolis e a Legnaro, rimarrete nel mio cuore per sempre.